Embankment as a carbon sink : a study on carbon sequestration pathways and mechanisms in topsoil and exposed subsoil

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Embankment as a carbon sink: a study on carbon sequestration pathways and mechanisms in topsoil and exposed subsoil

Présentée par Lorenzo MW Rossi
Le 09 Décembres 2019

Sous la direction de Alexia Stokes
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"Se l'organicazione del carbonio non si svolgesse quotidianamente attorno a noi, [...], dovunque affiori il verde di una foglia, le spetterebbe di pieno diritto il nome di miracolo."

Primo Levi, 'La tavola periodica' , Einaudi, 1975, pp. 261

"If the elaboration of carbon were not a common daily occurrence, […], wherever the green of a leaf appears, it would by full right deserve to be called a miracle."

Primo Levi, 'The periodic table', Einaudi, 1975, pp. 261

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DISCLAIMER

Résumé

La séquestration du carbone (C) fait l'objet d'une attention scientifique et politique croissante dans le cadre de la réduction des gaz à effet de serre. Cependant, les sols géotechniques ont été négligés en raison de leur potentiel de séquestration du carbone, et l'attention mondiale étant concentrée sur les sols agricoles et naturels. Dans le présent projet de thèse, nous visons à évaluer le potentiel des talus géotechniques comme puits de carbone et, par l'étude des espèces végétales et des sols présentant des caractéristiques contrastées, à mettre en lumière les mécanismes de séquestration du carbone organique et les rôles des différents acteurs impliqués. Nous visons non seulement à quantifier le C gagné et perdu dans le sol, mais aussi son origine (nouveau C frais et ancien C préexistant) et comment il est réparti dans différents pools de C qui montrent une stabilité du C différente (qualité du C stocké). Tout d'abord, nous avons évalué la séquestration du carbone dans différents pools de carbone sous un sol semé de 12 espèces herbacées différentes dans une expérience de 10 mois. En évaluant les différentes caractéristiques des racines, nous nous sommes concentrés sur leurs corrélations avec le stockage du C dans différents bassins de C du sol.. Nous avons montré que les espèces dont les caractéristiques racinaires sont associées à une production élevée de C labile entraînent une augmentation plus élevée de C dans le pool stable de SILT+CLAY (<20µm). Les espèces dont les traits racinaires sont associés à un faible apport de C récalcitrant favorisent plutôt l'accumulation dans la fraction POM instable. Ensuite, grâce à une expérience de marquage isotopique stable de 183 jours (CO_2 constamment enrichi en ^{13}C), nous avons pu étudier la dynamique du C dans différents pools de C sous deux espèces (*Lolium perenne* and *Medicago sativa*) sur deux sols (terre végétale, profondeur 0-30 cm et sol remonté, profondeur 110-140 cm) aux caractéristiques opposées. Nous avons mis en évidence le grand intérêt de faire le pont entre l'origine du C et les pools de C lors de l'étude des destins du C du sol, ce qui permet de dévoiler des processus que les méthodes plus traditionnelles cachent. Le nouveau C et l'ancien C présentaient une covariation synergique, avec des pertes plus faibles de l'ancien C associées à de nouvelles entrées de C plus élevées. Une part plus importante de nouveau C utilisé par les communautés microbiennes comme substrat peut expliquer ce comportement synergique. La théorie d'une plus grande quantité de nouveau C minéralisé par les communautés microbiennes a également été validée par l'étude de "priming effect" et de la respiration du sol, qui a montré que la concentration de C provenaient par le plantes dans le CO_2 inhalé par le sol était plus élevée lorsque l'apport de C par les plantes était élevé, au contraire augmentant la concentration de C provenaient par la minéralisation de l'anciennes C lorsque les input de C par le plants étaient faibles, c.-à-d. en sous sol.. Nous avons observé de nouveaux apports significatifs de C d'origine végétale dans la fraction SILT+CLAY (<20µm, très stable). Ces résultats viennent étayer le rôle des communautés microbiennes dans la consommation et le transfert de C dans cette fraction fine protégée sous forme de nécromasse et d'exopolysaccharides, comme le confirment les fortes corrélations positives constatées entre l'activité microbienne et l'augmentation de C dans la fraction SILT+CLAY.. L'effet de l'espèce s'est produit principalement sur les intrants de nouveaux C, mais il a été maîtrisé par l'effet du sol, avec un stockage de C plus faible dans un sol de faible qualité (faible activité et biomasse d'azote et microbienne). En général, les conditions microbiologiques ont été le principal moteur de la nouvelle accumulation de C et de l'ancienne perte de C et ont aidé à expliquer pourquoi aucun effet de la saturation en C du sol - une théorie centrale dans des études récentes sur la séquestration de C - n'a été trouvé dans le carbone protégé. Cette compréhension fondamentale des interactions plantes-sol nous aide à mieux optimiser la gestion des sols et de la végétation pour la revégétalisation des talus des routes.

Abstract

Carbon (C) sequestration is receiving increasing scientific and political attention in a framework of greenhouse gasses mitigation. However, geotechnical soils have been neglected for their C sequestration potential, with the global attention focusing on agricultural and natural soils. In the present thesis project, we aim to assess the potential of geotechnical embankments as C sink, and, through the study of plant species and soils showing contrasting features, shed light on C sequestration mechanisms and the role of the different actors involved. We aim not only to quantify the C gained and lost in soil, but even its origin (fresh new C input or old preexistent C) and how it is partitioned in different C pools characterized by different C stability (quality of stored C). First, we evaluated the C storage in different pools under soil sowed with 12 different herbaceous species in a 10 months experiment. Assessing different root traits we focused on their correlations with C storage in different soil C pools. We showed how traits linked to high labile C are linked to a higher C increase in the stable SILT+CLAY pool (<20µm). Root traits related to a low input of recalcitrant carbon, instead, favor accumulation in the unstable POM fraction. We designed a 183 days stable isotope labelling experiment (CO_2 constantly enriched with ^{13}C) and we were able to study the C dynamics in different C pools under two species (*Lolium perenne* and *Medicago sativa*) sowed on two soil (topsoil, 0-30cm depth and subsoil brought to the surface, 110-140 cm depth) showing contrasting characteristics. We evidenced the importance of bridging C origin and C pools when studying soil C fates, allowing unveiling processes those more traditional methods would hide. New C and old C showed synergetic covariation, with lower old C losses associated to higher new C inputs. A higher share of new C utilized by microbial communities as substrate can explain this synergetic behavior. The theory of a higher amount of new C mineralized by microbial communities was also validated with the study of priming effect and soil respiration, that showed higher plant derived C in respired CO_2 when plant C input was high, while increasing old C mineralization when plant C input was low, i.e. in subsoil. We observed significant plant derived new C input in the SILT+CLAY fraction (<20µm, highly stable). These results are supporting evidences for the role of microbial communities in consuming and transferring C in this protected fine fraction in form of necromass and exopolysaccharides, as confirmed by the strong positive correlations found between microbial activity and C increase in SILT+CLAY fraction. The species effect mainly occurred on new C input, but it was overpowered by the soil effect, with lower C storage in low quality soil (low nitrogen and microbial biomass and activity). In general, microbiological conditions were the main driver for new C accumulation and old C loss, and helped to explain why no effect of soil C saturation – a central theory in recent studies on C sequestration - was found in the protected carbon. Such fundamental understanding of plant-soil interactions helps us to better optimize soil and vegetation management for road embankment revegetation

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CHAPTER I: General introduction

1.1. CONTEXT

Soil holds the second largest terrestrial carbon (C) pool (1500 to 2400 GtC to a depth of one meter, IPCC 2014; Adams et al. 1990; Anderson 1992; Eswaran et al. 1993; Batjes 1996) with possibly another 900Gt at a depth of 1-2 m (Batjes 1996, Jobbagy et al. 2000), after the lithosphere but in front of vegetation (350 to 550 GtC, mainly in forests) and atmosphere (829 GtC, IPCC 2014). Soil shares the common interface with all the other spheres and thus plays a key role in driving the global C cycle. How to prevent C loss from soil and how to sequester more C into soils has become one of the most important scientific and political quests in global change biology (Sauerbeck 2001; Lal 2004). The European Union is actively involved in this issue, and the topsoil soil organic C content is an official indicator for the EU sustainable Development Goals (EU-SDG, 2018), leading to the funding and supervision of several programs focused on soil conservation and soil C increase. Some examples, among others, that involve assessment of soil organic carbon and potential sequestration, showing the interest and importance of this topic, are the CIRCASA project (https://www.circasa-project.eu/), LANDMARK project (http://landmark2020.eu/), iSQAPER project (http://www.isqaper-project.eu/), and LUCAS project (https://esdac.jrc.ec.europa.eu/projects/lucas), all funded in the framework of Horizon2020. The FAO is also involved in numerous projects focusing on soil health, that among other things underline the importance of soil C increase for climate change mitigation, like GSOCmap (http://54.229.242.119/GSOCmap/) or the Intergovernmental Technical Panel on Soils (ITPS) (http://www.fao.org/global-soil-partnership/intergovernmental-technical-panel-soils/en/). Similarly, on a national basis, different projects have been developed focusing on the potential of soil C storage for climate change mitigation. One of the most striking examples is the 4p1000 initiative (https://www.4p1000.org/), launched by France on 1 December 2015 at the COP 21, stating that increasing by 4 ‰ the soil C stock in agricultural soils would completely remove the excess of CO_2 in the atmosphere produce by anthropic actions. However, studies on strategies of C sequestration in soils are mainly limited to “green systems” (e.g. forests, grasslands, plantations, croplands, wetland etc.), where the soils are considered to be or potentially to be, a C sink. We argue that, in an irrevocable era of industrialization and urbanization, soil in “grey systems” connected with geotechnical infrastructure industry must be taken into consideration for soil C sequestration. There are two main reasons for that: i) the high environmental impact of geotechnical industry, in particular on CO_2 emissions, that needs to be mitigated, and ii) the drastic increase of geotechnical infrastructures, in particular road and railroads, which means that soils connected to geotechnical infrastructures can no longer be ignored for their potential ecosystem services, among which is soil C sequestration.

1.1.1. Impact of geotechnical structures on greenhouse gasses emissions and TERRE project

It is well known how construction activities and practices commonly related to geotechnical engineering have a high environmental impact, negatively influencing climate change, soil sealing, erosion, deforestation, desertification, ozone depletion and general air/water/soil pollution (Kibert 2008; Misra and Basu, 2011). Regarding the impact of these practices on CO_2 emissions, numbers can vary according to different sources, but there is general agreement that construction and infrastructure have a high impact on global greenhouse gas emissions. Global CO_2 emissions from construction work are attested in a range of 25-40% of the total CO_2 emissions (Dixit et al. 2010, O’Riordan et al. 2011). It is well established among researchers, policy makers and practitioners how a switch towards sustainable geotechnical solutions is not only desirable but absolutely vital to face the challenges of climate change mitigation, and to move toward a sustainable future (Dejong et al., 2011; Misra and Basu, 2011; Gallipoli and Mendes, 2017). In this optic, the current thesis is financed by the Marie Skłodowska-Curie Innovative Training Networks (ITN-ETN) TERRE (http://www.terre-etn.com/): Training Engineers and Researchers to Rethink geotechnical Engineering for a low carbon future. The aim of the TERRE project is to develop new geo-technologies to address the challenge of a low carbon impact European construction industry. In the TERRE action, multiple interdisciplinary projects have been developed under a wide umbrella of practices: optimization of energy requirement for construction, role of plants to increase soil stability via root reinforcement and hydraulic suction, new low C impact materials for construction, etc. The present thesis project aims to investigate the role of geotechnical embankments as C sinks.

1.1.2. Road and railroad development

In the last decades, highways and railroad systems have dramatically increased their surface. Especially in developing and emerging countries, major investments have been made to increase and expand the infrastructure systems, since connections among countries and cities are one of the fundamental aspects of economic growth. Globally, the railroad system increased its length by 100000km in the last year (https://data.worldbank.org/topic/infrastructure). The Chinese public roads passed from 3.5 to 4.8 million km in the last 10 years (http://statista.com). In India, in the last 4 years, the length of the highways increased by 60000km, and other 200000km of highways are expected to be finished by 2022 (https://www.ibef.org/industry/roads-india.aspx). Another striking example of the massive future infrastructure development is the China's ‘Belt and Road’ initiative, planning to connect via a complex system of roads and maritime route, Asia, Africa and Europe. Together with the development of the Trans-African Highway, consisting of 60000km of highways started in 1971 by United Nations Economic Commission for Africa and not yet completed, we have a picture of the dramatic increase of the global infrastructure system. This overview clearly shows how the soils connected with geotechnical work, in particular with the construction of road and railroad infrastructures (hereafter ‘geotechnical soils’), are increasingly important and any potential benefits and ecosystem services need to be explored. Road and railroad embankments play a pivotal role in the interactions between environment and infrastructure, and a correct design could increase the ecosystem services they can provide.

1.1.3. Development of sustainable geotechnical practices

Efforts to increase the sustainability of geotechnical structures have already been made. Practices included the use of alternative ecofriendly materials, use of bio-engineering on slopes, reuse and restauration of older structures, underground energy storage, and use of geothermal energy (Misra and Basu, 2011). However, all these practices are based on the reduction of CO_2 emissions. Such a framework relies on a passive role of geotechnical structures (new technologies to reduce CO_2 emission), but ignoring the potential active role that geotechnical soil can have in reducing atmospheric CO_2 concentration via soil organic C sequestration.

1.1.4. Geotechnical embankments: a new hotspot for soil carbon sequestration?

Geotechnical soils present some unique features that could potentially make them achieve efficient soil carbon sequestration. The main general feature is that geotechnical soils do not present specific constraints regarding their use. The objective of agricultural soils is the production of food and goods for direct consumption or to be placed on the market. Therefore, agricultural soils have an “economical constraint”, and the objective of stakeholders, even in a framework of sustainability, is to increase or maintain production without depleting soils. In a natural ecosystem, it is possible to talk about an ‘ecological constraint’, in the sense that it is not possible to modify the environment to increase soil C storage without disturbing the ecological balance and networks of the systems, affecting the health of the system itself and, ultimately, the ecosystem services that it provides to the community.

Geotechnical soils, instead, are heavily anthropized soils, where the ecological balance has already been disturbed. Moreover, soils are frequently moved from other areas or dug and brought to the surface, changing the soil composition, microbiology, fertility and, ultimately, their ecological value. Vegetation planted on geotechnical soils, especially on embankments, is not used for agricultural production. Re-vegetation is therefore artificially implanted, and there are few ecological or economic constraints. These soils and the plants used for revegetation can be chosen and planned to promote regulating, supporting and cultural ecosystem services, including embankment stability maintenance, erosion control, noise dissipation, traffic air pollution isolation, biodiversity conservation and aesthetical effect against driver fatigue. Among these ecosystem services, in particular, we argue that, contrary to agricultural and natural systems, geotechnical soils can be actively designed for CO_2 sequestration. Dejong et al. (2011) advocated the possibility of using geotechnical soils to efficiently store C by i) selecting plants that efficiently fix and move C into soil, ii) study different microbial communities that influence soil C cycle and the potential of inoculation, iii) selection of different soils with higher potential for organomineral interaction and C protection, and iv) using soil improvers (like recycled concrete and furnace slag) to increase C sequestration. However, no specific studies have been implemented to really investigate the C sequestration potential of geotechnical soils and how to maximize it. Therefore, in this thesis I aim to start investigating the potential for designing efficient C sequestering embankments, starting with the main issue of soil and plant selection.

1.1.5. Embankment design

When designing an embankment, the structure is based on a core of clay soil compacted according to a Proctor compaction test, to achieve maximum dry density (Standard Australia, 2003). This compacted soil core is usually covered with a 30-50 cm layer of uncompacted soil for revegetation (Fig. 1). The construction and design of the embankment is outside the scope of this research, where only the soil layer used for revegetation is considered for potential soil C storage. This soil layer is usually stripped topsoil (≅ 30cm) collected in the area and conserved, while the clay core is usually subsoil excavated, mixed and, if needed, adjusted with additional soils or soil improvers to achieve the optimal density level to support the structure. However, often the layer of topsoil is collected and transported to the construction site from other fertile areas, with a high impact on CO_2 emissions (for the transport) and on environment (for the ecological value of fertile topsoil). We argue that, to improve the sustainability of the embankments, instead of using valuable topsoil for revegetation, mineral subsoil (> 1m depth) collected in the area can be prepared and used for revegetation. Compared to stripped topsoil, subsoil embankments are more economically interesting, but usually demand higher constraints in plant selection due to their less favorable growing conditions (although recent soil inoculation techniques can improve this). Herbaceous plants are essential materials for embankment revegetation. Herbaceous plants usually demand low maintenance cost and intensity, with one or two cuts per year to maintain vegetation vigor. The choice of soil (organic topsoil *versus* mineral subsoil) and vegetation will deeply influence the potential for C sequestration.

However, to effectively enhance C sequestration in geotechnical soils, a better understanding of the mechanisms behind the plant-soil C-cycle is necessary. There is a need to understand the influence of different plants on soil C sequestration and their relationships with soil and microbiological communities, to allow the design of the best practices for soil C sequestration, in geotechnical and non-geotechnical soils.

1.1.6. Plants: the primary source of carbon input in soil

Plants act as conduits to transport C from atmosphere to soil (Fig. 2). Plants regulate the uptake and fixation of CO_2 in different organic forms via photosynthesis, using water and atmospheric CO_2 as ‘raw materials’ and light as an energy source (Chan, 2008). Plants also regulate the input of C in soil via two main processes: 1) plant biomass from roots and shoots in the form of litter, forming soil particulate organic matter (POM) and 2) root exudates and other labile C compounds released by roots during plant growth (Hungate et al. 1997; Lal, 2004) (Fig. 2).

With regard to C input, the first process strongly influencing the C-cycle is C input in forms of residues derived from vegetation. The selection of plants can considerably influence the C input in soil in terms of quantity (biomass production) and quality. Biomass production and related amount of C input is not the only driver for soil organic C accumulation. It has now been observed that the litter quality, especially regarding the C:N ratio of plant tissues, will strongly influence the decomposability of POM and its residence time in the soil (Castellano et al. 2015). C from exudates also represents a major amount of C that plants transfer from the atmosphere to soil (Balesdent and Balabane, 1996). Estimates vary from more conservative values, such as 5 - 33% of daily photoassimilates (De Deyn et al. 2008), to 40-60% (Högberg et al. 2001; Clemmensen et al. 2013; Keiluweit et al. 2015;) to up to 80% of photosynthetically assimilated C moved in 10 days via exudates in soil (Reid and Mexal, 1977). The input from exudates has traditionally been seen as the 'labile C input' that is consumed and respired quickly in the soil system. However, recent studies showed how C protected via organomineral complexation on minerals and in aggregates mainly derive from plant exudates or microbiological exudates and exopolysaccharides, that in turn originate from plant labile C input consumption and complexation (Lorenz and Lal 2005; Six et al. 2006; Cotrufo et al., 2013; Vidal et al., 2018).

In a recent review, Poirier *et al*., (2018) has argued that the root traits that most influence C stabilization are those related to chemical composition, root exudation and the presence of symbionts (mycorrhizas and dinitrogen (N_2) -fixing *Rhizobium*), whereas the role of morphological traits is not yet clear. More specifically, root traits increasing recalcitrance promote short-term C stabilization by slowing decomposition rates, but traits that reduce recalcitrance contribute to long-term C stabilization via the reaction of microbial products with mineral surfaces. Although several studies have analyzed the link between plant functional traits, microbial activity and C accumulation (Chapin 2003; Lavorel *et al*., 2007; De Deyn *et al*., 2008; Poirier et al. 2018), as yet, no study has focused on how root growth and specific traits can alter the accumulation and potential persistence of different soil C pools, that are linked to the physical structure of soil itself (see Cardinael et al., 2015; Fujisaki *et al*. 2018).

C entering the soil can face two main fates: be consumed by microorganisms and leave the soil pool via microbiological respiration, or be stored in the soil for different periods of time, often after being processed by microorganisms.

1.1.7. Microbiological communities: the carbon pump in different soil fractions

Microbiological communities can be identified as a further main actor for the C storage in soil (Fig. 2). Soil organic C consumption by microorganisms will mainly depend by their substrate use efficiency, meaning the proportion of the C used by microorganisms for biomass growth or enzyme production (part of the C stock in soil) and the C respired or mineralized (Lekkerkerk et al., 1990). The balance between these two fluxes, accumulation in biomass and/or via microbial exudation and loss via respiration, will depend on different factors related to substrate quality, (C:N ratio, molecular complexity, molecular weight and solubility) and the efficiency of different microbiological communities to degrade organic C in soil (Lekkerkerk et al., 1990, Cotrufo et al, 2013) which can vary by microbiological abundance, composition and partition between bacterial and fungal communities (Six et al., 2006). Microorganisms are also mainly responsible for C transformation in soil, consuming C input of plants in form of exudates or root debris, and 'pumping' it into the soil structures and in contact with mineral surfaces, in the form of microbial exudates and exopolysaccharides (Cotrufo et al., 2013; Vidal et al.; 2018). This active role of microbes have been formalized by the conceptual framework of 'microbial C pump' by Liang et al. (2017). However, this framework does not consider the destination of microbial derived C in different soil C pools. When in symbiosis with plants, the C substrates that are assimilated by microorganisms at the root apex are utilized rapidly for respiration and growth, or lost as microbial exudates or exopolysaccharides that are used as a substrate for subsequent microbial communities. Certain microbial species, such as *Rhizobium*, present in nodules of N_2-fixing species, produce large amounts of exopolysaccharides (Downie, 2010) that can also be adsorbed onto fine silt and clay particles (Fehrmann and Weaver, 1978).

1.1.8. Soil: responsible for carbon protection

Finally, the last main actor to consider in the determination of the fate of soil organic C is the soil itself (Fig. 2). The residence time of C is controlled by the protection mechanisms that contribute to stabilize it (Luo et al. 2004; Jastrow et al. 2005). C in soil can be divided into three main pools: an unprotected C pool, referring to the labile particulate organic matter (POM) in the soil, a biochemically protected pool (BPC) (Fig. 3), when C is moved in soil in recalcitrant forms and is difficult for microorganisms to consume it, and a physically protected pool (PPC), when C is protected inside aggregates or absorbed on clay/silt particles and cannot easily be consumed by microorganisms (Fig. 3). The POM and BPC pool fate depends on the nature of the organic matter and the microbiological communities, as discussed previously. The PPC is considered to be the most stable C pool, and therefore the most important for soil C storage (Rumpel et al. 2012). Regarding the PPC, it is particularly worthwhile to explore the linkage between PPC and labile C from plants, i.e. C from exudates. Exudates were usually considered to be immediately consumed by microorganisms and to play a marginal role in C sequestration (van Veen et al. 1991; Van Geijn et al. 1993; Hungate et al. 1997). This assumption is now questioned by more recent studies that state that labile soil C compounds are just partially consumed, and dissolved labile organic C can be protected by soil absorption inside aggregates or on clay and silt (see review by Kalbitz and Kaiser, 2008). Moreover, it has been demonstrated that microbiological exudates and exopolysaccharides are the main precursor of organomineral protected C (Cotrufo et al., 2013; Vidal et al., 2018). To understand the fate of C and increase the PPC, the two main mechanisms of C protection need to be investigated.

1.1.9. Soil structure and carbon physical protection in aggregates

Aggregate protection of C is due to the C physical protection from microorganisms by occlusion of C in the smaller pores, limiting the gas and nutrient diffusion and, therefore, microbiological activity, and separating enzymes from substrates on mineral and humic surfaces (O'Brien and Jastrow, 2013). Aggregates are formed by binding of soil particles by fine roots and fungal hyphae (Tisdall and Oades 1982) and cementation by microbiological and plants exudates, like glycoproteins, polysaccharides, and mucilage, directly influencing the stability of aggregates (Tisdall and Oades 1982; Caesar-Tonthat 2002; Nichols and Wright 2005).The formation of aggregates is different in regards to their size. Abiotic factor, such as ligand exchange and polyvalent cation bridging promoted by drying-rewetting cycles (Bronick and Lal, 2005; Keil and Mayer, 2014), are known to form stable microaggregates. Microaggregates are then complexed in small macroaggregates thanks to the biotic action and cementation from microbes that produce extracellular polymeric substances acting as glues to connect soil aggregates (Blankinship et al., 2016). Fine roots and hyphae of fungi further complex aggregates in bigger structures thanks to their enmeshing action (Tisdall and Oades, 1982; Blankinship et al., 2016). The silt and clay particles are connected with the formation of microaggregates, while sand particles are mostly associated with macro- aggregates (Blagodatskaya and Kuzyakov, 2008). The stability of aggregates is an important factor influencing C protection since it will directly influence the aggregation and disaggregation processes in soils. However, aggregation is a dynamic process, with aggregates continuously forming and being destroyed by natural cycles and animal or anthropogenic disturbance (Eyles et al. 2015). Aggregate size is another important characteristic influencing C protection: Jastrow (2006) states that C turnover is higher in macroaggregates (>212 µm) compared to microaggregates (53-212 µm), implying that microaggregates have a higher C protection potential.

1.1.10. Organomineral interactions with fine silt and clay minerals and soil carbon saturation

The other main mechanism for C protection is organomineral interactions with cations in soil that decrease the soil C lability (Eyles et al., 2015; O'Brien and Jastrow, 2013). This process can happen inside aggregates or in loose soil and relies on chemical sorption on mineral surfaces, polyvalent cation bridging and layered chemical binding on mineral surface, of microbiological products primarily adsorbed on minerals and covered by exudates (Cotrufo et al., 2015; Kleber et al. 2007; O'Brien and Jastrow, 2013). The soil potential for organomineral interactions relies on the amount of fine elements in the soil (especially clay particles), cations of different reactive elements, in particular Fe and Al (Swanston et al. 2009), and, particularly interesting for this study, C saturation level of the soil. The concept of soil C saturation has been highlighted after some studies reported no increase of soil organic C in soils even after further increase of C input (e.g. Campbell et al. 1991, Solberg et al. 1997, Gill et al. 2002). To explain this behavior of soil, Six et al. (2002) introduced the concept of 'soil C saturation', where it was suggested that the different C pools have different saturation points after which they cannot effectively store C anymore. The capacity of these pools to store C depends on their nature. For example, the physically protected C pool relies on the surface area of particles, meaning that after the available areas are occupied by adsorbed C and further C input will not be associated anymore and therefore not protected (Six et al. 2002). This concept was further elaborated by Stewart et al. (2007), who stated that the soil C pool can be saturated with respect to the C inputs and that a linear model cannot efficiently describe the input-storage behavior of a soil (Fig. 4). In this respect, Stewart et al (2007) conclude that a soil poor in C, can store C more efficiently than a soil rich in organic C and, therefore, closer to its C saturation threshold (Fig. 4), depending on the content of clay/silt of the soils, the aggregation capacity, and their adsorption capacity. Several studies suggested that subsoil might protect C more efficiently in fine soil fractions due to lower C saturation that increase the possibility for organomineral interactions (Rasse et al., 2005; Lorenz and Lal 2005; Thomas et al. 2007; Horrocks et al. 2010;Rumpel et al., 2012). However, to our knowledge, no studies focused on the potential of subsoil revegetation and the influence on the C-cycle and organomineral interactions.

1.1.11. Soil carbon pools associated to different soil fractions

When analyzing C content in soil, it is difficult to assess the different pools of C present in the soil and their protection (biological protection determined by recalcitrance or physical protection from aggregate occlusion or organomineral interactions). A method commonly used to assess C protection in soil is to fractionate the soil and analyze the C in each fraction (Fig. 5). These soil C pools relay on different protection mechanisms, and the degree of stability increases with decreasing fraction size. These pools are defined as: i) coarse particulate organic matter (coarse POM, soil fraction > 200µm) (Fig. 5a), that is free in the soil at different levels of degradation ii) fine POM (soil fraction 50-200µm) (Fig. 5b), that comprises organic C occluded in soil aggregates. These two pools are mostly derived from the decomposition of roots and shoots (Kögel-Knabner, 2002) and their C protection from microbial consumption relies mainly on the recalcitrance of their lignocellulose C structures (Six *et al.,* 2002). Finally, iii) C protected in the coarse silt (20-50 µm) (Fig. 5c) and iv) in fine silt+clay pools (<20µm) (Fig. 5d). C protected in these pools is mostly derived from labile rhizospheric and microbial compounds (Cotrufo *et al*., 2013, Vidal *et al*., 2018). C in these pools is highly degraded via decomposition and mineralized by microbial metabolism, and it is protected from microbial consumption via occlusion in microaggregates and through organo-mineral complexation with clay particles and metals.

1.1.12. Short – term changes in soil organic carbon mineralization due to vegetation: the priming effect

Although the positive effect of revegetating soils in terms of C input and soil C accumulation potential is well established, the influence of plants on the C-cycle can also have negative impacts on soil C sequestration. As already mentioned, the potential of a soil system in respect to C sequestration is determined by the balance between input of photosynthetically absorbed C in soil and output of CO_2 via soil heterotrophic respiration (Smith et al., 2000; De Deyn et al., 2008). The soil heterotrophic respiration is determined mainly by microbial communities and their activity, and their consumption of C in soil (Jones *et al*., 2009; Kuzyakov and Larionova, 2006). One of the effect of plant C input is to influence the microbial communities structure and activity and the consequent consumption of pre-existent soil C, that is commonly known as 'priming effect' (Broadbent and Nakashima, 1974; Sørensen, 1974; Wu et al., 1993; Kuzyakov et al., 2000). The priming effect is defined as strong short – term changes in C mineralization due to vegetation (Kuzyakov et al., 2000). We talk about 'positive priming effect' when the input of labile C increases the activity of microbial communities and the mineralization of pre-existent C in soil (Fontaine et al., 2003). The positive priming effect has an adverse effect on soil C storage. However, if microbial communities in soil switch from consuming pre-existent C to mineralizing fresh C input, the mineralization of soil C will decrease (Kuzyakov et al., 2000). In this case we talk about a 'negative priming effect', beneficial to C storage in soil. The magnitude of the priming effect and its direction (positive or negative) results from a complicated series of interactions between soil, plants and microbial communities (Cheng and Kuzyakov, 2005). The first mechanism is known as the 'competition hypothesis' (Jackson et al., 1989; Schimel et al., 1989; Kaye and Hart, 1997; Hodge et al., 2000; Cheng and Kuzyakov, 2005) and postulates that competition for mineral N will determine the direction of priming. If the soil is poor in N, then the priming effect is negative due to competition between plants and microbes. In the long run, plants have a higher efficiency for N mining, and they will reduce the nutrient sources for microbial communities, decreasing their C consumption (Cheng and Kuzyakov, 2005). Instead, when mineral nutrients are not limiting and there is no competition between plants and microbes, rhizodeposition will increase microbial activity resulting in increased soil C consumption and a positive priming effect (Cheng and Kuzyakov, 2005). These mechanisms hold when microbial communities need to mine C for nutrients and energy, and are usually observed in studies involving poor soils (pine forests and dry grasslands) (Ehrenfeld et al., 1997; Schimel et al., 1989; Cheng and Kuzyakov, 2005).

When mineral nutrients are not limiting and the input of labile C is high, the priming effect might be controlled by the preference of microbes for labile root derived C compared to nutrient rich soil C (Cheng and Kuzyakov, 2005). If no nutrient limitation is present, microbes will prefer labile derived C as an abundant and ready available source of energy (Cheng, 1999; Cheng and Kuzyakov, 2005). In this case, a switch of substrate utilized will decrease the C consumption and result in a negative priming effect, favouring soil C storage (Cheng and Kuzyakov, 2005, De Graaf et al. 2010). These effects are regulated by microbial metabolism (Cheng and Coleman, 1990). Increased microbial biomass is linked with positive priming, while negative priming is usually correlated to decreased microbial biomass (Cheng and Coleman, 1990; Reid and Goss, 1982; 1983; Sallih and Bottner, 1988). However, De Graaaf et al. (2010) showed how different levels of labile C input can influence microbial dynamics and consequent priming effect. Low input of labile C ($\cong$0.7 mgC g^{-1} soil) will increase microbial activity and soil C mining, resulting in a positive priming effect. Instead, high labile C input (> 7.2 mgC g^{-1}) increases microbial biomass but induce microbes to switch preference of substrate consumption, from old C present in the soil to the fresh C inputted from plants substrate utilization switch, decreasing old C consumption compared to unvegetated soil and resulting in a negative priming effect (De Graaf et al., 2010).

1.1.13. Possible impacts of revegetating geotechnical soils on the priming effect

In geotechnical works, soils are often heavily managed and revegetated. Environmental conditions are perturbed and it is not uncommon that subsoil is excavated, brought to the surface and revegetated. Subsoils have a high C stability given by i) low microbial biomass (Taylor et al. 2002; Andersen and Domsche 1989; Ekklund et al. 2001) and activity (Fang and Moncrieff 2005), ii) oxygen limitation (Rumpel and Kögel-Knabner, 2010), iii) energy limitation due to reduced labile C inputs (Fontaine et al. 2007) and iv) spatial heterogeneity of organic C in subsoil and consequent separation from microbes (Von Lützow et al. 2006; Holden and Fierer 2005). Fontaine et al. (2007) showed how a supply of fresh C in deep soil can decrease the stability of pre-existent old C and increase positive priming. However, to our knowledge, no in vivo experiment has been implemented on this topic, and, more importantly, no studies are available on the effects of excavating and revegetating subsoil on the priming effect. Excavating, crushing, mixing, and revegetating soil will have a major impact on the factors determining the stability of C in subsoil, and possibly a high priming effect.

1.2. GENERAL KNOWLEDGE GAPS

As stated above, soil embankments represent an interesting structure for C sequestration due to two features: 1) plants can be chosen to vegetate the embankments, and therefore the C input in the system, and 2) soil can be managed and chosen to optimize C sequestration. Embankments are constituted of a core of compacted soil, usually excavated from a depth of >1m and with a high percentage of clay, and they can be covered by a layer of stripped topsoil to be revegetated. The choice of revegetating organic topsoil (down to 30 cm depth) stripped and used to cover the embankment, or directly on an uncompacted surface layer of mineral subsoil (>1 m depth), will deeply influence the soil C storage potential of the geotechnical structure. However, no studies have been developed in depth on the effects of revegetating subsoil brought to the surface on C storage, and their potential as C sink. There is a need of comparing C storage potential of different plants and soils to design the most efficient C storage system in geotechnical soils, a potential that have been hypothesized before but never adressed (Dejong et al., 2011).

The study of two soils showing diverse characteristics (fertility, microbial communities, C saturation levels), and the use of plant species that have contrasting root traits connected with higher recalcitrance or lability, allows to tackle fundamental knowledge gaps regarding the actors and mechanisms driving C sequestration in soil. The next paragraphs give an overview of the knowledge gaps addressed in each chapter of the thesis.

1.2.1. Plant carbon input: influence of root traits and carbon accumulation in different soil C pools

Rhizosphere is considered as the main pathway for C to enter the soil, however few studies have tackled the relationships between root traits and C storage. The studies that have indeed explored the effect of the root economics spectrum on C storage (e.g. De Deyn et al., 2008; Bardgett et al., 2014; Poirer et al., 2018; Henneron et al., 2019) considered the C storage in bulk soil, without exploring the effect of root traits on C quality, i.e. the accumulation of C in different pools. Moreover, among the different explored root traits, the root elongation rate has never been studied in relationship to C storage. We state that root economics spectrum is lacking an important trait, since changes in root elongation rate affect the production and the spatial distribution of root exudates, the main precursor of C stored in SILT+CLAY pool (Cotrufo et al., 2013; Holz *et al.,* 2018).

1.2.2. Subsoil brought to the surface: effect on C fluxes and actors involved in C-cycle

Soil C stock within a defined time frame is the balance between input and transformation of newly photosynthesized C from plants to soil (new C) and losses of existing soil organic C (old C) (Kuzyakov and Domansky, 2000; Fontaine et al., 2004). Moreover, the balance between new C and old C is far from being the whole story, as increasing studies have highlighted the equal importance of quality of soil C, as C stored in different C pools (Cardinael et al.,2015). To our best knowledge, no study has ever bridged the link between C pools and the fates of new C and old C. Besides the exploration of the fates of soil new C and old C, as well as their associations with C pool, another significant knowledge gap comes to the predictability of the fates of soil new C and old C using plant and soil features. More specifically, no studies investigated the effect of root traits, microbial communities and soil characteristics (with an eye of attention to the C saturation theory) on new input and old c changes in different C pools.

1.2.3. Subsoil brought to the surface: what is the effect of revegetation on the priming effect

Revegetating subsoil could have a high impact on pre-existent old C stability and the priming effect. C in subsoil is highly stable, and perturbation of the environmental conditions could deeply influence the stability and protection of this pool. Studies on priming of subsoil have been conducted (Fierer et al., 2003; Fontaine et al., 2007; Wang et al. 2014), however no studies investigated the effect of bringing subsoil to the surface. Understanding the priming effect at soil fraction level may also bring us new insight on the vulnerability of soil C pools to fresh C input.

1.3. STRUCTURE OF THE THESIS: OBJECTIVES AND HYPOTHESES

Figure 6 shows the different research questions tackled in the research and discussed in each chapter, plus their link with the main factors and processes discussed in each chapter. In this theses I and the research team collaborating in this project aim to tackle the following general objectives:

i - Understanding the effect of plant and soil features on soil C sequestration in terms of quantity and quality (fundamental objective)

ii - Identifying possible plant and soil practices that can be implemented to increase soil C storage in embankments and, possibly, in grey soils from geotechnical work (applied objective)

The above two objectives regarding the fundamental mechanisms of C-cycle will be tackled in every chapter of the thesis.

1.3.1. Chapter II: Pathway to persistence: plant root traits alter C accumulation in different soil carbon pools through microbial mediation

i - Objective 1: Understand what are the relationships between root traits and C accumulation in different soil C pools for 12 different herbaceous species commonly used in embankment revegetation (Fig. 7).

Hypothesis 1: We hypothesize that traits related to labile C input (root elongation rate, hemicellulose content, root biomass) promote C accumulation in the protected coarse silt and fine silt + clay C pools, since these traits are expected to favor rhizodeposition and microbial activity, whereas root traits related to recalcitrance (high lignin and cellulose content, high C:N ratio) promote C accumulation in the unprotected coarse POM pool.

ii - Objective 2: What is the effect of species selection on the C sequestration in different soil C pools

Hypothesis. 2: We hypothesize that N_2-fixing species favor C accumulation in the protected fine silt+clay pools since they have traits more related to labile C input, while non N_2-fixing species will favor C accumulation in the POM fraction.

1.3.2. Chapter III: The fates of fresh new carbon and old soil carbon differ in topsoil and newly exposed subsoil and are explained by root, microbial, and soil particle size

i - Objective 1: Quantify the fluxes of new C and old C in different soil pools;

Hypothesis 1: We hypothesize that soil particle size fractions associated C pools can regulate the fates of old C and new C in the C sequestration process;

ii - Objective 2: Examine the pattern of covariation between new C input and changes of old C in different C pools

Hypothesis 2: The fate of new C and old C will show independent patterns

iii - Objective 3: Investigate if the different actors involved in C storage, and the influence that plant and soil have on them, can explain the patterns of new C and old C fluxes in different soil C pools

Hypothesis 3: We hypothesize that plant traits related to chemical composition and recalcitrance will be driving POM accumulation in new C and consumption in old C, while traits related with high C input will drive storage in protected fractions via microbiological consumption and deposition. We expect aggregate stability to be positively correlated with new C and old C accumulation in fine POM and coarse silt fractions due to physical protection of aggregates. We expect that soil N content positively correlates with new C input. Fine fraction in soil is believed to be positively correlated with the new C storage in fine silt+clay fraction due to organomineral interactions, and new C storage in fine silt+clay is expected higher in subsoil than in topsoil due to lower soil C saturation levels. Finally we expect microbial activity, diversity and abundance to be strongly linked with the amount of new C deposited in the protected coarse silt and silt+clay fractions, and with the consumption and transformation of old C in the unprotected coarse POM and fine POM fractions due to mineralization from microbial communities.

1.3.3. Chapter IV: Soil quality drives the priming effect and plant species refine it

i - Objective 1: Quantify the changes in C and the input of new C in soil to determine the losses of old C in revegetated topsoil and subsoil brought to the surface (Fig. 8) and the priming effect of revegetating with N_2-fixing (*Medicago sativa*) and a non N_2-fixing species (*Lolium perenne*) species (Fig. 9).

Hypothesis 1: Our hypothesis is that topsoil will have higher losses of old C due to higher microbial biomass and activity. However, due to the higher protection of old C in subsoil and the changes in environmental conditions given by revegetation, we hypothesize that subsoil will have higher old C losses compared to bare soil, meaning a higher positive priming effect compared to topsoil.

ii - Objective 2: Quantify the priming effect in different C pools related to granulometric soil fractions.

Hypothesis 2: Given the higher protection of C in the finer soil fraction (silt and silt +clay fractions) we hypothesis that the priming will occur in the unprotected particulate organic matter fractions (POM and finePOM).

iii - Objective 3: Study the evolution over time of the sources of respired C in the system (represented by the abundance of ^{13}C) and its correlations with old C losses, new C input and priming.

Hypothesis 3: We hypothesize that the source of respiration in the system will switch more towards labelled plant inputs over time, along with plant development. We believe new C input to be positively correlated with the abundance of ^{13}C in respired CO_2 ($A^{13}C$). However, we expect different behaviours in the two soils regarding the old C losses. In topsoil we suggest that $A^{13}C$ will be negatively correlated with old C losses, due to switch in microbiological substrate preference, while in subsoil $A^{13}C$ will be positively correlated with old C losses, due to increased microbial activity. In the same way, priming will be negatively correlated to $A^{13}C$ in topsoil, while being positively correlated in subsoil.

1.3.4. Chapter V: general discussion, guidelines and prospective for carbon storage in geotechnical embankments

In Chapter V I intend to delineate a more comprehensive view on the effect of soil and plant selection on C storage in embankments based on the results of this study. I want to discuss the potential benefits of embankments for C storage and propose guidelines for embankments revegetation, more specifically: i) possible management options to increase C storage in these geotechnical soils and ii) perspectives for future studies on C sequestration.

1.3.5. Annex I: Perspectives: the influence of vegetation on soil microstructure and its implications on soil carbon sequestration: a geotechnical approach

Annex one is an overview of an ongoing research with UNICAS regarding soil structure. More specifically we investigate the influence of vegetation on soil microstructure and its implications on soil C storage and protection. I propose a multidisciplinary approach including geotechnical engineering and soil science/ecological methods to investigate soil structure in terms of i) soil porosity and void ratio, ii) aggregate stability and C protection, and iii) new C input in different aggregate classes. These results will allow a more comprehensive view on aggregate formation and C protection in revegetated topsoil and subsoil brought to the surface, and understand the role of porosity and void ratio in relation to C protection. Research questions, methodology and preliminary results are outlined in Annex I.

REFERENCES

Adams, J.M., Faure, H., Faure-Denard, L., McGlade, J.M., Woodward, F.I. 1990. Increases in terrestrial carbon storage from the Last Glacial Maximum to the present. Nature 348: 711–714

Andersen, T.H., Domsche, K.H. 1989. Ratios of microbial biomass carbon to total organic carbon in arable soils. Soil Biology and Biochemistry 21, 471–479

Anderson, J.M. 1992. Responses of soils to climate change. Advances in Ecological Research 22, 163–210

Balesdent J and Balabane M. 1996. Major contribution of roots to soil carbon storage inferred from maize cultivated soils. Soil Biology and Biochemistry9, 1261–1263.

Bardgett, R.D., Mommer, L., De Vries, F.T., 2014. Going underground : root traits as drivers of ecosystem processes. Trends in Ecology & Evolution 29, 692–699.

Batjes, N.H. 1996. Total carbon and nitrogen in the soils of the world. European Journal of Soil Science 47: 151–163

Blagodatskaya, E., Kuzyakov, Y., 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: Critical review. Biology and Fertility of Soils 45, 115–131.

Blankinship, J.C., Fonte, S.J., Six, J., Schimel, J.P., 2016. Plant versus microbial controls on soil aggregate stability in a seasonally dry ecosystem. Geoderma 272, 39–50.

Broadbent, F.E., Nakashima, T., 1974. Mineralisation of carbon and nitrogen in soil amended with carbon-13 and nitrogen-15 labeled plant material. Soil Science Society of America Journal 38, 313–315.

Bronick, C.J., Lal, R., 2005. Soil structure and management: a review. Geoderma 124, 3–22.

Caesar-Tonthat, T.C. 2002. Soil binding properties of mucilage produced by a basidiomycete fungus in a model system. Mycological Research 106, 930–937.

Campbell, C.A., Lafond, G.P., Zentner, R.P., Biederbeck, V.O., 1991. Influence of fertilizer and straw baling on soil organic matter in a thick black chernozem in Western Canada. Soil Biology and Biochemistry 23, 443–446.

Cardinael, R., Chevallier, T., Barthès, B.G., Saby, N.P.A., Parent, T., Dupraz, C., Bernoux, M., Chenu, C., 2015. Geoderma Impact of alley cropping agroforestry on stocks , forms and spatial distribution of soil organic carbon — A case study in a Mediterranean context. Geoderma 259–260, 288–299.

Castellano, M. J., Mueller, K. E., Olk, D. C., Sawyer, J. E., & Six, J. 2015.. Integrating plant litter quality, soil organic matter stabilization, and the carbon saturation concept. Global Change Biology, 21(9), 3200–3209.

Chan, Y. 2008. Increasing soil organic carbon of agricultural land. Primefact 735, (JANUARY), 1–5. Retrieved from http://www.dpi.nsw.gov.au/__data/assets/pdf_file/0003/210756/Increasing-soil-organic-carbon.pdf

Chapin, F.S. 2003. Effects of plant traits on ecosystem and regional processes: a conceptual framework for predicting the consequences of global change. Annulas of Botany, 91, 455–463.

Cheng, W. 1999. Rhizosphere feedbacks in elevated CO2. Tree Physiology 19, 313–320

Cheng, W., Kuzyakov, Y., 2005. Root effects on soil organic matter decomposition. In: S. Wright, S., Zobel, R. (Eds.), Roots and Soil Management: Interactions Between Roots and the Soil. Agronomy Monograph No. 48, American Society of Agronomy, Madison, Wisconsin, USA 119–143.

Cheng,W., and D.C. Coleman. 1990. Effect of living roots on soil organic matter decomposition. Soil Biology and Biochemistry 22, 781–787.

Clemmensen, K. E. et al. 2013. Roots and associated fungi drive long-term carbon sequestration in boreal forest. Science 339, 1615–1618.

Cotrufo, M. F., Soong, J. L., Horton, A. J., Campbell, E. E., Haddix, M. L., Wall, D. H., & Parton, W. J. 2015. Formation of soil organic matter via biochemical and physical pathways of litter mass loss. Nature Geoscience, 8(10, 776–779.

Cotrufo, M. F., Wallenstein, M. D., Boot, C. M., Denef, K., & Paul, E. 2013. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: Do labile plant inputs form stable soil organic matter? Global Change Biology, 19(4), 988–995.

De Deyn, G. B., Cornelissen, J. H. C., & Bardgett, R. D. 2008. Plant functional traits and soil carbon sequestration in contrasting biomes. Ecology Letters, 11(5), 516–531.

De Graaff, M.A., Classen, A.T., Castro, H.F., Schadt, C.W., 2010. Labile soil carbon inputs mediate the soil microbial community composition and plant residue decomposition rates. New Phytologist 188, 1055–1064.

Dejong, J. T., Soga, K., Banwart, S. A., Whalley, W. R., Ginn, T. R., Nelson, D. C., … Barkouki, T. 2011. Soil engineering in vivo : harnessing natural biogeochemical systems for engineering solutions, Journal of The Royal Society Interface 8(54), 1-15

Dixit, M.K., Fernandez-Solis, J.L., Lavy, S. and Culp, C.H. 2010 Identification of parameters for embodied energy measurement: A literature review. Energy and Buildings, 42, 1238-1247

Dixit, M.K., Fernandez-Solis, J.L., Lavy, S. and Culp, C.H. 2010. “Identification of parameters for embodied energy measurement: A literature review”, Energy and Buildings, 42, 1238-1247.

Downie, J.A., 2010. The roles of extracellular proteins, polysaccharides and signals in the interactions of rhizobia with legume roots 34, 150–170.

Ehrenfeld, J.G.,W.F.J. Parsons, X. Han, R.W. Parmelee, and W. Zhu. 1997. Live and dead roots in forest soil horizons: Contrasting effects on nitrogen dynamics. Ecology 78, 348–362.

Ekklund, F., Ronn, R., Christensen, S., 2001 Distribution with depth of protozoa, bacteria and fungi in soil profiles from three Danish forest sites. Soil Biology and Biochemistry 33, 475–481

Eswaran, H., van den Berg, E., Reich, P. 1993 Organic carbon in soils of the world. Soil Science Society of America Journal 57: 192–194

EU-SDG 2018 Sustainable development in the European Union - monitoring report on progress towards the SDGs in an EU context. Luxembourg: Publications Office of the European Union, 2018

Eyles, A., Coghlan, G., Hardie, M., Hovenden, M., & Bridle, K. 2015. Soil carbon sequestration in cool-temperate dryland pastures: Mechanisms and management options. Soil Research, 53(4), 349–365.

Fang, C., Moncrieff, J.B. 2005 The variation of soil microbial respiration with depth in relation to soil carbon composition. Plant and Soil 268, 243–253

Fehrmann, R., Weaver, R., 1978. Scanning electron microscopy of Rhizobium spp. ad- hering to fine silt particles. Soil Science Society of America Journal 42, 279–281.

Fierer, N., Allen, A.S., Schimel, J.P., Holden, P.A., 2003. Controls on microbial CO2 production: A comparison of surface and subsurface soil horizons. Global Change Biology 9, 1322–1332.

Fontaine, S., Bardoux, G., Abbadie, L., Mariotti, A., 2004. Carbon input to soil may decrease soil carbon content. Ecology Letters 7, 314–320.

Fontaine, S., Barot, S., Barré, P., Bdioui, N., Mary, B., Rumpel, C., 2007. Stability of organic carbon in deep soil layers controlled by fresh carbon supply. Nature 450, 277–280.

Fontaine, S., Mariotti, A., Abbadie, L., 2003. The priming effect of organic matter: A question of microbial competition? Soil Biology and Biochemistry 35, 837–843.

Fujisaki, K., Chevallier, T., Chapuis-Lardy, L., Albrecht, A., Razafimbelo, T., Masse, D., Ndour, B.Y., Chotte, J.L. 2018. Soil carbon stock changes in tropical croplands are mainly driven by carbon inputs: A synthesis. Agriculture, Ecosystems & Environment, 259, 147-158

Gallipoli, D., & Mendes, J. 2017. A geotechnical perspective of raw earth building, 463–478.

Gill RA, Polley HW, Johnson HB et al. 2002 Nonlinear grassland responses to past and future atmospheric CO2. Nature, 417, 279–282.

Henneron, L., Picon-cochard, C., 2019. Plant economic strategies of grassland species control soil carbon dynamics through rhizodeposition.

Hodge, A., D. Robinson, and A. Fitter. 2000. Are microorganisms more effective than plants at competing for nitrogen? Trends in Plant Science 5, 304–308

Högberg P, Nordgren A, Buchmann N, Taylor AFS, Ekblad A, Högberg MN, Nyberg G, Ottosson-Löfvenius M, Read DJ 2001 Large-scale girdling experiment shows that current photosynthesis drives soil respiration. Nature 411:789–792

Holden, P.A., Fierer, N. 2005 Microbial processes in the vadose zone. Vadose Yone Journal 4:1–21

Holz, M., Zarebanadkouki, M., Kaestner, A., Kuzyakov, Y., Carminati, A., 2018. Rhizodeposition under drought is controlled by root growth rate and rhizosphere water content 429–442.

Horrocks, A., Thomas, S., Tregurtha, C., Beare, M.H., Meeken, E., 2010. Implications for dry matter production and nitrogen management as soils develop following ‘humping and hollowing’ on the West Coast. Proceedings of the New Zealand Grassland Association 72, 103–108.

Hungate, B. A., Holland, E. A., Jackson, R. B., Chapin, F. S., Mooney, H. A., & Field, C. B. 1997. The fate of carbon in grasslands under carbon dioxide enrichment. Nature, 388(6642), 576–579.

IPCC. 2014. Climate change 2014: Mitigation of climate change. Contribution of working group III to the fifth assessment report of the intergovernmental panel on climate change (O. P. Edenhofer et al., Eds.). Cambridge, UK and New York, NY, USA: Cambridge University Press

Jackson, L.E., J.P. Schimel, and M.K. Firestone. 1989. Short-term partitioning of ammonium and nitrate between plants and microbes in an annual grassland. Soil Biology and Biochemistry 21, 409–415.

Jastrow, J. D., Miller, R. M., Matamala, R., Norby, R. J., Boutton, T. W., Rice, C. W., & Owensby, C. E. 2005. Elevated atmospheric carbon dioxide increases soil carbon. Global Change Biology, 11(12), 2057–2064.

Jastrow, J.D., 2006. Soil aggregate formation and the accrual of particulate and mineral-associated organic matter. Soil Biology and Biochemistry, 28, 665-676

Jones, D.L., Nguyen, C., Finlay, R.D., 2009. Carbon flow in the rhizosphere: Carbon trading at the soil-root interface. Plant and Soil 321, 5–33.

Kalbitz, K., & Kaiser, K. 2008. Contribution of dissolved organic matter to carbon storage in forest mineral soils. Journal of Plant Nutrition and Soil Science, 171(1), 52–60.

Kaye, J.P., and S.C. Hart. 1997. Competition for nitrogen between plants and soil microorganisms. Trends in Ecological Evolution 12, 139–143

Keil, R.G., Mayer, L.M., 2014. Mineral matrices and organic matter. Treatise on Geochemistry 12, 337–359.

Keiluweit, M., Bougoure, J. J., Nico, P. S., Pett-Ridge, J., Weber, P. K., & Kleber, M. 2015. Mineral protection of soil carbon counteracted by root exudates. Nature Climate Change, 5(6), 588–595.

Kibert, C.J. 2008. Sustainable Construction, 2nd Edition – John Wiley and Sons Inc., New Jersey,

Kleber, M., Sollins, P., & Sutton, R. 2007. A conceptual model of organo-mineral interactions in soils: Self-assembly of organic molecular fragments into zonal structures on mineral surfaces. Biogeochemistry, 85(1), 9–24.

Kögel-Knabner, I., 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biology and Biochemistry34, 139–162

Kuzyakov, Y. V., Larionova, A.A., 2006. Contribution of rhizomicrobial and root respiration to the CO2 emission from soil (A review). Eurasian Soil Science 39, 753–764.

Kuzyakov, Y., Domanski, G., 2000. Carbon input by plants into the soil. Review. Journal of Plant Nutrition and Soil Science 163, 421–431.

Kuzyakov, Y., Friedel, J.K., Stahr, K., 2000. Review of mechanisms and quantification of priming effects. Soil Biology & Biochemistry 32, 1485-1498

Lal, R. 2004. Soil carbon sequestration impacts on global change and food security. Science 304: 1623-1627., 304(June).

Lavorel, S., Dı´az, S., Cornelissen, J.H.C., Garnier, E., Harrison, S.P., McIntyre, S. et al. 2007. Plant functional types: are we getting any closer to the Holy Grail? In: Terrestrial Ecosystems in a Changing World (eds Canadell, J., Pitelka, L.F. & Pataki, D.). Springer, Berlin, pp. 171–186

Lekkerkerk, L.J.A., Van De Geijn, S.C., Van Veen, J.A. 1990 Effects of elevated atmospheric CO2-1evels on the carbon economy of a soil planted with wheat. In Soils and the Greenhouse Effect. Ed. A F Bouwman. pp 423-429. Wiley and Sons, Chichester, UK

Liang, C., Schimel, J.P., Jastrow, J.D., 2017. The importance of anabolism in microbial control over soil carbon storage. Nature Microbiology 2, 1–6.

Lorenz, K. and Lal, R. 2005. The depth distribution of soil organic carbon in relation to land use and management and the potential of carbon sequestration in subsoil horizons. Advanced Agronomy, 88, 35–66.

Luo Y, Su B, Currie WS et al. 2004 Progressive nitrogen limita- tion of ecosystem responses to rising atmospheric carbon dioxide. BioScience, 54, 731–739.

Misra, A., Basu, D., 2011. Sustainability in geotechnical engineering. Internal Geotechnical Report 2011-2" Technical Reports. http://digitalcommons.uconn.edu/cee_techreports/1

Nichols, K.A., Wright, S.F. 2005 Comparison of glomalin and humic acid in eight native U.S. soils. Soil Science 170, 985–997.

O'Brien, S. L., & Jastrow, J. D. 2013. Physical and chemical protection in hierarchical soil aggregates regulates soil carbon and nitrogen recovery in restored perennial grasslands. Soil Biology and Biochemistry, 61, 1–13.

O'Riordan, N. O., Nicholson, D., Hughes, L., Phear, A., & Group, A. 2011. Technical Paper Examining the carbon footprint and reducing the environmental impact of slope engineering options, Ground engineering

Poirier, V., Roumet, C., Munson, A.D., 2018. The root of the matter: Linking root traits and soil organic matter stabilization processes. Soil Biology and Biochemistry 120, 246–259.

Rasse, D.P., Rumpel, C., Dignac, M., 2005. Is soil carbon mostly root carbon ? Mechanisms for specific stabilization Is soil carbon mostly root carbon ? Mechanisms for a specific stabilisation.

Reid, C. P. P., & Mexal, J. G. 1977. Water stress effects on root exudation by lodgepole pine. Soil Biology and Biochemistry, 9(6), 417–421.

Reid, J.B., and M.J. Goss. 1982. Suppression of decomposition of 14C-labelled plant roots in the presence of living roots of maize and perennial ryegrass. Journal of Soil Science. 33, 387–395

Reid, J.B., and M.J. Goss. 1983. Growing crops and transformations of 14C-labelled soil organic matter. Soil Biology and Biochemistry 15, 687–691.

Riordan, N.O., Nicholson, D., Hughes, L., Phear, A., Group, A., 2011. technical Paper Examining the carbon footprint and reducing the environmental impact of slope engineering options.

Rumpel, C., Chabbi, A., and Marschner, B. 2012. Carbon storage and sequestration in subsoil horizons: knowledge, gaps and potentials. in Recarbonization of the Biosphere, eds R. Lal, K. Lorenz, R. Hüttl, B. Schneider, and J. von Braun (Dordrecht: Springer)

Rumpel, C., Kögel-Knabner, I., 2011. Deep soil organic matter-a key but poorly understood component of terrestrial C cycle. Plant and Soil 338, 143–158.

Sallih, Z., and P. Bottner. 1988. Effect of wheat (Triticum aestivum) roots on mineralization rates of soil organic matter. Biology and Fertility of Soils 7, 67–70.

Sauerbeck, D.R., 2001. CO2emissions and C sequestration by agriculture - Perspectives and limitations. Nutrient Cycling in Agroecosystems 60, 253–266.

Schimel, J.P., L.E. Jackson, and M K. Firestone. 1989. Spatial and temporal effects on plant microbial competition for inorganic nitrogen in a California annual grassland. Soil Biology and Biochemistry 21, 1059– 1066.

Six, J., Feller, C., Denef, K., Ogle, S.M., Moraes Sa J.C., Albrech, A. 2002. Soil organic matter, biota and aggregation in temperate and tropical soils – effects of no-tillage. Agronomie 22, 755–775.

Six, J., Frey, S.D., Thiet, R.K., Batten, K.M. 2006 Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Science Society of America Journal, 70, 555–569.

Smith, P., Powlson, D.S., Smith, J.U., Falloon, P., Coleman, K., 2000. Meeting Europe’s climate change commitments: Quantitative estimates of the potential for carbon mitigation by agriculture. Global Change Biology 6, 525–539.

Solberg, E.D., M. Nyborg, R.C. Izaurralde, S.S. Malhi, H.H. Janzen, and M. Molina-Ayala. 1997. Carbon storage in soils under continuous cereal grain cropping: N fertilizer and straw. p. 235–254. In R. Lal et al. (ed.) Management of carbon sequestration in soil. CRC Press, Boca Raton, FL

Sørensen, L.H., 1974. Rate of decomposition of organic matter in soil as influenced by repeated air drying-rewetting and repeated additions of organic material. Soil Biology and Biochemistry 6, 287–292.

Standards Australia, 2003. Methods of testing soils for engineering purposes – soil compaction and density tests – determination of the dry density/moisture content relation of a soil using standard compactive effort, AS 1289.5.1.1, Sydney

Stewart, C. E., Paustian, K., Conant, R. T., Plante, A. F., & Six, J. 2007. Soil carbon saturation: Concept, evidence and evaluation. Biogeochemistry, 86(1), 19–31.

Swanston, C. W., Castanha, C., Berkeley, L., & Trumbore, S. E. 2009. Storage and Turnover of Organic Matter in Soil, In book: Biophysico-Chemical Processes Involving Natural Nonliving Organic Matter in Environmental System John Wiley & Sons, Inc.

Taylor, J.P., Wilson, B., Mills, M.S., Burns, R.G., 2002. Comparison of microbial numbers and enzymatic activities in surface soils and subsoils using various techniques 34.

Thomas, S.M., Beare, M.H., Rietveld, V., 2007. Changes in soil quality following humping/hollowing and flipping of pakihi soils on the West Coast, South Island New Zealand. New Zealand Grassland Association Sixty-Ninth Conference 265–270.

Tisdall, J. M., & Oades, J. M. 1982. Organic matter and water-stable aggregates in soils. Journal of Soil Science, 33, 141–163.

Van de Geijn , S.C., J. Goudriaan, J. Van der Eerden, and J. Rozema, 1993: Problems and approaches to integrating the concurrent impacts of ele- vated carbon dioxide, temperature, ultraviolet-B radiation, and ozone on crop production. In: International Crop Science , vol. I. Crop Science Society of America, Madison, WI, pp. 333-338

Van Veen, J.A., E. Liljeroth, L.J.A. Lekkerkerk, and S.C. Van de Geijn. 1991. Carbon fluxes in plant- soil systems at elevated atmospheric CO2 levels. Ecological applications, 1:175–181

Vidal, A., Hirte, J., Bender, S. F., Mayer, J., Gattinger, A., Höschen, C., … Mueller, C. W. 2018. Linking 3D Soil Structure and Plant-Microbe-Soil Carbon Transfer in the Rhizosphere. Frontiers in Environmental Science, 6(February), 1–14.

von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B., Flessa, H. 2006. Stabilization of organic matter in temperate soils: Mechanisms and their relevance under different soil conditions—a review. European Journal of Soil Science 57, 426–445

Wang, J., Chapman, S.J., Yao, H., 2014 The effect of storage on microbial activity and bacterial community structure of drained and flooded paddy soil, J Soils Sediments, 15, 880–889

Wu, J., Brookes, P.C., Jenkinson, D.S., 1993. Formation and destruction of microbial biomass during decomposition of glucose and ryegrass in soil. Soil Biology and Biochemistry 25, 1435–1441.

Yin, L., Dijkstra, F.A., Wang, P., Zhu, B., Cheng, W., 2018. Rhizosphere priming effects on soil carbon and nitrogen dynamics among tree species with and without intraspecific competition. New Phytologist 218, 1036–1048.

FIGURES AND TABLES

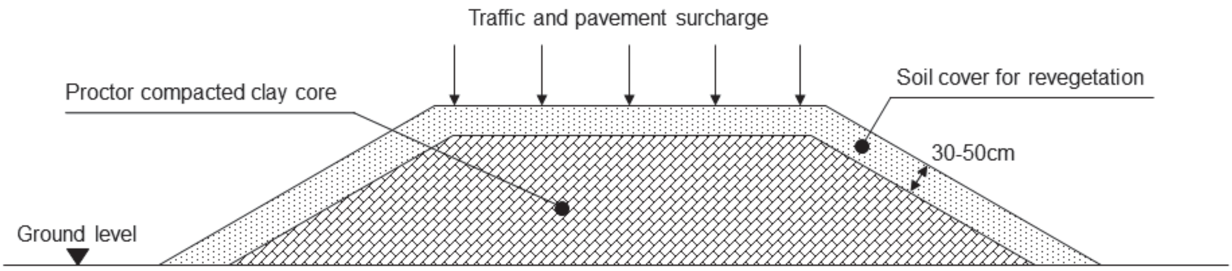


Figure 1: Section of a geotechnical embankments comprehensive of proctor compacted clay core and cover of soil for revegetation purpuses

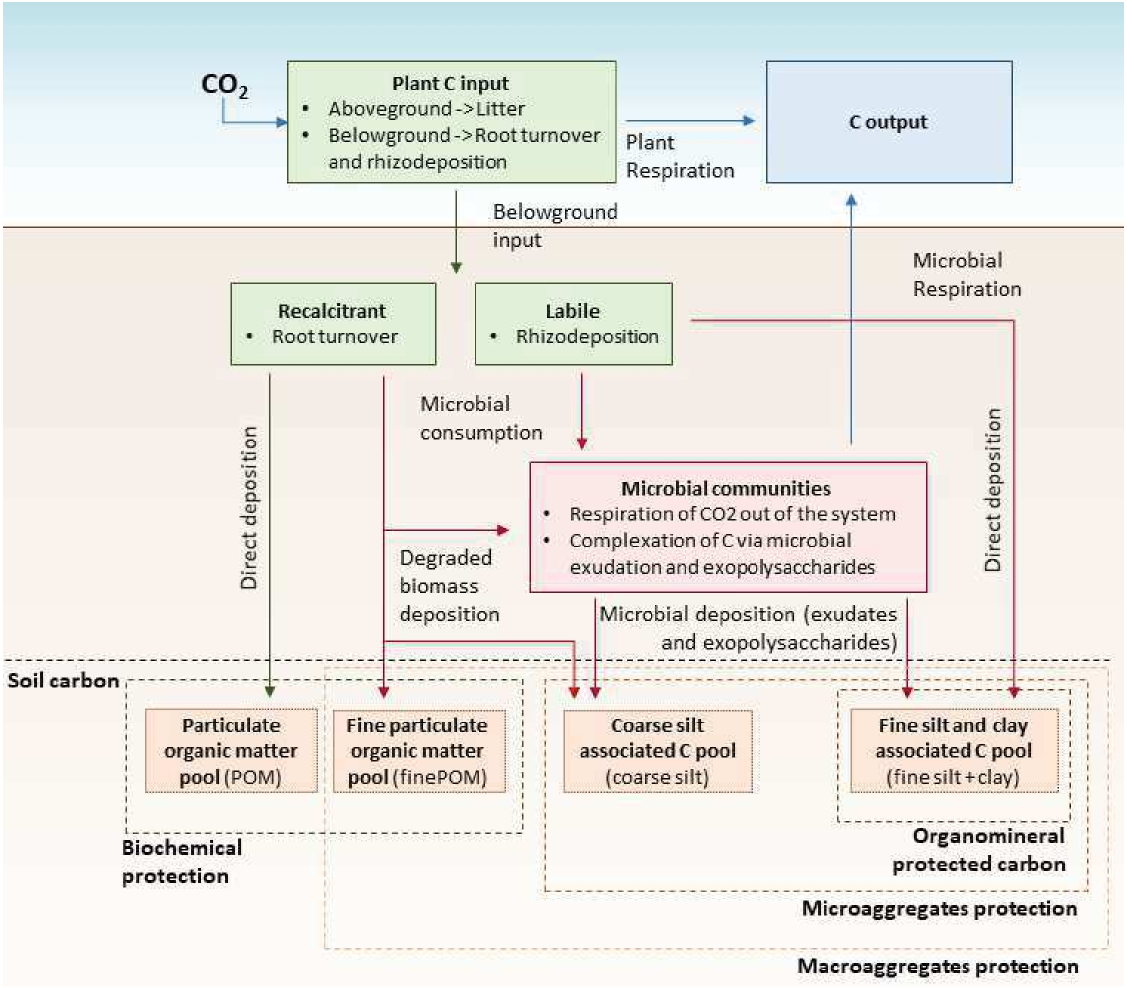


Figure 2: Flow chart of soil carbon (C) cycle and deposition/complexation in different soil fractions. In green square boxes, C input from plants is depicted, and green arrow signifies deposition of direct plant inputs. The red box symbolizes the microbial communities and the red arrows the consumption and deposition of C by microbial communities. Brown boxes represent the different soil C pools. The green dashed line represents the biochemical protection of free particulate organic matter (POM) in soil, while the brown dotted lines the soil protection via aggregate complexation and organomineral absorption on fine silt and clay minerals.

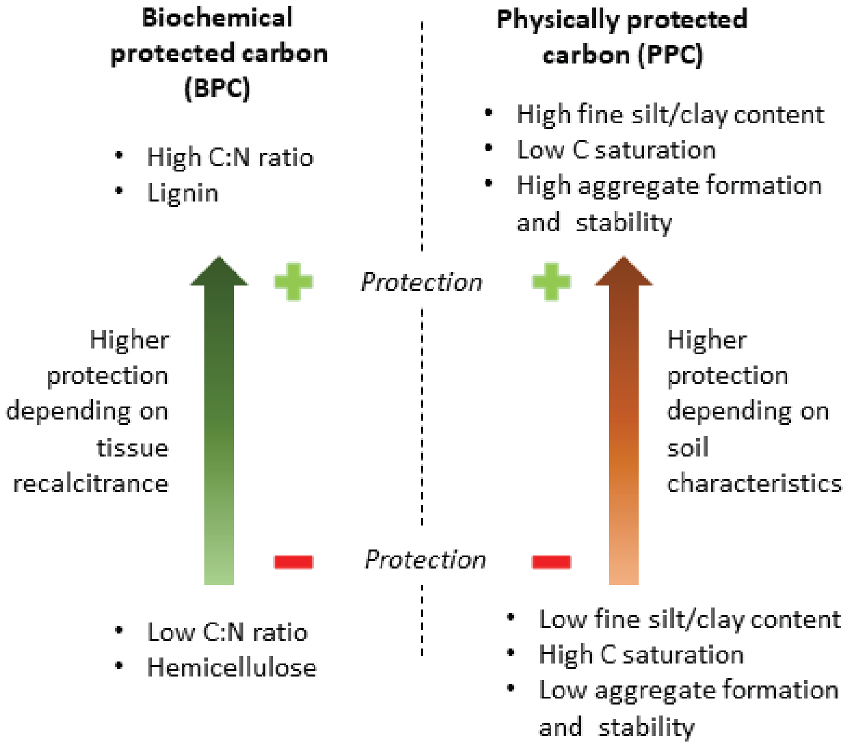


Figure 3: Representation of different types of carbon (C) protection in soil. Biochemically protected carbon (BPC, left) protection depends on the chemical composition of plant tissues, with recalcitrant C having a lower turnover. Physically protected carbon (PPC) depends on soil properties, more specifically on the aggregate formation and the resulting stability, the fine soil fraction in soil and soil C saturation levels.

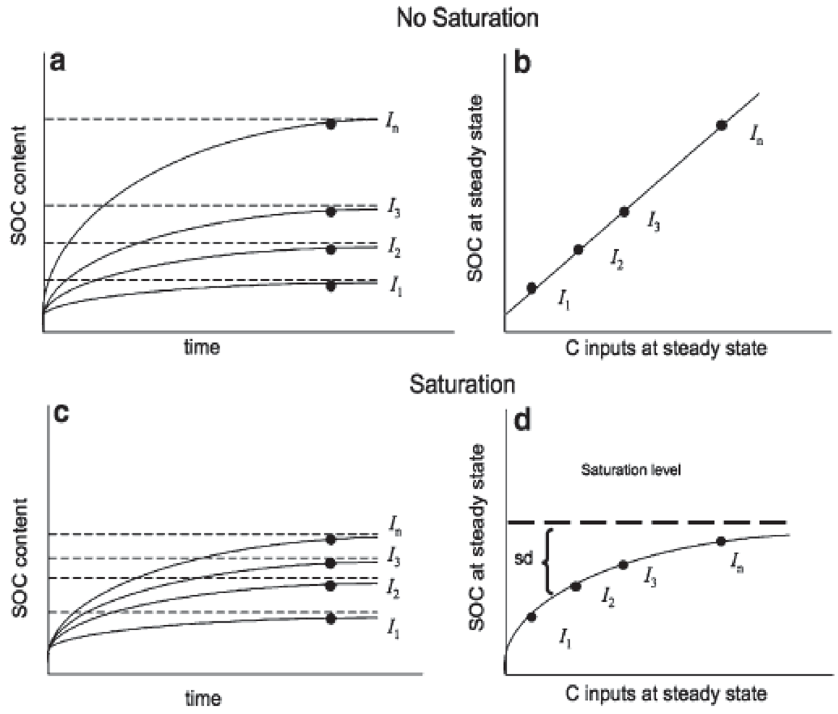


Figure 4 : Different soil organic C (SOC) evolutions with a constant carbon (C) input for two conditions: a,b, not considering the effect of C saturation, and c, d including the C saturation effect. Under unsaturated conditions, (a) a steady state soil C accumulation over time will express a linear relationship if expressed (b) over C input. If the relationship between SOC and time is analyzed for (c) a C saturated soil it will not be proportional, meaning that a C input increase will not result in a linear SOC accumulation over time, but (d) in a asymptotic relationship (after Stewart et al. 2007).

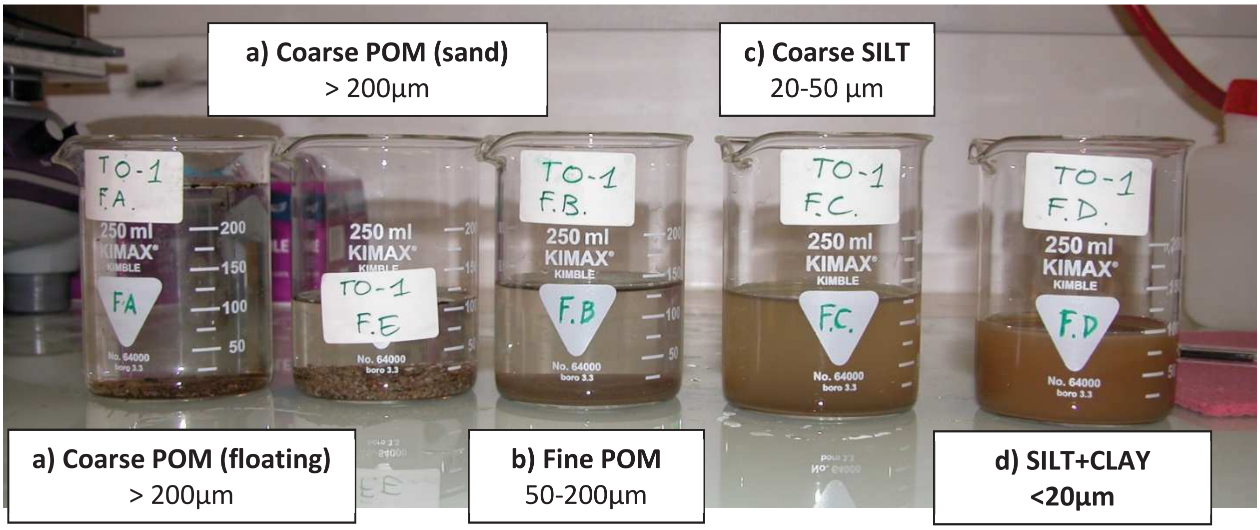
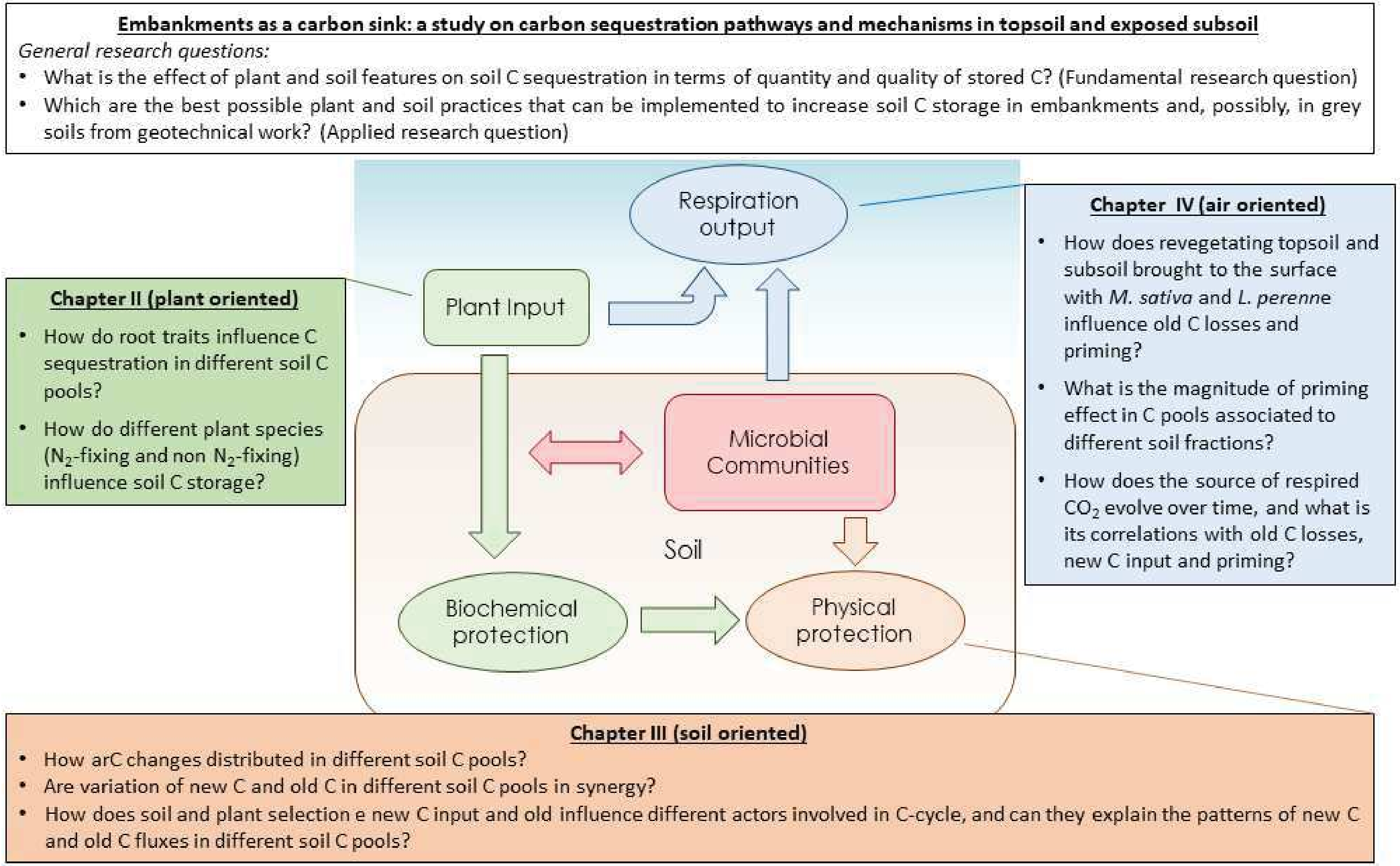


Figure 5: Example of soil fractionation following the Gavinelli et al. (2005) methodology. a) coarse POM soil fraction > 200µm (+ sand fraction), b) finePOM, soil fraction 50-200µm, c) the coarse silt fraction 20-50 µm, d) silt+clay fraction, <20µm.



Embankments as a carbon sink: a study on carbon sequestration pathways and mechanisms in topsoil and exposed subsoil

General research questions:

- What is the effect of plant and soil features on soil C sequestration in terms of quantity and quality of stored C? (Fundamental research question)
- Which are the best possible plant and soil practices that can be implemented to increase soil C storage in embankments and, possibly, in grey soils from geotechnical work? (Applied research question)

Chapter II (plant oriented)

- How do root traits influence C sequestration in different soil C pools?
- How do different plant species (N_2-fixing and non N_2-fixing) influence soil C storage?

Chapter IV (air oriented)

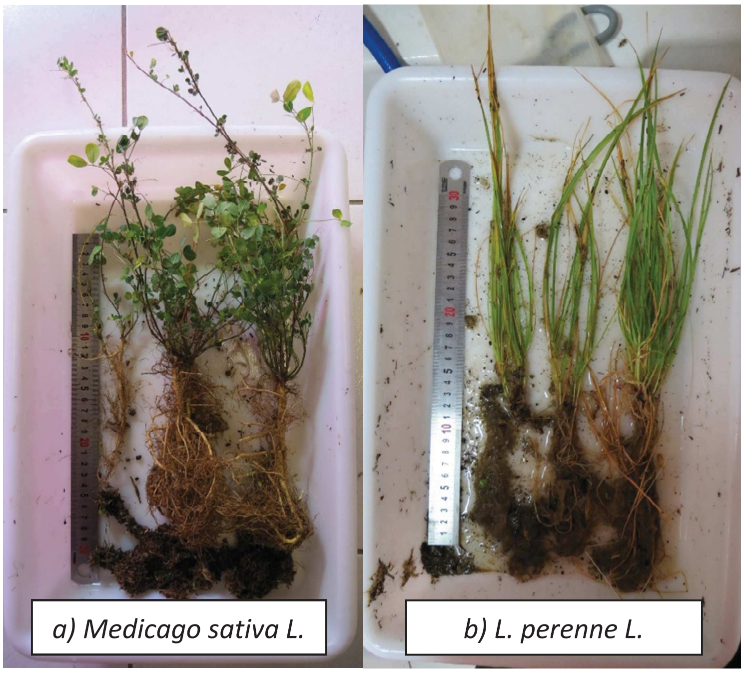
- How does revegetating topsoil and subsoil brought to the surface with *M. sativa* and *L. perenne* influence old C losses and priming?
- What is the magnitude of priming effect in C pools associated to different soil fractions?
- How does the source of respired CO_2 evolve over time, and what is its correlations with old C losses, new C input and priming?

Chapter III (soil oriented)

- How arC changes distributed in different soil C pools?
- Are variation of new C and old C in different soil C pools in synergy?
- How does soil and plant selection e new C input and old influence different actors involved in C-cycle, and can they explain the patterns of new C and old C fluxes in different soil C pools?

Figure 6: scheme of thesis structure and related research questions (RQ). Applied RQ are presented in the first box, together with the title of the thesis. Fundamental RQ are displayed in the different boxes related to the different chapters of the thesis. The scheme in the middle represents a simplified version of Figure 1. The squared boxes represent the main actors in C-cycle (green plants, red microbs, brown soil) while the circle the pools of C: soil carbon (biochemically and physically protected) and the atmospheric C in CO_2.

Figure 7: Experimental set up with 12 species grown in monoculture in grow-boxes. The picture shows two of the three blocks of growboxes present in the experiment (Chapter II)



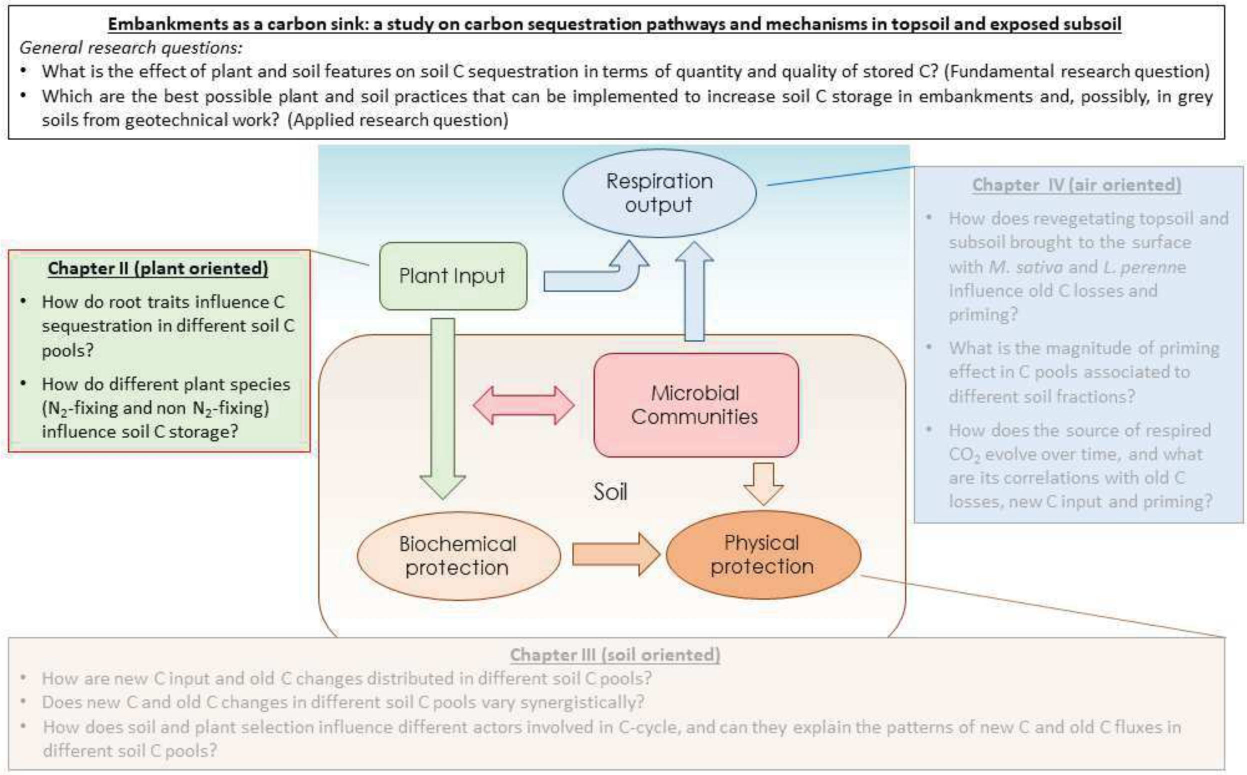
a) Medicago sativa L.

b) L. perenne L.

Figure 8: Species grown in ^{13}C constant labelling experiment sampled after 6 months for root traits assessment (Chapter III and IV).

Figure 9: Excavation in Pisciotta (SA), Italy, to collect the soil for the experiment described in Chapter III and IV. Topsoil excavated from 0-30cm depth, subsoil from 110-140 cm depth. On the far right a picture with the main components of soil structure visually isolated.

CHAPTER II: Pathway to persistence: plant root traits alter carbon accumulation in different soil carbon pools through microbial mediation



Embankments as a carbon sink: a study on carbon sequestration pathways and mechanisms in topsoil and exposed subsoil

General research questions:

- What is the effect of plant and soil features on soil C sequestration in terms of quantity and quality of stored C? (Fundamental research question)
- Which are the best possible plant and soil practices that can be implemented to increase soil C storage in embankments and, possibly, in grey soils from geotechnical work? (Applied research question)

Chapter II (plant oriented)

- How do root traits influence C sequestration in different soil C pools?
- How do different plant species (N_2-fixing and non N_2-fixing) influence soil C storage?

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- How does revegetating topsoil and subsoil brought to the surface with *M. sativa* and *L. perenne* influence old C losses and priming?
- What is the magnitude of priming effect in C pools associated to different soil fractions?
- How does the source of respired CO_2 evolve over time, and what are its correlations with old C losses, new C input and priming?

Chapter III (soil oriented)

- How are new C input and old C changes distributed in different soil C pools?
- Does new C and old C changes in different soil C pools vary synergistically?
- How does soil and plant selection influence different actors involved in C-cycle, and can they explain the patterns of new C and old C fluxes in different soil C pools?

Plant input is the first step in soil C sequestration. Plant choice influences the final C storage in soil by providing different amounts and quality of C input. In this chapter, we aim to quantify this effect by assessing the C changes in different soil C pools associated with different soil size particle fractions, and relating them to contrasting root traits characterizing 12 different herbaceous species used for embankment revegetation in south of France.

Pathways to persistence: plant root traits alter carbon accumulation in different soil carbon pools

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Keywords*: particulate organic matter, mineral-associated organic matter, carbon stabilization, physical and density soil fractionation, root biomass, root elongation rate, substrate induced respiration, microbial biomass*

ABSTRACT

Aims: Mineral-associated organic matter, mainly derived from microbial by-products, persists longer in soil compared to particulate organic matter (POM). POM is highly recalcitrant and originates largely from decomposing root and shoot litter. Theory suggests that root traits and growth dynamics should affect carbon (C) accumulation into these different pools, but the specific traits driving this accumulation are not clearly identified.

Methods: Twelve herbaceous species were grown for 37 weeks in monocultures. Root elongation rate (RER) was measured throughout the experiment. At the end of the experiment, we determined morphological and chemical root traits, as well as substrate induced respiration (SIR) as a proxy for microbial activity. Carbon was measured in four different soil fractions, following particle-size and density fractionation.

Results: In N_2-fixing Fabaceae species, root biomass, RER, root diameter, hemicellulose content and SIR, were all positively correlated with increased C in the coarse silt fraction. Root diameter and hemicellulose content were also negatively correlated with C in the POM fraction, that was greater under non N_2-fixing Poaceae species, characterized by lignin-rich roots with a high carbon:nitrogen ratio that grew slowly. The accumulation of C in different soil pools was mediated by microbial activity.

Conclusions: Our results show that root traits determine C input into different soil pools, mediated primarily by microbial activity, thus determining the fate of soil organic C. We also highlight that C in different soil pools, and not only total soil organic C, should be reported in future studies to better understand its origin, fate and dynamics.

Abbreviations

Abbreviation	*Meaning*
C	Carbon
POM	Particulate organic matter
C:N	Carbon – nitrogen ratio in plant tissue and/or soil
N_2-fixing	Dinitrogen fixing
t0	Time zero, beginning of the experiment
t37	Time 37 weeks, end of the experiment
ΔC	Delta carbon, as difference between carbon at time 0 and carbon at time 37, in different fractions (mg C g^{-1} soil)
C_{POM}	Carbon in the coarse POM 200-2000 µm fraction (mg C g^{-1} soil)
$C_{finePOM}$	Carbon in the fine POM 50-200 µm fraction (mg C g^{-1} soil)
C_{SILT}	Carbon in the 20-50 µm coarse silt fraction (mg C g^{-1} soil)
$C_{SILT+CLAY}$	Carbon in the fine silt+clay <20 µm fraction (mg C g^{-1} soil)
ΔC_{SUM}	Sum of delta carbon in different fractions, $\Delta C_{SUM} = \Delta C_{POM} + \Delta C_{finePOM} + \Delta C_{SILT} + \Delta C_{SILT+CLAY}$ (mg C g^{-1} soil)
RER	Root elongation rate (mm d^{-1})
RLP	Root length production (m)
RER_{NEW}, RLP_{NEW}	RER and RLP of 'new' roots initiated during the 2 weeks interval between measurements

RER_{OLD}, RLP_{OLD}	RER and RLP of ‘old’ roots, initiated more than 2 weeks before the measurement
SIR	Substrate induced respiration (µg C-CO_2 g^{-1} soil h^{-1})
PCA	Principal component analysis

2.1. INTRODUCTION

Given the current climate change emergency, several international initiatives have been launched to unlock the potential of soils to sequester atmospheric carbon (C) (e.g. 4 per Thousand Initiative, Minasny et al. 2017). Better understanding the interactions between vegetation and soil has become central for sequestering C into anthropogenically disturbed soil, such as agricultural fields, mining waste soil, road embankments and technosols (Paustian et al. 2016; Griscom et al. 2017). Plants act as a major conduit for transferring C into soils via litterfall, root mortality and exudation (Six et al. 2004; Derrien et al. 2016; Sokol et al. 2019). Some C is transformed by soil microbes and released back into the atmosphere by respiration (Jones et al. 2009; Kuzyakov and Larionova, 2005), but C can also be stabilized in soil, increasing its residence time (Besnard et al. 1996; Lal, 2004; Rasse et al. 2005; Bardgett et al. 2014 ; Vidal et al. 2018; Sokol et al. 2019). Carbon persists in soil at different time scales based on recalcitrance (short-term preservation), spatial inaccessibility to decomposers due to occlusion in soil aggregates, and adsorption to mineral and metal surfaces (Kleber et al. 2011, Schmidt et al. 2011, Poirier et al. 2018). These mechanisms are influenced by abiotic and biotic factors and especially by plant roots, since their C is preferentially stabilized compared to aerial parts (Balesdent and Balabane, 1996; Rasse et al. 2005, Kätterer et al. 2011). In a recent review, Poirier et al. (2018) argued that the root traits most influencing C stabilization are those related to chemical composition, root exudation and the presence of symbionts (e.g. mycorrhizas and *Rhizobium* bacteria), whereas the role of morphological traits is not yet clear. More specifically, root traits increasing chemical recalcitrance promote short-term C stabilization by slowing root decomposition rates, whereas root traits increasing exudation rate promote long-term C stabilization faster. Several studies have analysed the link between plant functional traits, microbial activity and C accumulation (Chapin 2003; Lavorel et al. 2007; De Deyn et al. 2008; Poirier et al. 2018). However, as yet, it is not understood how root traits can alter the accumulation and potential persistence of C.

Through differences in chemical and physiological traits, roots should affect C accumulation into different C pools depending on soil texture. These pools are defined as: i) coarse particulate organic matter (coarse POM, > 200μm fraction), that is free in the soil at different levels of degradation, ii) fine POM (50-200μm fraction), that comprises free organic C and organic C occluded in soil macroaggregates. These two pools are mostly derived from the decomposition of roots and shoots (Kögel-Knabner, 2002), and their short-term C protection from microbial consumption relies mainly on the recalcitrance of their lignocellulose C structures and the physical protection given by macroaggregate structure (Six et al. 2002). Finally, iii) C protected in the coarse silt and fine silt+clay pools (20-50μm and <20μm fractions, respectively); that is highly processed and protected from microbial consumption via occlusion in microaggregates and through organo-mineral adsorption to clay particles and metals. This C is mostly derived from dissolved organic C originating from the degradation of above and belowground plant C input (Bird et al. 2008; Rubino et al. 2010; Sanderman et al. 2014), from root exudation of labile rhizospheric compounds and from microbial compounds (Simpson et al., 2007; Mambelli et al., 2011; Cotrufo et al. 2013; 2014; Vidal et al. 2018; Rossi 2019). It is now generally accepted that labile low molecular weight compounds persist in soil longer than chemically recalcitrant C structures, when protected by organo-mineral adsorption (Mikutta et al. 2006; Kleber et al. 2015; King et al. 2019; Robertson et al. 2019; Sokol et al. 2019). The stability of sequestered C in soil is therefore linked to the fraction of soil to which it is associated, with a greater stability of C pools associated with finer fractions (Torn et al., 2009).

As C accumulation into the coarse POM pool is related to the amount of recalcitrant matter present, it should therefore be greater in soils containing roots with high cellulose, lignin and carbon:nitrogen ratio (C:N) (Poirier et al. 2018). However, it is C-rich exudates produced during fine root elongation that promote long-term C stabilization in the coarse silt and fine silt+clay fractions (Mikutta et al. 2006; King et al. 2019; Robertson et al. 2019; Sokol et al. 2019), and together with mucilage and border cells (shed during growth), are important substrates for microbial communities (Dennis et al., 2010). These C substrates that are assimilated by microorganisms close to the root apex are utilized rapidly for respiration and growth, or lost as microbial exudates or exopolysaccharides that are then used as a substrate for subsequent microbial communities. Since microbial byproducts (from activity in any soil C pool) are believed to be the main precursor of protected C due to organo-mineral interactions (Simpson et al., 2007; Mambelli et al., 2011; Cotrufo et al., 2013; Rossi 2019), root exudation should influence the C storage in this fraction. It is however important to mention that exudation and the resulting microbial activiy can also negatively influence soil C storage, increasing the consumption of preexistent soil C (i.e. priming effect; Hamer and Marschner, 2005; Shahzad et al. 2017). Root traits related to exudation are however poorly understood; the few studies available showed contrasted results and relate to root morphological traits measured at the whole root system level (Roumet et al. 2006; Guyonnet et al. 2018). Because root exudates are mainly released at the elongating root tip, where rhizospheric microbial activity is high (Jones et al. 2009; Canarini et al. 2019), it can be expected that root elongation rate (RER) is a powerful predictor of C deposits in coarse silt and fine silt+clay C pools (Holz et al. 2018). Root elongation rate is affected principally by local abiotic soil conditions such as soil temperature, moisture, and compaction, but also differs among species, although most known data is related to woody species (Steinaker et al. 2011; Mohamed et al. 2016; Wang et al. 2018). Fast growing species with small diameter fine roots, high specific root length and N uptake rate usually have high RER (Larson and Funk 2016).

Determining plant traits that increase C accumulation in different soil C pools should therefore enable the selection of species that promote C persistence in soil. Recent debate has focused on the ability of dinitrogen fixing (N_2-fixing) species to sequester large amounts of C in soil (Plaza-Bonilla et al. 2016; King et al. 2018). Bacteria such as *Rhizobium*, present in nodules of N_2-fixing species, produce large amounts of exopolysaccharides (Downie, 2010; Sasse et al. 2018), that are adsorbed onto fine silt and clay particles (Fehrmann and Weaver, 1978). Also, N_2-fixing species have roots that are easily degradable with a high content of hemicelluloses (Hernández et al. 2017) and low C:N ratio (Warembourg et al. 2003; Roumet et al. 2005), therefore enhancing microbial activity (Poirer et al. 2018). However, it is not known whether N_2-fixing species promote greater C accumulation in the fine silt and clay soil fractions, thereby enhancing C persistence in soil.

We explored the effect of root traits on C accumulation into different soil C pools beneath 12 herbaceous species grown in monocultures for 37 weeks. These species had diverse root traits in terms of morphology, chemical composition, and elongation rate and belonged to different plant families: five N_2-fixing Fabaceae, five Poaceae, one Rosaceae and one Plantaginaceae. Our main hypothesis is that C accumulation into different soil C pools is driven by root traits and their effects on microbial activity and biomass. More specifically, we hypothesize that (i) traits related to high RER promote C accumulation in the coarse silt and fine silt + clay C pools, since these traits are expected to favour exudation and subsequent microbial activity, whereas (ii) root traits related to chemical recalcitrance (high lignin and cellulose content and high C:N ratio), promote C accumulation in the unprotected POM pool, and iii) N_2-fixing species favour C accumulation in the coarse silt and fine silt + clay pools. Results should enable us to disentangle the relationships between root growth, traits and the accumulation and stabilization of C in different soil C pools, between different families (Poaceae and Fabaceae) and N_2 and non N_2-fixing species.

2.2. MATERIALS AND METHODS

2.2.1. *Experimental setup*

The experiment was set up in the experimental garden of CEFE-CNRS Montpellier, France (43.6389° N°, 3.864125° E and lasted 37 weeks (from t0: Sept-2016 to t36: July-2017). Twelve herbaceous species were grown as monocultures in steel boxes (0.7 m length x 0.7 m width x 0.3 m depth): five N_2-fixing species from the Fabaceae family and seven non N_2-fixing species, including five Poaceae, one Plantaginaceae and one Rosaceae species (Table 1). A weather station was set up permanently in the experimental garden, and air humidity, air temperature (minimum, maximum and mean daily) and solar irradiation (measured daily) were monitored throughout the experiment.

Seventy-eight boxes were prepared: six replicate boxes per species and six additional boxes of bare soil used as controls. Boxes were organized in three blocks with two rows of 13 adjacent boxes in each block, and with a distance of 50 cm between each box. Each row comprised 12 monocultures (one per species) and a bare soil, randomly arranged in each row (Fig S1). Boxes of the first row were used for destructive plant and soil sampling, while the boxes of the second row were equipped with rhizotrons for the study of root elongation (Section 1.1, Fig. S1). In the second block, each box was equipped with soil temperature and humidity sensors placed at a depth of 0.1 m. Soil temperature was recorded every 4 hours with an i-button sensor (iButtonLink, Wisconsin, USA); soil relative humidity was recorded every hour with moisture sensors (Waterscout SM100, Spectrum Technologies Inc.) and a datalogger (WatchDog weather station 200 series, Spectrum Technologies Inc.). These boxes were undisturbed for the duration of the experiment. Rhizotrons comprised a 0.2 m width x 0.3 m depth x 0.05 m thick pane of transparent plexiglass set into the lower walls of the boxes, through which roots were observed and root elongation rate (RER, in mm root^{-1} day^{-1}) and root length production (RLP, in mm mm^{-2} day^{-1}), were calculated (Fig. S2). For RER and RLP, only one replicate box per species was analyzed, because the analysis of root images was extremely time consuming.

Boxes were inclined at 20° relative to the horizon to encourage the positive geotropism of roots when they came into contact with rhizotron windows (Huck and Taylor, 1982). Boxes were filled with soil sieved to 8 mm. Five layers of soil were successively added and manually compacted to attain a volume of 0.113 m^3, i.e., a total of 190 kg of soil per box (bulk density = 1.70 ± 0.02 g cm^{-3}). The soil, excavated in Villefort (France; 44°26’25” N, 3°55’58” E), was sandy-loam (62.6 % sand, 26.1 % silt, 11.3 % clay); with 1.36 g kg^{-1} of total N, 16.9 g kg^{-1} of total C, 0.069 g kg^{-1} of phosphorus (P Olsen), pH in water was 7.06, and cation-exchange capacity (CEC) was 7.98 cmol$_c$ kg^{-1}.

On 17-19 October 2016, 72 boxes were sown as monocultures (12 species x 6 replicates with one replicate species per row). Seeds of each species were sown in lines, the distance between lines was 75 mm and the distance between plants within a line was 75 mm, leading to a final plant density of 155 plants m^{-2}. Once seeds had germinated, each box was inoculated with a purified solution of local *Rhizobium* bacteria strains (Incolum Valorhiz™, France) and was netted to avoid birds disturbing seeds. During the experiment, mean air temperature was 13 C° (Figure S3) with a maximum of 30 °C and a minimum of -0.4 °C (Figure S3a), and the cumulative precipitation was 349 mm (Fig S3 for additional information on climatic conditions over the 37 weeks of the experiment). Soil temperature in the boxes followed closely the air temperature over the 37 weeks period, with a mean of 13.5 °C, a maximum of 25.9 °C and a minimum of 3 °C (Figure S3a). Air humidity ranged from 53 to 87%, with a mean value of 74% (Figure S3a) and solar irradiation ranged from 320 to 897 W m^{-2} with a mean value of 568 W m^{-2} (Figure S3b). During the experiment, boxes were carefully weeded by hand and plants were cut to ground level every 4 months to maintain a regular aerial cover. In addition, each box was watered with sprinklers when required.

2.2.2. Analysis of carbon content in different soil fractions

Soil C content was measured before filling the boxes, as a reference for time 0 (t0), on three samples from the initial homogenized soil batch, and at the end of the experiment, i.e. at 37 weeks (t37) after sowing. At t37, soil samples were taken at 0-200 mm depth using a soil corer (75 mm in diameter) in each box dedicated to soil and plant sampling. All soil samples were separated into two depths (0-100 mm and 100-200 mm), air dried and separately sieved to 2 mm. A subsample of 40 g of soil was collected at a depth of 0-100 mm for subsequent fractioning into POM fractions (coarse POM: 200-2000 μm and fine POM: 50-200 μm), coarse silt (20-50 μm), fine silt + clay (<20 μm) fractions. Soil fractionation was carried out using the method from Gavinelli et al. (1995). Soil samples were presoaked overnight in 300 ml of deionized water at 4°C with 0.5 g of hexametaphosphate to enhance disaggregation. Soil was then shaken at 300 rpm (digital orbital shaker, Intertek) with five agate marbles for 2 h (i.e., the time suggested for sandy soils, to avoid the transfer of C into finer fractions, Gavinelli et al. 1995). The soil was wet sieved with a 200 µm sieve, and the resulting 200-2000 µm fraction was then transferred into a separate container and soaked in deionized water. The floating coarse particulate organic matter (POM) was then carefully collected. The remaining 200-2000 µm fraction represented the coarse sand fraction in soil and was carefully collected by washing the content of the sieve in a beaker using deionized water. Then, the remaining fraction was sieved with a 50 µm sieve, to separate and collect the fine sand fraction and the fine POM fraction (50-200 µm). The remaining fraction <50 µm was sonicated with a 1510E-MT Bransonic sonicator for 10 minutes to break microaggregates before sieving at 20 µm. The 20-50 µm fraction (coarse silt) was collected and the resulting solution of deionized water and <20 µm fraction collected in a beaker and filled up to 1.0 L. This solution was tumbled 30 times to homogenize it and an aliquot of 100 mL was collected with the aid of a syringe, representing the fine silt + clay fraction. All the fractions were oven dried at 40°C until all the water evaporated. The dried fractions were weighed to check that the sum of the fraction's weight did not differ from +/-5% the initial 40 g total weight. The quality of the soil particle dispersion was checked and did not differ +/-5% compared to the soil texture analysis, being 62.6% in the sand fraction and 37.4% in the fine silt + clay fraction.

Carbon content in each of the soil fractions (C_{POM} - carbon in the coarse POM fraction; $C_{finePOM}$ – carbon in the fine POM fraction; C_{SILT} - carbon in the coarse silt fraction and $C_{SILT+CLAY}$ - carbon in the fine silt+clay fraction), was analyzed using an elemental analyzer (CHN model EA 1108; Carlo Erba Instruments, Milan, Italy) to assess the amount of C present in each pool. A subsample of 0.1 g was taken from each 40 g sample and analyzed without fractioning to determine the total C in the bulk sample. The difference between total C in bulk soil and the sum of C in the different soil fractions was used to assess the correctness of the fractionation (+/-5%) and was equal to 97.3% (SD=22%; n=34).

We calculated soil C changes (ΔC) in each soil fraction as the difference between C content in mg C g^{-1} soil at 0 and 37 weeks ($\Delta C = C_{t37} – C_{t0}$). The sum of ΔC in each soil fraction ($\Delta C_{SUM} = \Delta C_{POM} + \Delta C_{finePOM} + \Delta C_{SILT} + \Delta C_{SILT+CLAY}$) was also calculated to investigate the variation in the totality of the soil fractions. Note that ΔC can be either positive (accumulation) or negative (depletion due to the positive priming effect, that is the increase of pre-existing soil C consumption and losses due to vegetation, Kuzyakov, 2002).

All the raw data can be found in the Harvard Dataverse 'Embankment as a carbon sink: a study on carbon sequestration pathways and mechanisms in topsoil and exposed subsoil', DOI: 10.7910/DVN/QTFLVE.

2.2.3. Measurement of root elongation rate (RER) and root length production (RLP)

As soon as the first root of a given species was visible in a rhizotron, roots of that species were scanned every 2 weeks for the experiment experiment (i.e. n = 19 measurement dates) using a smartphone scanner application CamScanner (INTSIG Information Co., Ltd, Shanghai, China; version 3.9.5). A smartphone (Samsung Xcover3, Samsung Electronics, Korea) was kept at a fixed distance from the rhizotron (0.3 m) and a ruler was included in the picture to set the scale (Mohamed et al. 2017). Images were then analyzed with the SmartRoot software (Lobet et al. 2011), a freeware plugin of ImageJ software (Schneider et al. 2012). The images acquired were converted into 8-bit grey scale and, when necessary, color- inverted, so that roots were dark on a lighter background. SmartRoot allows the semi-automatic tracing of roots by clicking on the basal point of each root (Fig. S4a). Data extracted include the length and diameter of the roots. The resulting traced image of roots could then be imported and superimposed onto a new image, allowing analysis of subsequent images and creating a time-dependent dataset acquiring root length at different time steps.

Root elongation rate (RER; mm d^{-1}) is indicative of primary root growth and is defined as the difference in individual root length measured between two dates. RER is a frequent but punctual observation of root dynamics over time. As soil C storage is a cumulative process, root length production (RLP; m) after 37 weeks was also calculated for up to 60 roots (randomly chosen) per rhizotron. RLP is the total length of all roots produced in a specific period of time (Mommer et al. 2015). Of these 60 roots measured, 30 were selected from the 'new roots', i.e., the roots that were not present in the previous scan, and so had formed in the previous two weeks. Of the 30 'new roots', 20 were primary and first order roots and ten were second order laterals (Fig. S4a, according to the developmental centrifugal protocol of root topology; Berntson, 1997). Then, 30 'old roots' were selected at each subsequent sampling date. The 'old roots' were the roots already present in the previous scan (again, 20 primary axes and ten second order lateral roots). Fig. S5 shows an example of rhizotron analysis for new and old roots. To have a more representative sample of the 'old roots,' ten primary roots were selected from the 20 primary 'old roots' of the previous scan, ten were selected from the 20 newly emerged roots of the previous scan, five were chosen from the ten second order lateral 'old roots' of the previous scan and five were selected from the newly emerged second order laterals of the previous scan. This method was used to select roots at each subsequent sampling date. If one or more roots had: 1) reached the boundaries of the rhizotron, or 2) were in a bundle and not distinguishable (Fig. S4b), or 3) could not be analyzed for any other reason (e.g. soil masking the root), they were discarded and different roots were then selected.

The mean daily RER was calculated by subtracting from the length of a root (L_{t2}) the length of the root acquired at the previous sampling date (L_{t1}). This result was then standardized dividing by the number of days between the two sampling dates (t) to have the mean elongation rate of a single root:

$$RER(t) = (L_{t2} - L_{t1})/[t2 - t1] \qquad \text{Equation 1}$$

Root length production (RLP) of roots over the 37 weeks was chosen as a cumulative indicator for root dynamics, adapted from Mommer et al. (2015):

$$RLP = \sum_{t=1}^{T} \sum_{r=1}^{R} (\mathrm{RER(t)} \quad * \frac{R_{30}}{R}) \qquad \text{Equation 2}$$

Where *t* represents the sampling date ; $_{(t)}$; RER(t) is the daily RER; R the real number of roots analyzed in that interval. Since the number of analyzed roots varied depending on dates and species, we decided to standardize the analysis of RLP for R_{30} = 30 roots.

To refine the understanding of root dynamics, the RER and RLP were calculated separately for the new roots (RER_{NEW} and $RLP_{NEW,}$ i.e. roots initiated during the 2 weeks interval between measurements), old roots (RER_{OLD} and RLP_{OLD}, i.e. roots older than 2 weeks), and also the total root system, regardless of root age. For all species, RER was high during the first two samplings after their initiation and then decreased rapidly or stopped. Therefore, mean RER could be biased by the development of new roots, justifying our decision to separate roots based on age and order for the statistical analysis.

2.2.4. Analysis of root traits

After 37 weeks, a soil core (75 mm diameter, 200 mm depth), centered on one individual plant per species and per box was collected. In each core, roots were separated from the aboveground part and washed. Roots were sorted into absorptive roots, typically the first, second and third root orders (defined as the most distal root orders), and transport roots, that were higher order roots (all orders above third order roots, following McCormack et al. 2015). A subsample of absorptive roots (0.1 g dry mass on average) was selected, stained in a solution of methyl violet (0.5 g L^{-1}), spread into a transparent water filled tray and scanned at 800 dpi (Epson Expression 1680, Canada).

The software Winrhizo Pro (Regent Instruments, Quebec, Canada) was used to determine the root diameter (from 0 to 2 mm, with a 0.1 mm diameter interval) of absorptive roots. Roots were then oven dried at 40°C for 3 days and weighed to determine the total root dry mass for each core.

For each species, determination of root chemical composition was conducted on three subsamples of absorptive roots reserved for chemical analyses. C and N concentrations were determined on ground material using an elemental analyser (CHN model EA 1108; Carlo Erba Instruments, Milan, Italy). Concentrations of water-soluble compounds + hemicelluloses, cellulose and lignin were obtained following the Van Soest method (Van Soest, 1963) and using a fiber analyser (Fibersac 24; Ankom, Macedon, NJ, USA). This method consists of measuring the various plant tissue constituents by sequential extraction with neutral detergent, acid detergent and sulfuric acid (76%).

Substrate-induced respiration (SIR) was used as a proxy for potential soil microbial respiration and activity, according to Beare et al. (1990). Briefly, 20 g air-dried 2 mm sieved soil samples were incubated in 150 mL sealed serum flasks with 1.5 mg C-glucose g^{-1} soil, at 80 % field capacity and at 25°C. A 200 µL aliquot of the flask headspace was analyzed for CO_2 concentration after 2 and 6 hours using a microcatharometer (MicroGC Serie S, SRA Industries, Marcy l'Etoile, France), equipped with a PoraPlot column (Agilent, Santa Clara, United States). Substrate induced respiration rates were calculated as the mass of C-glucose converted to C-CO_2 per g of soil dry weight and per hour (in µg C-CO_2 g^{-1} soil h^{-1}).

2.2.5. *Statistical analysis*

First, a one-way analysis of variance (ANOVA) and post-hoc Tukey honestly significance difference (HSD) tests were performed to test the effects of species on mean RER, mean RLP, root traits and ΔC sequestration in soil C pools. Secondly, one way ANOVAs were conducted on the five Poaceae species and the five Fabaceae species, i.e excluding *P. lanceolata* and *S. minor* (hypothesis 3). In order to select the environmental parameters to be included in the constrained ordination, an initial db-RDA including all parameters was performed followed by a stepwise model selection using Generalized Akaike Information Criterion (AIC, ordistep function with a backward direction). The normal distribution of residuals was verified using a Shapiro-Wilk test ($p = 0.05$). If the data were not normally distributed, one way ANOVA was substituted with a Kruskal-Walls test. Finally, the same procedure using one way ANOVA was performed to compare the mean effect of N_2-fixing and non N_2-fixing species (for the latter, grouping together Poaceae, *P. lanceolata* and *S. minor*) on root traits and C storage (hypothesis 3).

A principal component analysis (PCA) was performed on 12 variables (six root traits, four ΔC of each soil C pool plus their sum, and SIR) using the mean for three replicate boxes ($n = 12$) to investigate the effect of root traits at the species level. RER and RLP were not included in the PCA since they were measured on one replicate box per species. Then, Pearson's correlation coefficients were calculated to study the relationships between root traits and ΔC in each soil C pool (hypotheses 1 and 2) and linear models of the significant correlations were analyzed to study the data dispersion. To deepen the undesrtanding of these correlations, Pearson's correlation analysis and a study of the linear models were performed on raw data ($n = 34$) to study relationships at the individual level.

To investigate the effect of abiotic factors on root growth dynamics, Pearson's correlation coefficients were calculated between mean daily RER, mean RLP, mean daily soil and air temperatures, mean daily soil humidity and mean daily solar irradiation ($n = 12$ for each variable). Means of daily climate data were calculated for the 2 weeks preceding the measurement of RER.

All the statistical analyses were performed in the open-source statistical environment R, version 3.4.3 (R Development Core Team, 2017) using the packages *Hmisc* (Harrel 2007) and *vegan* (Oksanen et al. 2019).

2.3. RESULTS

Effect of plant species on soil carbon accumulation (ΔC) in different C pools associated with soil fractions

Plant species did not significantly influence the accumulation of C in different pools, nor in the sum of C pools (Fig. 1). The mean ΔC_{SUM} increase was 1.72 ± 1.45 mg C g^{-1} soil, and was highest in soil beneath *L. corniculatus* (3.60 ± 0.70 mg C g^{-1} soil) compared to the bare soil control (0.21 ± 3.87 mg C g^{-1} soil, Fig. 1a). The mean increase in the coarse pool ΔC_{POM} was 0.58 ± 0.34 mg C g^{-1} soil (Fig. 1b) and in the $\Delta C_{finePOM}$ was 1.21 ± 0.74 mg C g^{-1} soil (Fig. 1c). In the protected C_{SILT} pool, the ΔC mean increase was 0.57 ± 0.34 mg C g^{-1} soil (Fig. 1d), while the $\Delta C_{SILT+CLAY}$ decreased by -0.50 ± 0.77 mg C g^{-1} soil (Fig. 1e). However, no significant differences were found between any species and bare soil with regard to any C pool (Fig. S6, C data in different soil C pools for each species at t37).

Significant differences in mean ΔC between N_2-fixing Fabaceae and non N_2-fixing Poaceae were found with regard to C_{POM} and C_{SILT}. Mean C_{POM} was significantly higher in soil beneath Poaceae species (ANOVA, p = 0.024, Tukey HSD test, Fig. 2a), whilst C_{SILT} was significantly higher in Fabaceae species (ANOVA, p = 0.060, Tukey HSD test, Fig. 2b), and no significant differences were found in C_{SILT} between Poaceae and bare soil. When grouping the data for all the non N_2-fixing species (i.e., Poaceae, *P. lanceolata* and *S. minor*), mean C_{POM} was higher compared to N_2-fixing Fabaceae (ANOVA, p = 0.06, F = 3.61) but C_{silt} was lower (ANOVA, p =0.01, F = 7.01) (Fig. 2), although a Tukey HSD test did not find significant differences between N_2-fixing and non N_2-fixing species.

2.3.1. Root elongation rate (RER) and root length production (RLP)

More than a threefold variation in mean daily RER_{TOT} occurred among species, ranging from 0.23 mm d^{-1} (*F. rubra*) to 0.75 mm d^{-1} (*T. repens*) (Table 1). Mean daily RER_{TOT} did not differ between N_2-fixing Fabaceae (0.57 ± 0.08 mm d^{-1}on average) and non N_2-fixing Poaceae (0.42 ± 0.13 mm d^{-1}, ANOVA, p = 0.221, Table 1), even when grouped with non N_2-fixing species (0.46±0.14 mm d^{-1}, ANOVA, p = 0.075). Mean daily RER_{TOT} peaked at 0.75 mm d^{-1} in mid-February for Poaceae and then decreased, attaining a value of 0.4 mm d^{-1} from April to June 2017 (Figs. S7). For Fabaceae species, mean daily RER_{TOT} peaked at 1.1 mm d^{-1} in May 2017, before decreasing sharply in June 2017 (Fig. S7, mean RER_{TOT} for Fabaceae and Poaceae species).

The mean daily RER for new roots (RER_{NEW}, 0.83 ± 0.22 mm d^{-1}) was significantly higher than that of old roots (RER_{OLD}, 0.17 ± 0.09 mm d^{-1}, ANOVA, p < 0.001). Mean daily RER_{NEW} ranged from 0.32 mm d^{-1} (*F. rubra*) to 1.13 mm d^{-1} (*D. glomerata*) whereas RER_{OLD} ranged from 0.05 mm d^{-1} (*P. pratensis*) to 0.40 mm d^{-1} (*T. pratense*). Mean daily RER_{NEW} did not differ in N_2-fixing Fabaceae compared to non N_2-fixing Poaceae or all non N_2-fixing species grouped together. However, mean daily RER_{OLD} was greater in N_2-

fixing Fabaceae (0.25 ± 0.09 mm d^{-1}) than in non N_2-fixing Poaceae (0.13 ± 0.03 mm d^{-1}, ANOVA, p = 0.020) or all non N_2-fixing species grouped together (0.12 ± 0.04 mm d^{-1}, ANOVA, p = 0.005, Table 1).

After 37 weeks, the highest cumulative RLP_{TOT} was observed in in *O. viciifolia* (3.62 m) and the lowest in *F. rubra* (1.19 m) (Table 1). N_2-fixing Fabaceae species possessed a greater RLP_{TOT} (3.37 ± 2.32 m) compared to non N_2-fixing Poaceae (2.32 ± 0.70 m, ANOVA, p = 0.032), as well as all the N_2-fixing species grouped together (2.42 ± 0.63 m, ANOVA, p = 0.009). Root dynamics of only three species were correlated with climate factors. In *L. corniculatus,* mean daily RER_{TOT}, RER_{OLD}, RER_{NEW}, RLP_{TOT}, RLP_{OLD} and RLP_{NEW} were all positively correlated with soil and air temperature and solar irradiation (Tables S1, S2). In *T. repens,* RER_{TOT} and RER_{NEW}, RLP_{NEW} were significantly and positively correlated with soil and air temperature (Tables S1, S2). With regard to Poaceae species, mean RER_{NEW} of *D. glomerata* was negatively correlated with soil and air temperature and solar irradiation (Table S1). In *O. viciifolia*, RLP_{TOT} was slightly and positively correlated with solar irradiation (Table S2). In *D. glomerata*, RLP_{NEW} only, was negatively correlated with soil and air temperature (Table S2).

2.3.2. Root biomass, diameter and chemical composition

At 37 weeks, *M. sativa* had significantly greater mean root biomass (4.23 ± 0.42 g) compared to all other species (Tukey HSD test, Table 1). In general, N_2-fixing Fabaceae species had a significantly higher mean root biomass (2.08 ± 1.33 g) compared to non N_2-fixing Poaceae (0.62 ± 0.11 g) and all the non N_2-fixing species grouped together (0.65 ± 0.17 g, ANOVA, $p < 0.001$). The mean diameter of absorptive roots differed significantly between species, with *O. viciifolia* having the thickest absorptive roots and *D. glomerata* the thinnest (0.21 ± 0.14 mm, Table 1). Species from the N_2-fixing Fabaceae family had significantly thicker absorptive roots (0.39 ± 0.11 mm) compared to non N_2-fixing Poaceae (0.23 ± 0.03 mm) or all the non N_2-fixing species grouped together (0.25 ± 0.03 mm, ANOVA, $p < 0.001$).

The chemical composition of absorptive roots strongly varied among species and between N_2-fixing Fabaceae and non N_2-fixing Poaceae or all the non N_2-fixing species grouped together (Table 1). Absorptive roots of N_2-fixing Fabaceae possessed more hemicelluloses + water-soluble compounds (705 ± 74 mg g^{-1}) than non N_2-fixing Poaceae (543 ± 33 mg g^{-1}) or all non N_2-fixing species grouped together (583 ± 69 mg g^{-1}), a lower mean lignin content (N_2-fixing Fabaceae: 173 ± 56 mg g^{-1}, non N_2-fixing Poaceae: 302 ± 59 mg g^{-1}, all non N_2-fixing species grouped together: 264.18 ± 79.06 mg g^{-1}), and a lower mean C:N ratio (N_2-fixing Fabaceae: 19.15 ± 3.07, non N_2-fixing Poaceae: 58.67 ± 6.34 , and all non N_2-fixing species grouped together: 62.04 ± 7.41). Mean root cellulose content did not differ either among species or between N_2-fixing Fabaceae and non N_2-fixing Poaceae. However, when all the non

N_2-fixing species were grouped together, absorptive roots had a significantly higher mean cellulose content compared to N_2-fixing Fabaceae (Table 1).

2.3.3. *Soil substrate induced respiration (SIR)*

Mean SIR for soil microbial communities varied significantly among species and between N_2-fixing Fabaceae (5.28 ±1 µg C-CO_2 g^{-1} soil h^{-1}) and non N_2-fixing Poaceae (3.12 ± 0.41 µg C-CO_2 g^{-1} soil h^{-1}, ANOVA, $p < 0.001$, Table 1). Mean SIR ranged from 2.47 ± 0.34 µg C-CO_2 g^{-1} soil h^{-1} (beneath *B. erectus)* to 6.41 ± 0.56 µg C-CO_2 g^{-1} soil h^{-1} (beneath *M. sativa*). When grouping all the non N_2-fixing species together, mean SIR was still significantly lower (3.1 ± 0.46 µg C-CO_2 g^{-1} soil h^{-1}) compared to N_2-fixing Fabaceae (5.28 ± 1.02 µg C-CO_2 g^{-1} soil h^{-1}, ANOVA, $p < 0.001$, Table 1).

2.3.4. *Relationships between soil carbon accumulation (ΔC), root growth dynamics, root traits, and substrate induced respiration (SIR)*

The PCA conducted on the ΔC in the different C pools, SIR and root traits explained 64.6% of the variance of the variables analyzed (Fig. 3). The first PCA axis (horizontal), accounting for 44.4% of the variation, opposed ΔC_{POM} (negative) and ΔC_{SILT} (positive), while the remaining C pools, as well as the sum of C pools, covaried and were quite orthogonal to ΔC_{POM} and ΔC_{SILT} and related to the second PCA axis, that accounted for 20.2% of the variation. SIR and root biomass, diameter, and hemicelluloses + water soluble compounds content of absorptive roots all went along the 1st axis (positive) together with ΔC_{SILT}. Root traits linked with recalcitrance, lignin, cellulose and C:N ratio, went along the 1st axis (negative) together with ΔC_{POM}. Convex hull polygons reflecting intraspecific variations generally had small areas and were segregated over the biplot (Fig. 3). The PCA strongly discriminated Poaceae from Fabaceae. Poaceae were all on the negative end of the first axis and were characterized by high lignin and cellulose contents, high C:N and accumulation of C in the coarse POM fraction. Fabaceae species were at far right of the first axis and were characterized by a higher biomass and thicker roots that were rich in hemicelluloses, favoring accumulation of C in the coarse silt fraction. The two other non N_2-fixing species were situated in intermediate positions on the axis.

When analyzing the species effect of root traits on C storage (n = 12) regression analyses showed that mean ΔC_{POM} was not related to RER, but was slightly significantly and negatively related to two traits: diameter and hemicelluloses + water-soluble compounds content of absorptive roots (Table S3a, Figure 4a,b). Mean ΔC_{SILT} was significantly and positively correlated with mean daily $RER_{OLD\ and}$ RLP_{OLD} and with the mean diameter of absorptive roots, root biomass, hemicellulose + water-soluble compounds of absorptive roots, and SIR (Table S3s, Figures 5a,b,c,d,f, h), whereas mean ΔC_{SILT} was negatively correlated with mean lignin and C:N ratio (Table S3s, Figures 5e,g). Linear regressions of mean ΔC_{SILT} and C:N ratio show two segregated clusters of points: one with low C:N related to N_2-fixing species and one with non N_2-fixing species having a high C:N ratio and low accumulation in ΔC_{SILT} (Figure 5g). Variations in mean ΔC_{SUM}, $\Delta C_{finePOM}$ and $\Delta C_{SILT+CLAY}$ were not explained by any variables. Mean SIR was significantly and positively correlated to mean RER_{OLD} and RLP_{OLD}, root biomass and hemicelluloses + water-soluble compounds (Table S3, Fig. 6a,b,c,e), but negatively correlated with mean lignin and C:N ratio (Table S3a, Figures 6d,f). Mean hemicelluloses + water-soluble compounds were significantly and negatively correlated with mean lignin content and C:N ratio (Table S3a).

When considering Pearson's correlations at the individual level (n = 36), significant correlations were found only between absorptive root diameter and ΔC_{POM}, SIR and ΔC_{SILT} (Table S3b). Correlations between root traits and mean SIR were similar compared to correlatins of raw data (Table S3b). The data dispersion in linear models showed that at the individual level, even if R^2 was low, the tendency remained the same as that when mean data were used for ΔC_{POM} (Fig. S9), ΔC_{SILT} (Fig. S10), and SIR (Fig. S11).

2.4. DISCUSSION

Total C accumulation in soil did not differ among plant species (Fig. 1), but as expected, C accumulation was significantly greater in the C_{SILT} pool beneath N_2-fixing Fabaceae, whereas in soil beneath non N_2-fixing Poaceae species, C accumulation was greater in the C_{POM} pool (Fig. 2). In line with our hypotheses, the accumulation of C into different soil C fractions, specifically C_{POM} and C_{SILT}, was correlated with root traits (Fig. 4, 5, 6). The more rapid RER and greater RLP of older roots promoted C accumulation into the C_{SILT} pool, but smaller root diameter and low content of labile compounds (hemicelluloses and water soluble compounds) enhanced C accumulation into the C_{POM} pool. Although measuring total soil organic carbon can be an easy method to evaluate C storage, it is not as sensitive to short-term C dynamics or effect of plant species and families, as the C changes in different soil fractions. Studies of C sequestration should therefore focus on better estimating C input into different C pools associated with soil textural fractions (Wiesmeier et al. 2019).

2.4.1. Hypothesis 1: Root elongation rate and root length production are expected to favour carbon accumulation in the C_{SILT} and $C_{SILT+CLAY}$ fractions

We hypothesized that a fast RER would promote C accumulation in coarse silt and fine silt+clay soil fractions, through an increase in exudation and microbial activity along newly initiated roots. Interestingly, RER_{OLD} and RLP_{OLD} were significantly and positively correlated with soil microbial SIR and ΔC_{SILT} (Fig. 5a,b, Table S3a), but not with the RER and RLP of newly initiated roots, that had very high rates of growth. Dennis et al. (2010) hypothesized that rapidly elongating root tips grow quickly out of the main zone of microbial activity, that is established once root exudates have been consumed. These microbial communities then consume rhizodeposits from mucilage and cell senescence as well as exudates from roots growing in proximity. Therefore, slow growing older roots would be maintained in this zone of high microbial activity, and C accumulation in the coarse silt fraction would be higher, especially in N_2-fixing species with populations of bacteria distributed in nodules all along roots. N_2-fixing *Rhizobium* bacteria also increase root elongation (Garrido-Oter et al. 2018), likely inducing a feedback mechanism whereby a stimulated RER results in a higher exudation rate (Garcia et al. 2001), acting as a substrate for newly colonizing *Rhizobium* communities. Although the role of microbial communities is of utmost importance for C input into the soil, differences in the use of C within plants could also explain the lack of a relationship between RER_{NEW}, RLP_{NEW}, SIR and C_{SILT}. In fast-growing, newly initiated roots, we suggest that C in the form of non-structural carbohydrates (NSC, produced during photosynthesis), will be used preferentially for cell production and expansion, as found in a recent seasonal study of root elongation and NSC fluxes (Wang et al. 2018). In older roots with lower RER, less NSC is required for growth, and excess NSC would be freely exuded, reflected in the high SIR that we observed.

Contrary to our hypothesis, RER and RLP did not promote accumulation of C in the $C_{SILT+CLAY}$ pool. Surprisingly, the $C_{SILT+CLAY}$ pool was the only pool where C was actually lost over the 37 weeks, in both bare soil and beneath all plant species, and this mineralisation of C could not be explained by microbial activity or by any root traits. When soil was prepared in our study, its excavation, crushing and sieving would have disrupted soil aggregates (Franzluebbers, 1999). This increase in C mineralisation is higher in clays, as organic matter that was highly protected within the clay fraction will be released during disruption, providing a new pool of C available to microorganisms (Hassink, 1992). The presence of plant roots can also lead to an increased mineralization and loss of preexistent soil C due to an increased microbial activity (positive priming effect; Kuzyakov et al., 2000). In our study, the origin of C was not assessed, so it was not possible to quantify any priming effect. The decrease in C was mainly observed in the silt+clay fraction, challenging the assumption that the C pool associated to this soil fraction has greater C stability (Torn et al., 2009). However, these results are in line with the findings from Keiluweit et al. (2015) who observed a major priming effect in the organomineral associated C after the input of oxalic acid, a common component of root exudates. The soil disturbance, together with the input of highly degradable C, may well increase the release and priming of C in the organomineral associated fraction, thereby decreasing the C content in the silt+clay fraction, but further studies are required to better understand this phenomenon.

2.4.2. Hypothesis 2: more recalcitrant root traits are expected to favour the unprotected coarse POM fraction

Root traits linked to recalcitrance (high cellulose and lignin content and high C:N ratio) did not correlate to C accumulation in the coarse POM fraction (C_{POM}) (Fig. 4), but the PCA showed that this suite of traits was sharing similar coordinates with C_{POM} on axis 1 (Fig. 3). This result is mainly due to the Poaceae species that all have higher C accumulation in the C_{POM} pool, as well as recalcitrant root traits, compared to species from other families. Recalcitrant compounds have all been reported to decrease root decomposition rates (Silver and Miya, 2001; Aulen et al. 2012; Poirier et al. 2018). Lignin-carbohydrate complexes prevent polymer-hydrolyzing enzymes access to substrates, thus reducing the degradability of plant organic matter (Cornu et al. 1994, Malherbe and Cloete, 2002). SIR, as a proxy for microbial activity, was also strongly and negatively correlated to lignin content (Fig. 6f), probably because lignin reduces the accessibility of polysaccharides to microorganisms through the formation of links between lignin and polysaccharides (Bertrand et al. 2006). Products of lignin degradation can also react with ammonia or amino acids to form further recalcitrant complexes that are less available to microorganisms (Nömmik and Vahtras, 1982). The trend observed in the PCA (Fig. 3), that species with recalcitrant tissues were linked to higher C_{POM} accumulation, is in contradiction with the lack of significant correlations between ΔC_{POM} and lignin or C:N ratio. One reason for this lack of correlation might be that the experiment was shorter than the root life span of some or all species, and full accumulation of C in the C_{POM} pool had not yet occurred (Van der Krift et Berendse, 2002). Another reason may be because C_{POM} was derived from the input of fresh C from plants, as well as losses of older C that already existed in soil. While the accumulation of new C in this fraction is influenced by the chemical composition of the root system, the losses of older, pre-existing C are not. A C labeling approach would be helpful to assess the different fluxes of new and old C and to better explain the relationships between root traits and C storage.

Interestingly, C accumulation in the C_{SILT} pool was negatively correlated with recalcitrant root traits (lignin and C:N ratio, Fig. 5e,g), but positively with hemicelluloses content and root diameter (Fig. 5d,f).

Hemicelluloses comprise polysaccharides soluble in alkali and are easily degradable by microorganisms (Dekker, 1985). Hemicelluloses are usually produced to the detriment of lignin and enhance tissue degradability through higher accessibility to amorphous phases in the lignocellulose structure (Malherbe and Cloete, 2002). Microorganisms will use this easily degradable C for growth and respiration and then produce exudates and exopolysaccharides, that are used as a substrate for subsequent microbial communities (Dennis et al. 2010). These exopolysaccharides and low molecular weight compounds are belived to be the main precursors of C in the coarse silt pool (Simpson et al., 2007; Mambelli et al., 2011; Cotrufo et al. 2013, Vidal et al. 2018), probably explaining the high C_{SILT} we found beneath N_2-fixing species (Fig. 2) with high hemicelluloses + water soluble compounds and low lignin contents. Absorptive roots were negatively correlated with C accumulation in the C_{POM} pool (Fig. 4a) and positively correlated with C accumulation in the C_{SILT} pool (Fig. 5d). Absorptive roots generally have a higher turnover rate and undergo rapid transformation through microbial degradation (McCormack et al., 2015), explaining the low accumulation in the C_{POM} pool and the positive correlation with the C_{SILT} pool. However, this relationship may also be an artefact because Poaceae roots are inherently very fine compared to Fabaceae roots (Roumet et al. 2006; 2016), highlighting that the understanding of relationships between C accumulation and morphological traits is challenging because of their inherent nature and plasticity (Poirier et al. 2018).

2.4.3. Hypothesis 3: Fabaceae and Poaceae strongly differ in their influence on accumulation of C into different soil fractions

Contrary to that observed in previous studies (Binkley 2005; Fornara and Tilman, 2008; Plaza-Bonilla et al., 2016; King et al., 2018), we did not find evidence of a greater accumulation of total C (ΔC_{SUM}) in soil beneath N_2-fixing species, because variability was high within Fabaceae. However, we showed that N_2-fixing species and non N_2-fixing species (especially Poaceae and Fabaceae) strongly differed in their effect on the accumulation of C into different soil C pools. Roots of Poaceae, as compared to Fabaceae, had a lower RER and RLP. Poaceae produced thinner roots, rich in lignin and cellulose with a high C:N ratio. These more recalcitrant tissues slow down microbial activity and hence root decomposition rate (Roumet et al. 2016; Freschet et al. 2017). Due to their particular chemical composition, non N_2-fixing species, especially Poaceae species, promote C accumulation in the unprotected C_{POM} pool, and have a lower C accumulation in the more stable C_{SILT} pool (Fig. 4). On the other hand, roots of N_2-fixing Fabaceae grow faster and produce thick roots that are easily degradable, since they are rich in N (low C:N ratio) and in hemicelluloses and water soluble compounds. These traits favour the development of microbial biomass and enhance their activity, as observed from the SIR that was 40% higher beneath

Fabaceae than Poaceae species (see Fig. 7 for a conceptual model describing C accumulation in different pools).

Because of the lack of correlations between root traits and C accumulation in $C_{SILT+CLAY}$ pool, we cannot establish that the $C_{SILT+CLAY}$ pool increases when there is a higher input of labile C from N_2-fixing species. C accumulation is the result of the input of new C and losses of pre-existing C that can be influenced by the input of fresh C from plants (priming effect). The difference in behavior between these two pools could result in poor correlations between root traits and C accumulation in the $C_{SILT+CLAY}$ pool. An isotopic approach differentiating between the changes in new C and old C in different C pools, allowing us to assess the priming effect, would be fundamental to understanding the mechanisms behind soil C storage (Rossi 2019).

2.5. CONCLUSION

Our findings show that specific plant root traits influence the accumulation of C into different pools, largely through the mediation of microbial activity, shaping the C pathway in soil and, finally, its persistence. Our results showed that non N_2-fixing Poaceae species, characterized by high contents of lignin and cellulose and a high C:N, promoted accumulation of C in the unprotected coarse POM fraction, while root traits associated with high labile C input (high hemicelluloses + water soluble compound contents, high RER_{OLD} and RLP_{OLD}) and microbial activity, typical of N_2-fixing Fabaceae species, stimulated C accumulation in the protected coarse silt fraction. Root elongation rate and length production promoted microbial activity in older roots only, potentially suggesting either a spatial influence of root exudate accessibility on microbial communities, or a relationship between non-structural carbohydrate use in roots and available exudates for microbial consumption. The planting of vegetation in bare soil also led to a loss of C in the fine silt+clay fraction, commonly belived to be the most stable. Differentiating the source of C loss (pre-existing C in soil or fresh C from live plants), is a fundamental step to assess the priming effect and understand the mechanisms behind C loss in the finer soil fractions, and could be achieved through an isotope labelling approach. Longer term studies on C dynamics are needed to understand these species and root trait effects over time and the consequent C accumulation in different pools. Moreover, the influence of different soils and associated microbial communities need to be taken into consideration for a broader understanding of C pool dynamics. Our results will not only be useful for identifying plant species capable of enhancing long-term C storage in soil, but will also contribute significantly to the understanding of mechanistic processes within the C cycle.

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REFERENCES

Aulen M, Shipley B, Bradley R (2012) Prediction of in situ root decomposition rates in an interspecific context from chemical and morphological traits. Annals of Botany 109: 287-297

Balesdent J and Balabane M (1996) Major contribution of roots to soil carbon storage inferred from maize cultivated soils. Soil Biol Biochem 9: 1261–1263.

Bardgett RD, Mommer L, Vries FT De (2014) Going underground : root traits as drivers of ecosystem processes. Tends Ecol Evol 29: 692–699.

Beare MH, Neely CL, Coleman DC, Hargrove WL (1990) A substrate-induced respiration (SIR) method for measurement of fungal and bacterial biomass on plant residues. Soil Biol Biochem 22(5): 585-594

Berntson GM (1997) Topological scaling and plant root system architecture: developmental and functional hierarchies. New Phytol 135: 621–634

Bertrand I, Chabbert B, Kurek B, Recous S (2006) Can the biochemical features and histology ofwheat residues explain their decomposition in soil? Plant Soil 281:291–307

Besnard E, Chenu C, Balesdent J, Puget P, Arrouays D (1996) Fate of particulate organic matter in soil aggregates during cultivation. Eur J Soil Sci 47: 495–503.

Binkley D (2005) How Nitrogen-Fixing Trees Change Soil Carbon. In: Binkley D., Menyailo O. (eds) Tree Species Effects on Soils: Implications for Global Change. NATO Science Series IV: Earth Env Sci, vol 55. Springer, Dordrecht

Bird JA, Kleber M, Torn MS (2008) C-13 and N-15 stabilization dynamics in soil organic matter fractions during needle and fine root decomposition. Organic Geo- chemistry, 39, 465–477

Canarini A, Kaiser C, Merchant A, Richter A, Wanek W (2019) Root exudation of primary metabolites: mechanisms and their roles in plant responses to environmental stimuli. Front Plant Sci 10: 157

Chapin FS III (2003) Effects of plant traits on ecosystem and regional processes: a conceptual framework for predicting the consequences of global change. Ann Bot 91: 455–463.

Cornu A, Besle JM, Mosoni P, Grent E (1994) Lignin- carbohydrate complexes in forages: Structure and consequences in the ruminal degradation of cell-wall carbohydrates. Reprod Nutr Dev 24: 385–398

Cotrufo MF, Wallenstein MD, Boot CM, Denef K, Paul E (2013) The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? Global Change Biol 19: 988–995.

De Deyn GB, Cornelissen JHC, Bardgett RD (2008) Plant functional traits and soil carbon sequestration in contrasting biomes. Ecol Lett 11: 516–531.

Dekker M (1985) Biosynthesis and Biodegradation of Wood Components: Biodegradation of the Hemicelluloses. Ed. T. Higuchi pp. 505 – 533: Academic Press

Dennis PG, Miller AJ, Hirsch PR (2010) Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? FEMS Microbiol Ecol 72: 313–327.

Derrien D, Barot S, Chenu C, Chevallier T, Freschet GT, Garnier P, Guenet B, Hedde M, Klumpp K, Lashermes G, et al. (2016) Stocker du C dans les sols : Quels mécanismes, quelles pratiques agricoles, quels indicateurs ? Étude et Gestion des Sols 23: 193–224.

Downie JA (2010) The roles of extracellular proteins, polysaccharides and signals in the interactions of rhizobia with legume roots. Fed of Eur Microbiol Soc 34: 150–170.

Dungait JA, Hopkins DW, Gregory AS, Whitmore AP (2012) Soil organic matter turnover is governed by accessibility not recalcitrance. Global Change Biol 18: 1781–1796

Fehrmann RC and Weaver RW (1978) Scanning electron microscopy of Rhizobium sp. adhering to fine silt particles. Soil Sci Soc Am J 42: 279-281

Fornara D, Tilman D (2008) Plant functional composition influences rates of soil carbon and nitrogen accumulation. J Ecol 96: 314–322.

Franzluebbers AJ (1999) Potential C and N mineralization and microbial biomass from intact and increasingly disturbed soils of varying texture. Soil Biol Biochem 31: 1083-1090,

Fujisaki K, Chapuis-Lardy L, Albrecht A, Razafimbelo T, Chotte JL, Chevallier T (2018) Data synthesis of carbon distribution in particle size fractions of tropical soils: implications for soil carbon storage potential in croplands. Geoderma 313, 41-51.

Garcia JAL, Barbas C, Probanza A, Barrientos ML, Manero FJG (2001) Low molecular weight organic acids and fatty acids in root exudates of two Lupinus cultivars at flowering and fruiting stages. Phytochem Analysis 12: 305–311.

Garrido-Oter R, Nakano RT, Dombrowski N, Ma KW, AgBiome Team, McHardy AC, Schulze-Lefert P (2018) Modular traits of the Rhizobiales root microbiota and their evolutionary relationship with symbiotic rhizobia. Cell Host Microbe 24(1): 155-167.

Gavinelli E, Feller C, Larré-Larrouy MC, Bacye B, Djegui Z,Nzila JD (1995) Routine method to study soil organic matter by particle-size fractionation: examples for tropical soils. Comm Soil Sci Plan26(11&12): 1749-1760

Gleixner G, Poirier N, Bol R, Balesdent J (2002) Molecular dynamics of organic matter in a cultivated soil. Org Geochem 33: 357-366.

Griscom BW, Adams, J, Ellis PW, Houghton RA, Lomax G, Miteva DA, Schlesinger WH, Shoch D, Siikamäki J V, Smith P, Woodbury P, Zganjar C, Blackman A, Campari J, Conant RT, Delgado C, Elias P, Gopalakrishna T, Hamsik MR, Herrero M, Kiesecker J, Landis E, Laestadius L, Leavitt SM, Minnemeyer S, Polasky S, Potapov P, Putz FE, Sanderman J, Silvius M, Wollenberg E, Fargione J (2017) Natural climate solutions. Proceedings of the National Academy of Science of the United States of America. 114, 11645–11650

Hamer U, Marschner B, (2005) Priming effects in soils after combined and repeated substrate additions. Geoderma 128(1):38-51

Harrell FE (2007) Package 'Hmisc'. Harrell Miscellaneous

Hassink J (1992) Effects of soil texture and structure on carbon and nitrogen mineralization in grassland soils. Biol Fertil Soils 14 : 126–134.

Heinze , Gensch S, Weber E, Joshi J (2017) Soil temperature modifies effects of soil biota on plant growth. J Plant Ecol 10 (5): 808 – 821

Hernández MA, Romero J, Jaime C, León-pulido J (2017) Lignocellulosic biomass from fast-growing species in Colombia and their use as bioresources for biofuel production. Chem Eng Trans 58: 541–546.

Holz M, Zarebanadkouki M, Kaestner A, Kuzyakov Y, Carminati A (2018) Rhizodeposition under drought is controlled by root growth rate and rhizosphere water content. Plant Soil 423: 429–442.

Huck MG, Taylor HM (1982) The Rhizotron as a Tool for Root Research In Advances in Agronomy, ed. NC Brady, pp. 1-35: Academic Press

Huo C, Luo Y, Cheng W, (2017) Rhizosphere priming effect: A meta-analysis. Soil Biology and Biochemistry 111: 78–84

Jones DL, Nguyen C, Finlay RD (2009) Carbon flow in the rhizosphere: carbon trading at the soil-root interface. Plant Soil 321: 5–33.

Kätterer T, Bolinder MA, Andrén O, Kirchmann H, Menichetti L (2011) Roots contribute more to refractory soil organic matter than above-ground crop residues, as revealed by a long-term field experiment. Agric. Ecosyst. Environ. 141, 184–192

Keiluweit M, Bougoure JJ, Nico PS, Pett-Ridge J, Weber PK, Kleber M (2015) Mineral protection of soil carbon counteracted by root exudates. Nature Climate Change 5: 588-595

King AE, Blesh J, (2018) Crop rotations for increased soil carbon: Perenniality as a guiding principle. Ecol. Appl. 28, 249–261

King AE, Congreves KA, Deen B, Dun KE, Voroney RP, Wagner-riddle C (2019) Quantifying the relationships between soil fraction mass , fraction carbon, and total soil carbon to assess mechanisms of physical protection. Soil Biol Biochem 135: 95–107.

Kleber M, Nico PS, Plante A, Filley T, Kramer M, Swanston C, Sollins P (2011) Old and stable soil organic matter is not necessarily chemically recalcitrant: Implications for modeling concepts and temperature sensitivity. Glob. Chang. Biol. 17, 1097–1107

Kleber M, Eusterhues K, Keiluweit M, Mikutta C, Mikutta R, Nico PS (2015) Mineral-Organic Associations: Formation, Properties, and Relevance in Soil Environments, Advances in Agronomy. Elsevier Ltd.

Kogel-Knabner I (2002) The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biol Biochem 34: 139-162

Kuzyakov Y, Friedel JK, Stahr K, (2000) Review of mechanisms and quantifcation of priming effects. Soil

Biology and Biochemistry 32: 1485-1498

Kuzyakov Y, Larinova AA (2005) Root and rhizomicrobial respiration: a review of approaches to estimate respiration by autotrophic and heterotrophic organisms in soil. J Plant Nutr Soil Sci 168: 503 - 520

Lal R (2004) Soil carbon sequestration impacts on global change and food security. Science 304: 1623-1627.

Larson JE, Funk JL (2016) Seedling root responses to soil moisture and the identification of a belowground trait spectrum across three growth forms. New Phytol 210: 827–838.

Lavorel S, Dıaz S, Cornelissen JHC, Garnier E, Harrison SP, McIntyre S, Juli G, Pérez-Harguinde PS, Roumet C, Urcelay C (2007) Plant functional types: are we getting any closer to the Holy Grail? In: Terrestrial Ecosystems in a Changing World, eds Canadell, J., Pitelka, L.F. & Pataki, D. Springer, Berlin, pp. 171–186.

Lobet G, Pagès L, Draye X (2011) A novel image-analysis toolbox enabling quantitative analysis of root system architecture. Plant Physiol 157: 29-39

von Lutzow M, Kogel-Knabner I, Ekschmitt K, Matzner E, Guggenberger G, Marschner B, Flessa H (2006) Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions – a review. Eur J Soil Sci 57: 426–445.

Malherbe S, Cloete TE (2002) Lignocellulose biodegradation: fundamentals and applications. Rev Environ Sci Bio 1: 105–114,: 105–114.

Mambelli S, Bird JA, Gleixner G, Dawson TE, Torn MS (2011) Relative contribution of foliar and fine root pine litter to the molecular composition of soil organic matter after in situ degradation. Organic Geochemistry, 42: 1099–1108

McCormack LM, Dickie IA, Eissenstat DM, Fahey TJ, Fernandez CW, Guo D, Erik A, Iversen CM, Jackson RB.(2015) Redefining fine roots improves understanding ofbelow-ground contributions to terrestrial biosphere processes. New Phytol 207: 505–518.

Mikutta R, Kleber M, Torn MS, Jahn R (2006) Stabilization of soil organic matter: association with minerals or chemical recalcitrance?. Biogeochem 77: 25-56

Minasny B, Malone BP, McBratney AB, Angers DA, Arrouays D, Chambers A, Chaplot V, Chen ZS, Cheng K, Das BS, Field DJ, Gimona A, Hedley CB, Hong SY, Mandal B, Marchant BP, Martin M, McConkey BG, Mulder VL, O'Rourke S, Richer-de-Forges AC, Odeh I, Padarian J, Paustian K, Pan G, Poggio L, Savin I (2017) Soil carbon 4 per mille. Geoderma 292, 59–86.

Mohamed A, Monnier Y, Mao Z, Lobet G, Maeght JL, Ramel M, Stokes A (2017) An evaluation of inexpensive methods for root image acquisition when using rhizotrons. Plant Methods 13: 1–13.

Mohamed A, Stokes A, Mao Z, Jourdan C, Sabatier S, Pailler F, Fourtier S, Dufour L, Monnier Y (2018) Linking above- and belowground phenology of hybrid walnut growing along a climatic gradient in temperate agroforestry systems. Plant and Soil 424: 103

Mommer L, Padilla FM, van Ruijven J, de Caluwe H, Smit-Tiekstra A, Berendse F, de Kroon H (2015)

Diversity effects on root length production and loss in an experimental grassland community. Funct Ecol 29: 1560–1568.

Nguyen C (2003) Rhizodeposition of organic C by plants: mechanisms and controls. Agronomie 23, 375–396.

Nömmik H, Vahtras Z (1982) Retention and fixation of ammonium and ammonia in soils. In: Stevenson, F.J. (Ed.),. Nitrogen in agricultural soils. Agronomy monographs, No. 22. Agronomy Society of America, Madison, WI, USA.

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Henry M, Stevens H, Szoecs E, Wagner H (2019) Package 'vegan'. Community Ecology Package, R package version 1.17– 2. http://CRAN.R-project.org/package=vegan

Paustian K, Lehmann J, Ogle S, Reay D, Robertso GP, Smith P (2016) Climate-smart soils. Nature 532: 49–57.

Plaza-Bonilla D, Nolot JM, Passot S, Raffaillac D, Justes E, (2016) Grain legume-based rotations managed under conventional tillage need cover crops to mitigate soil organic matter losses. Soil Tillage Res. 156, 33–43

Poirier V, Roumet C, Munson AD (2018) The root of the matter: linking root traits and soil organic matter stabilization processes. Soil Biol Biochem 120: 246–259.

R Development Core Team (2013) R: A Language and Environment for Statistical Computing. Vienna, Austria

Rasse DP, Rumpel C, Dignac M (2005) Is soil carbon mostly root carbon ? Mechanisms for specific stabilization. Plant Soil 269: 341–356

Robertson AD, Paustian K, Ogle S, Wallenstein MD, Lugato E , Cotrufo FM (2019) Unifying soil organic matter formation and persistence frameworks: the MEMS model. Biogeosci 16: 1225-1248

Rossi LMW (2019) Embankment as a carbon sink: a study on carbon sequestration pathways and mechanisms in topsoil and exposed subsoil. Ph. D. Thesis, University of Montpellier, France

Roumet C, Urcelay C, Díaz S, Roumet C (2006) Suites of root traits differ between annual and perennial species growing in the field. New Phytol 170 : 357–368

Roumet C, Birouste M, Picon-Cochard C, Ghestem M, Osman N, Vrignon-Brenas S, Cao K-F, Stokes A (2016) Root structure-function relationships in 74 species: Evidence of a root economics spectrum related to carbon economy. New Phytol 210: 815–826.

Rubino M, Dungait JAJ, Evershed RP et al. (2010) Carbon input belowground is the major C flux contributing to leaf litter mass loss: evidences from a (13)C labelled- leaf litter experiment. Soil Biology & Biochemistry, 42: 1009–1016

Sanderman J, Maddern T, Baldock J, (2014) Similar composition but differential stability of mineral retained organic matter across four classes of clay minerals. Biogeochemistry 121: 409-424

Sasse J, Martinoia E, Northen T (2018) Feed Your Friends: Do Plant Exudates Shape the Root

Microbiome? Trends in Plants Science 23(1): 25-41

Schmidt MWI, Torn MS, Abiven S, Dittmar T, Guggenberger G, Janssens IA, Kleber M, Kögel-Knabner I, Lehmann J, Manning DAC, Nannipieri P, Rasse DP, Weiner S, Trumbore SE (2011) Persistence of soil organic matter as an ecosystem property. Nature 478, 49–56.

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9: 671–675

Shahzad T, Chenu C, Genet P, Barot S, Perveen N, Mougin C, and Fontaine S (2015) Contribution of exudates, arbuscular mycorrhizal fungi and litter depositions to the rhizosphere priming effect induced by grassland species. Soil Biology and Biochemistry, 80: 146–155

Silver WL, Miya RK (2001) Global patterns in root decomposition: comparisons of climate and litter quality effects. Oecologia 129:407-419

Simpson AJ, Simpson MJ, Smith E, Kelleher BP (2007) Microbially derived inputs to soil organic matter: are current estimates too low? Environmental Science & Technology, 41: 8070–8076

Six J, Bossuyt H, Degryze S, Denef K (2002) A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Soil Till Res 79: 7-31

Sokol NW, Kuebbing SE, Karlsen-ayala E, Bradford MA (2019) Evidence for the primacy of living root inputs, not root or shoot litter, in forming soil organic carbon. New Phytol 221: 233–246.

Sollins P, Hofmann P and Caldwell BA (1996) Stabilization and destabilization of soil organic matter: mechanisms and controls. Geoderma 74: 65–105.

Steinaker DF, Wilson SD, Peltzer DA (2010) Asynchronicity in root and shoot phenology in grasses and woody plants. Global Change Biol 16: 2241–2251

Torn MS, Swanston CW, Castanha C, Trumbore SE (2009) Storage and Turnover of Organic Matter in Soil. In: Biophysico-Chemical Processes Involving Natural Non-living Organic Matter in Environmental Systems, Publisher: John Wiley & Sons

Van Der Krift TAJ, Berends F (2002) Root life spans of four grass species from habitats differing in nutrient availability. Functional Ecology 16(2): 198 - 203

Van Soest PJ (1963) Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. J AOAC Int 46: 829–835.

Vidal A, Hirte J, Bender SF, Mayer J, Gattinger A, Höschen C, Schädler S, Iqbal TM, Mueller CW (2018) Linking 3D Soil Structure and Plant-Microbe-Soil Carbon Transfer in the Rhizosphere. Front Env Sci 6: 1–14.

Wang Y, Mao Z, Bakker MR, Kim JH, Brancheriau L, Buatois B, Leclerc R, Selli L, Rey H, Jourdan C, Stokes A (2018) Linking conifer root growth and production to soil temperature and carbon supply in temperate forests. Plant Soil 426: 33–50

Warembourg FR, Roumet C, Lafont F (2003) Differences in rhizosphere carbon-partitioning among plant species of different families. Plant Soil 256: 347–357.

Wiesmeier M, Urbanski L, Hobley E, Lang B, von Lützow A , Marin-Spiotta E, van Wesemael B, Rabot E, Ließ M, Garcia-Franco N, Wollschläger U, Vogel HJ, Kögel-Knabner I (2019) Soil organic carbon storage as a key function of soils - A review of drivers and indicators at various scales. Geoderma 333: 149-162,

Žifcˇáková L, Veˇtrovský T, Howe A, Baldrian P (2016) Microbial activity in forest soil reflects the changes in ecosystem properties between summer and winter. Env Microbiol 18: 288-301

FIGURES AND TABLES

Table 1: Plant root traits and microbial activity for the 12 herbaceous species. Mean data are also given for species from Fabaceae and Poaceae families.

Family	**Fabaceae**					**Poacea**					**Rosaceae**	**Plantaginaceae**	**Effect of species (ANOVA)**			**Fabaceae**	**Poaceae**	**Effect of families (ANOVA)**		
Species	*Lotus corniculatus*	*Trifolium repens*	*Trifolium pratense*	*Onobrychis viciifolia*	*Medicago sativa*	*Bromus erectus*	*Festuca rubra*	*Dactylis glomerata*	*Poa pratense*	*Lolium perenne*	*Sanguisorba minor*	*Plantago lanceolata*								
Acronym code	Lc	Tr	Tp	Ov	Ms	Be	Fr	Dg	Pp	Lp	Sm	Pl	Df	F	*p*			Df	F	*p*
RER$_{TOT}$ (mm d^{-1})	0.55±1.1 (a)	0.75±1.5 (a)	0.59±1 (a)	0.53±10 (a)	0.57±0.9 (a)	0.42±0.6 (a)	0.23±0.50 (a)	0.58±0.9 (a)	0.66±2.90 (a)	0.42±0.8 (a)	0.39±0.5 (a)	0.5±0.7 (a)	11,175	18.9	0.06[1]	0.57±0.08	0.42±0.13	2,9	1.8	0.22
RER$_{OLD}$ (mm d^{-1})	0.17±0.07 (a)	0.2±0.08 (a)	0.4±0.11 (a)	0.21±0.05 (a)	0.26±0.08 (a)	0.12±0.03 (a)	0.09±0.04 (a)	0.14±0.06 (a)	0.05±0.02 (a)	0.11±0.04 (a)	0.13±0.04 (a)	0.16±0.04 (a)	11,178	17.9	0.08[1]	0.25±0.09	0.13±0.03	2,9	6.3	**0.02**
RER$_{NEW}$ (mm d^{-1})	0.99±0.27 (a)	0.91±0.21 (a)	0.73±0.15 (a)	0.81±0.16 (a)	0.97±0.12 (a)	1.06±0.29 (a)	0.32±0.08 (a)	1.13±0.25 (a)	0.96±0.31 (a)	0.67±0.11 (a)	0.66±0.08	0.76±0.11	11,178	17.4	0.1[1]	0.88±0.11	0.79±0.33	2,9	0.37	0.7
RLP$_{TOT}$ (m)	3.03	3.26	3.36	3.62	3.61	2.55	1.19	2.76	2.16	2.95	2.31	3.04	-	-	-	3.37±2.32	2.32±0.7	2,9	5.17	**0.03**
RLP$_{OLD}$ (m)	4.51	4.91	4.26	4.96	5.23	3.47	1.05	3.30	2.76	4.29	3.32	4.50	-	-	-	1.33±0.61	0.61±0.2	2,9	7.5	**0.01**
RLP$_{NEW}$ (m)	0.89	1.05	1.78	1.35	1.60	0.78	0.53	0.81	0.32	0.62	0.47	0.99	-	-	-	4.78±2.97	2.97±1.21	2,9	5.12	**0.03**
Root biomass (g)	1.53±2.6 (bc)	0.55±1.13 (c)	2.01±0.62 (b)	2.06±1.1 (b)	4.23±0.42 (a)	0.65±0.08 (c)	0.70±1.6 (c)	0.58±0.51 (c)	0.60±0.83 (c)	0.57±0.57 (c)	0.91±2.13 (c)	0.49±0.60 (c)	11,24	27.3	**<0.001**	2.08±1.33	0.62±0.11	1,28	10.9	**<0.001[1]**
Diametre absorptive roots (mm)	0.47±0.05 (b)	0.28±0.05 (de)	0.32±0.04 (cd)	0.55±0.18 (a)	0.35±0.18 (c)	0.27±0.02 (def)	0.22±0.05 (fg)	0.21±0.14 (g)	0.22±0.19 (g)	0.22±0.05 (fg)	0.26±0.09 (efg)	0.28±0.14 (de)	11,23	98.7	**<0.001**	0.39±0.11	0.23±0.03	1,27	31.7	**<0.001**
Hemicell. +H_2O soluble compounds (mg g^{-1})	779.5±58.1 (a)	612±8.3 (bcde)	674.5±44.6 (abc)	704.1±78.8 (ab)	755.1±31 (a)	533.9±12.1 (de)	572.3±27.3 (cde)	530.4±57.6 (de)	562.4±31.2 (cde)	520.5±31.7 (e)	703.2±NA (ab)	639.4±12.7 (bcd)	11,21	17.4	**<0.001**	705.11±74.39	543.51±33.56	1,25	51.5	**<0.001**
Cellulose (mg g-1)	101.6±6.3 (a)	163.2±8.7 (a)	102.5±17.7 (a)	120±69.3 (a)	123.9±27.7 (a)	177.9±76.5 (a)	160.7±44.8 (a)	137.5±7.7 (a)	89.1±6 (a)	181.6±32.3 (a)	140±NA (a)	151.2±54 (a)	11,21	1.7	0.13	122.39±33.61	154.89±50.8	1,25	3.9	0.06
Lignine (mg g-1)	118.9±57.8 (c)	224.8±0.4 (abc)	223±33.7 (abc)	175.9±9.4 (abc)	120.9±3.5 (c)	288.3±88.5 (ab)	267±39.5 (ab)	332.1±65.3 (a)	348.4±25.1 (a)	297.9±62.1 (ab)	156.8±NA (bc)	209.3±66.7 (abc)	11,21	6.7	**<0.001**	172.5±56.53	301.6±59.02	1,25	33.7	**<0.001**
C:N ratio	15.4±0.8 (c)	21.2±1.1 (c)	17.6±3.20 (c)	20.5±3.2 (c)	21.4±2 (c)	50.8±7.6 (b)	61.3±2.6 (ab)	62.5±6.4 (ab)	59.1±4.1 (ab)	61±4.4 (ab)	69.8±3.2 (a)	68.9±3.1 (a)	11,21	96.4	**<0.001**	19.15±3.07	58.67±6.34	1,25	436	**<0.001**
SIR (µg C-CO_2 g^{-1} soil h^{-1})	5.37±0.46 (ab)	4.43±0.48 (bc)	6.11±0.35 (a)	4.07±0.18 (bcd)	6.41±0.56 (a)	2.47±0.34 (d)	3.22±0.11 (cd)	3.58±0.28 (cd)	3.15±0.15 (cd)	3.17±0.1 (cd)	3.16±0.23 (cd)	2.99±0.11 (cd)	9,20	16	**<0.001**	5.28±1	3.12±0.4	1,28	45.9	**<0.001**

[1] Distribution not normal, Kruskal-Walls test instead of ANOVA

Mean data ± standard deviation are given for species from N$_2$-fixing Fabaceae , N$_2$-fixing Poaceae families, and N$_2$-fixing species aggregated (Poaceae species, *P. lanceolate* and *S. minor*). For each species and for N$_2$-fixing Fabaceae , N$_2$-fixing Poaceae families, and N$_2$-fixing species aggregated, mean and standard deviation are given. Abbreviations: RER$_{TOT}$ – root elongation rate of the entire root system; RER$_{OLD}$ – of old roots older than 2 weeks; RER$_{NEW}$ – of new roots younger than 2 weeks; RLP_{TOT} root length production of the entire root system; RLP_{OLD} – of old roots; RLP_{NEW} – of new roots; Root biomass – total root biomass of a core sampled after 37 weeks; Diameter absorptive – mean diameter of absorptive roots after 37 weeks; Hemicell. + H_20 – concentration of hemicelluloses and water soluble compounds in absorptive roots; Cellulose, Lignin – concentrations of cellulose and lignin in absorptive roots; C:N – ratio of carbon and nitrogen in absorptive roots; SIR – microbial subsrate induced respiration. Different letters next to the average value indicate statistically significant differences ($p < 0.05$) between species or families according to Tukey HSD tests. DF – degree of freedom (number of species - 1, number of observations). Statistically significant values ($p < 0.05$) are indicated in bold text.

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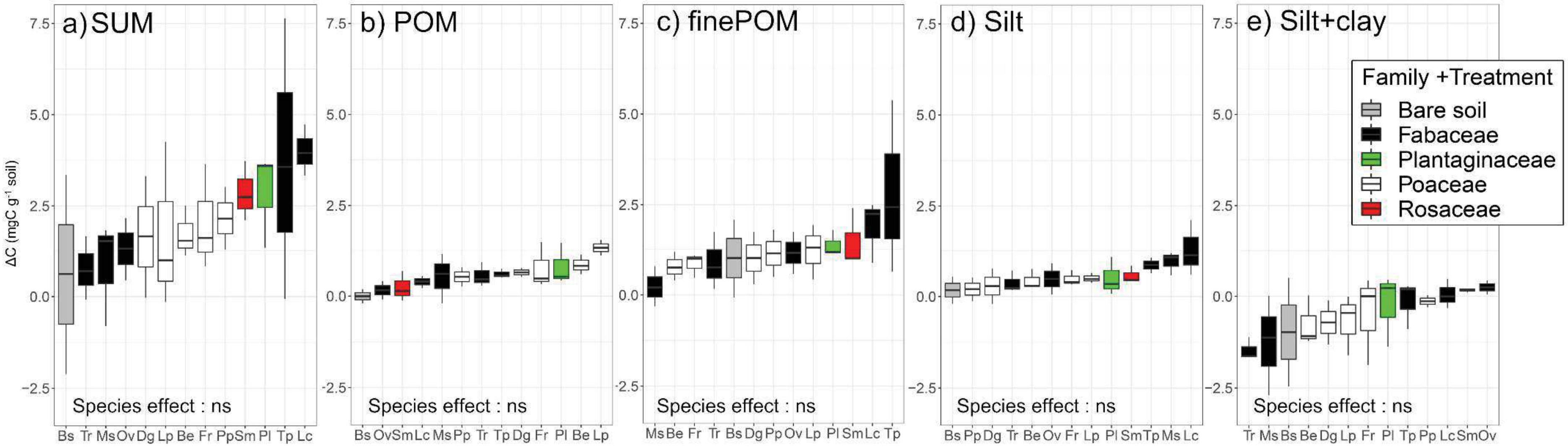

Fig.1: Comparison of the difference ($\Delta C = C_{t37} - C_{t0}$) in carbon (C) after 37 weeks between different soil fractions for each species. a) total C (ΔC_{SUM}), b) C in the coarse POM fraction (ΔC_{POM}), c) C in the fine POM fraction ($\Delta C_{finePOM}$), d) C in the coarse silt fraction (ΔC_{SILT}) and e) C in the fine silt+clay fraction ($\Delta C_{SILT+CLAY}$). In each boxplot, the lower edge of the box corresponds to the 25th percentile data point, while the top edge of the box corresponds to the 75th percentile data point. The line within the box represents the median.

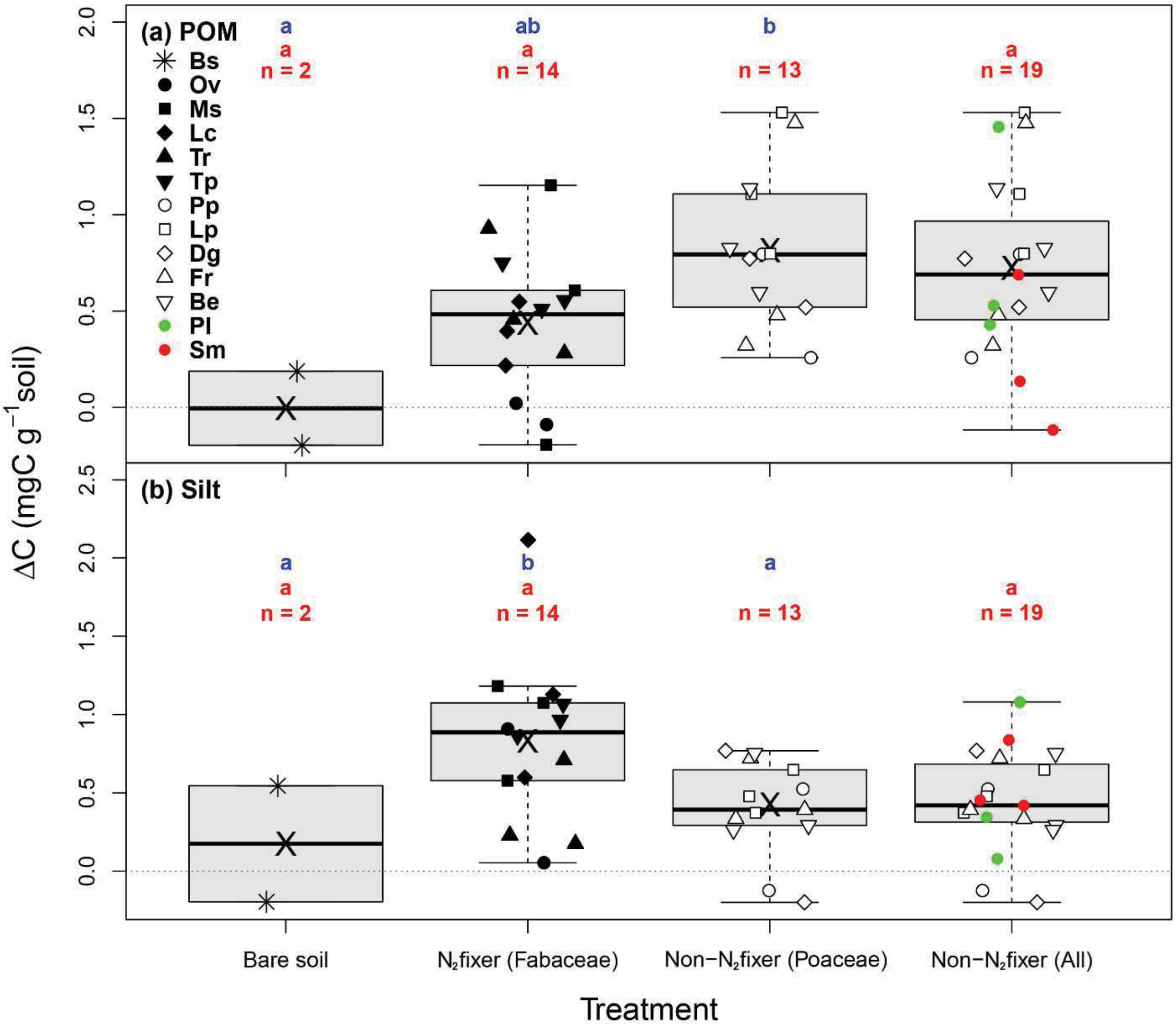

Fig.2: Comparison of the difference ($\Delta C = C_{t37} - C_{t0}$) in carbon (C) after 37 weeks among N_2-fixing Fabace, non N_2-fixing Poaceae species only and non N_2-fixing species aggregated (Poaceae, P. lanceolate, S. Minor), and control.a) C in the coarse POM fraction (ΔC_{POM}) and b) C in the silt fraction (ΔC_{silt}). No significant differences were found in total C (ΔC_{SUM}), C in the fine POM fraction ($\Delta C_{finePOM}$), or in C in the silt+clay fraction ($\Delta C_{silt+clay}$). In each boxplot, the lower edge of the box corresponds to the 25th percentile data point, while the top edge of the box corresponds to the 75th percentile data point. The line within the box represents the median and black dots indicate outliers. Different letters above the boxplots indicate statistically significant differences ($p < 0.05$) among families and control according to a Tukey HSD test. The blue leter refer to the Tuckey HSD test perfomed between control, N_2-fixing Fabaceae species and N_2-fixing Poaceae species only, while the red letters refer to the Tuckey HSD test performed between control, N_2-fixing Fabaceae species and N_2-fixing species aggregated together.Note the graduation differences in *y*-axis between a) and b).

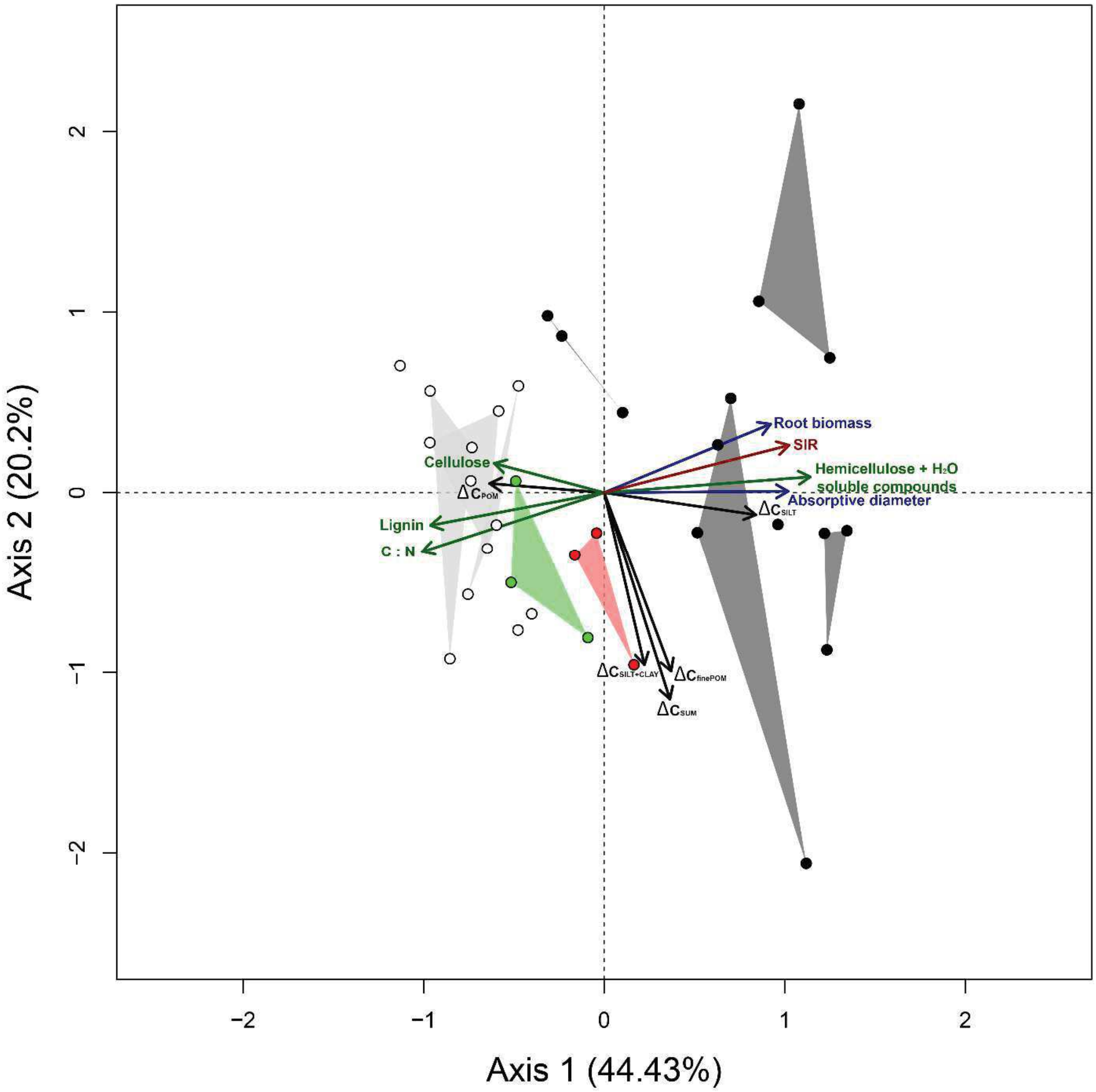

Fig.3: Principal Component Analysis of six soil variables (five carbon pool changes and SIR) and six root variables measured on 12 species. Black dots are Fabaceae, white dots are Poaceae, red dots are *Sanguisorba minor*, and green dots are *Plantago lanceolata*. The Hull polygons unify the different replicates for the same species. Abbreviations: SIR – microbial subsrate induced respiration; Hemicelluloses + $H_2$0 – concentration of hemicelluloses and water soluble compounds in absorptive roots, cellulose, lignin – concentrations of cellulose and lignin in absorptive roots; C:N – ratio of carbon and nitrogen in absorptive roots; ΔC_{POM} – difference ($\Delta C = C_{t37} - C_{t0}$) in carbon (C) after 37 weeks for the coarse POM C pool; $\Delta C_{finePOM}$ – for the fine POM C pool; ΔC_{SILT} – for the silt C pool; $\Delta C_{SILT+CLAY}$ – for the silt + clay f C pool; ΔC_{SUM} – sum of different fractions, ΔC as the total change in C concentration in soil.

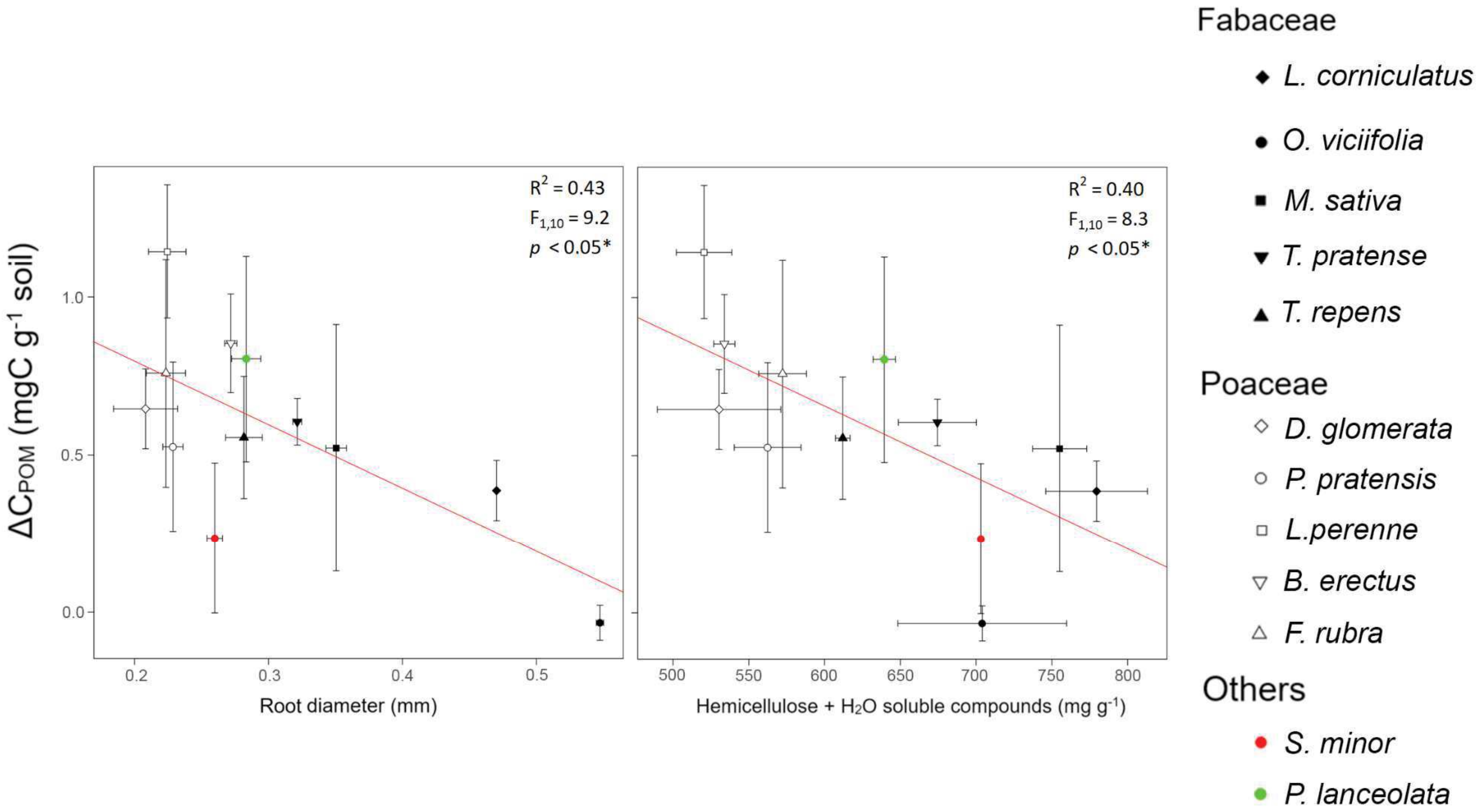

Fig. 4: Linear regression at species level (n=12) between ΔC_{POM} as the difference ($\Delta C = C_{t37} - C_{t0}$) in carbon (C) after 37 weeks for the coarse POM C pool and a) diameter of absorptive roots and b) hemicelluloses + water soluble compounds. The black symbols are the N_2-fixing Fabaceae species, the white symbols the non N_2-fixing Poaceae species, the red dots are *S. minor* and the green dots are *P. lanceolata*. The red line is the linear model function of the variables and R^2, F and *p* of the linear model are shown.

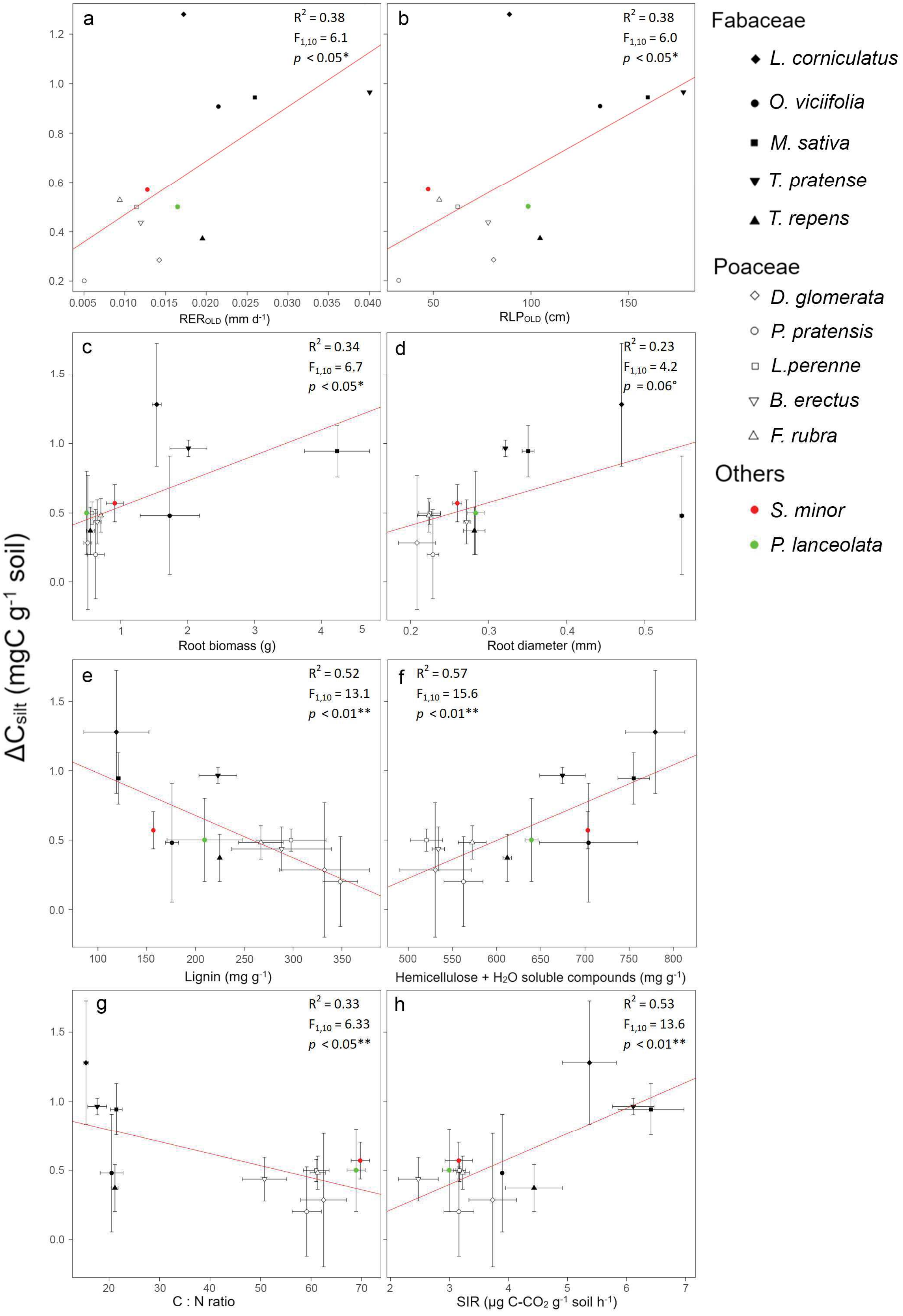
a
R² = 0.38
F1,10 = 6.1
p < 0.05*
1.2
1.0
0.8
0.6
0.4
0.2
0.005 0.010 0.015 0.020 0.025 0.030 0.035 0.040
RER_OLD (mm d-1)
b
R² = 0.38
F1,10 = 6.0
p < 0.05*
50 100 150
RLP_OLD (cm)
c
R² = 0.34
F1,10 = 6.7
p < 0.05*
1.5
1.0
0.5
0.0
1 2 3 4 5
Root biomass (g)
d
R² = 0.23
F1,10 = 4.2
p = 0.06°
0.2 0.3 0.4 0.5
Root diameter (mm)
e
R² = 0.52
F1,10 = 13.1
p < 0.01**
100 150 200 250 300 350
Lignin (mg g-1)
f
R² = 0.57
F1,10 = 15.6
p < 0.01**
500 550 600 650 700 750 800
Hemicellulose + H2O soluble compounds (mg g-1)
g
R² = 0.33
F1,10 = 6.33
p < 0.05**
20 30 40 50 60 70
C : N ratio
h
R² = 0.53
F1,10 = 13.6
p < 0.01**
2 3 4 5 6 7
SIR (µg C-CO2 g-1 soil h-1)
ΔCsilt (mgC g-1 soil)
Fabaceae
◆ L. corniculatus
● O. viciifolia
■ M. sativa
▼ T. pratense
▲ T. repens
Poaceae
◇ D. glomerata
○ P. pratensis
□ L.perenne
▽ B. erectus
△ F. rubra
Others
● S. minor
● P. lanceolata

Fig. 5: Linear regression at species level (n=12) between ΔC_{silt} as difference ($\Delta C = C_{t37} - C_{t0}$) in carbon (C) after 37 weeks for the coarse silt C pool and a) root elongation rate of old roots (RER_{OLD}), b) root length production of old roots (RLP_{OLD}), c) root biomass, d) diameter of absorptive roots, e) lignin content, f) hemicelluloses + water soluble compounds, g) C:N ratio and h) substrate induced respiration rate (SIR). The black symbols are the N_2-fixing Fabaceae species, the white symbols the non N_2-fixing Poaceae species, the red dots are *S. minor* and the green dots are *P. lanceolata*. The red line is the linear model function of the variables and R^2, F and *p* of the linear model are shown.

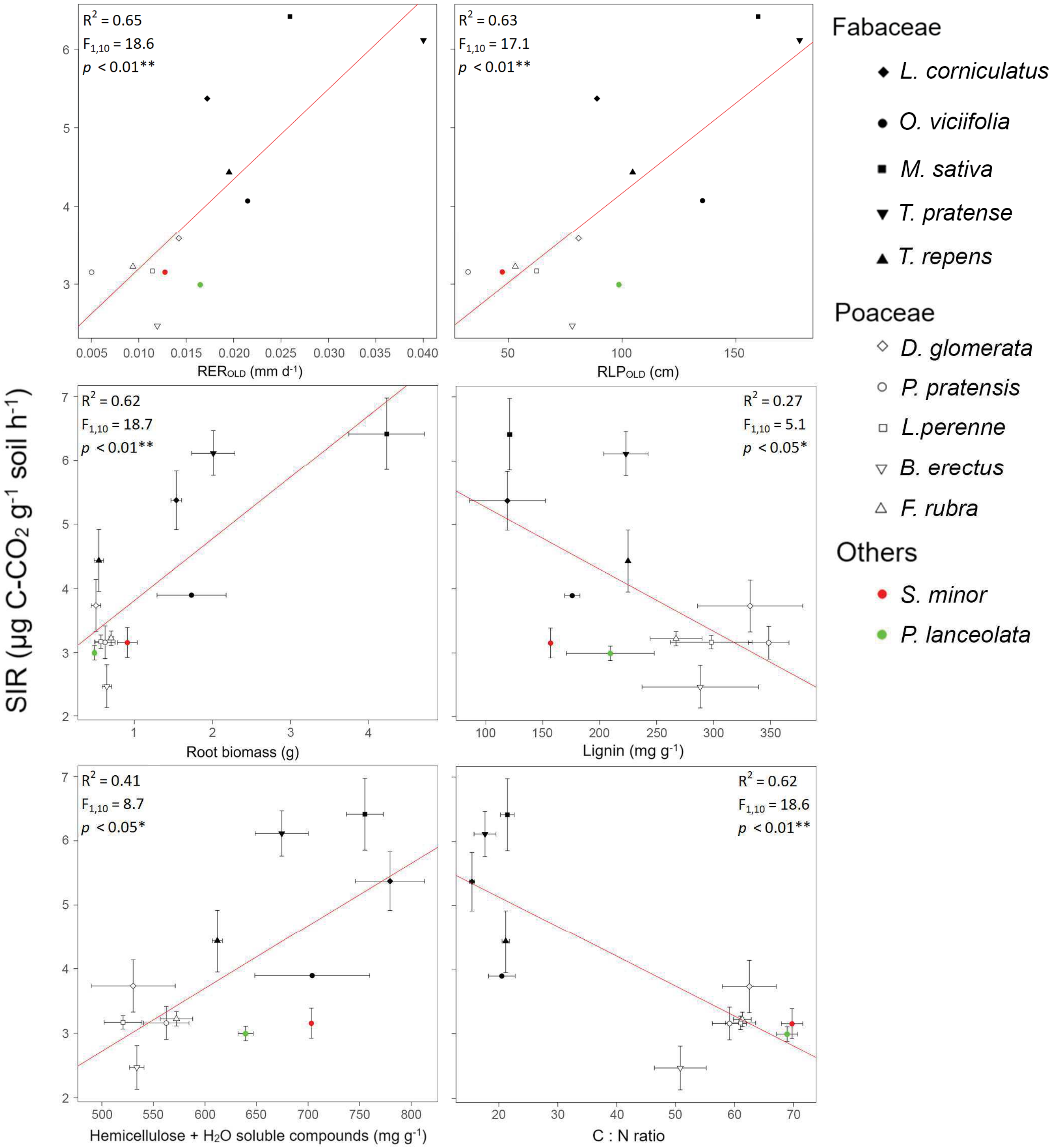

Fig. 6: Linear regression at species level (n=12) between substrate induced respiration rate (SIR) and a) root elongation rate of old roots (RER_{OLD}), b) root length production of old roots (RLP_{OLD}), c) root biomass, d) lignin content, e) hemicelluloses + water soluble compounds, f) C:N ratio. The black symbols are the N_2-fixing Fabaceae species, the white symbols the non N_2-fixing Poaceae species, the red dots *S. minor* and the green dots *P. lanceolata*. The red line is the linear model function of the variables and R^2, F and *p* of the linear model are shown.

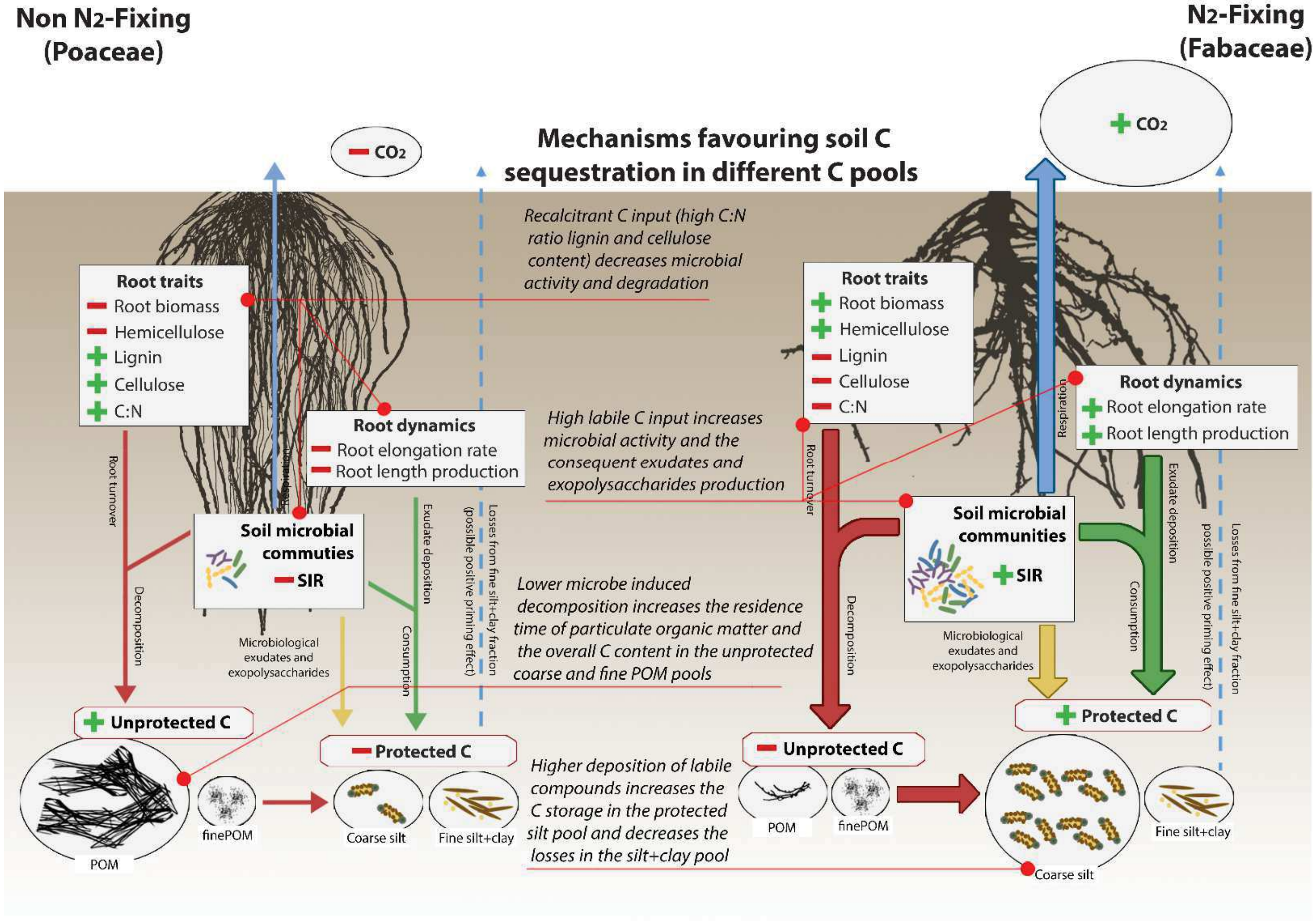

Fig.7: Conceptual scheme of carbon (C) sequestration mechanisms into different soil pools under N2-fixing (Fabaceae) and non N2-fixing (Poaceae) species. Square boxes refer to the major factors affecting C sequestration. Ellipses show the destination of C into unprotected pools (POM and finePOM) and protected pools (coarse silt and fine silt+clay). Text in the central column describes the mechanisms favoring C sequestration into soil C pools. The arrows symbolize the processes of transformation or transport of C into different pools. Arrow colors represent the nature of the C: red C derived from root turnover, green C from rhizodeposition, yellow C from microbial exudates and exopolysaccharides and blue the C respired back into the atmosphere as CO2. The thickness of the arrows is qualitative, with wider arrows reflecting higher C fluxes. The signs: "+" (in green) means an increase and "–" (in red) means a decrease

Supplementary Materials

Article: Pathways to persistence: plant root traits alter carbon accumulation in different soil carbon pools

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Table S1: Pearson's correlation coefficients (r) showing relationships between mean root elongation rate and climatic variables.

Variables tested include: mean daily soil temperature, mean daily air temperature, minimum daily air temperature, maximum daily air temperature, mean daily solar radiation and a) RER_{TOT}: mean root elongation rate of a single root, b) RER_{OLD}: mean root elongation rate of a single root that was already present at the previous sampling date (i.e. that were older than 14 days), c) RER_{NEW}: mean root elongation rate of a single newly initiated root (i.e. that were aged 1 to 14 days). The correlations were performed for data from each RER sampling date, i.e. every 2 weeks for each species over the 10 month period.

a - Correlation between climate factors and RER_{TOT} of the **total root system**

		Soil temperature	Mean air temperature	Minimum air temperature	Maximum air teperature	Mean solar radiation
Poaceae	*Dactylis glomerata*	-0.23	-0.21	-0.1	-0.18	-0.1
	Lolium perenne	0.01	-0.04	-0.03	0.09	0.38
	Festuca rubra	-0.21	-0.27	-0.34	-0.2	0.15
	Bromus erectus	-0.02	-0.05	-0.01	0.02	0.29
	Poa pratensis	0.1	0.08	0.34	0.1	0.43
Fabaceae	*Trifolium repens*	**0.76*****	**0.81*****	0.45	**0.68****	0.33
	Trifolium pratense	0.02	-0.02	-0.02	0.09	0.4
	Lotus corniculatus	**0.70****	**0.70****	0.44	**0.68****	**0.72****
	Onobrychis viciifolia	0.13	0.09	0.3	0.17	**0.54***
	Medicago sativa	0.01	0.01	-0.05	0.02	0.22
Rosaceae	*Sanguisorba minor*	-0.06	-0.06	-0.06	-0.04	0.16
Plantaginaceae	*Plantago lanceolata*	0	-0.04	0	0.04	0.3

b - Correlation between climate factors and RER_{OLD} of the **old roots**

Family	Species	Soil temperature	Mean air temperature	Minimum air temperature	Maximum air teperature	Mean solar radiation
Poaceae	*Dactylis glomerata*	-0.27	-0.26	-0.17	-0.22	-0.12
	Lolium perenne	0.14	0.13	0.31	0.09	0.21
	Festuca rubra	-0.25	-0.3	-0.41	-0.25	0.05
	Bromus erectus	0.17	0.28	-0.07	0.2	0.29
	Poa pratensis	-0.03	0	0.4	0.04	0.21
Fabaceae	*Trifolium repens*	0.28	0.28	0.15	0.4	0.45
	Trifolium pratense	0.18	0.25	-0.07	0.24	0.38
	Lotus corniculatus	**0.73****	**0.71****	0.51	**0.74****	**0.89*****
	Onobrychis viciifolia	0.04	0.02	0.26	0.07	0.36
	Medicago sativa	0.03	0.01	-0.05	0.03	0.23
Rosaceae	*Sanguisorba minor*	0.13	0.2	-0.08	0.1	-0.33
Plantaginaceae	*Plantago lanceolata*	-0.05	-0.07	-0.05	-0.03	0.21

c - Correlation between climate factors and RER_{NEW} of **new roots**

		Soil temperature	Mean air temperature	Minimum air temperature	Maximum air teperature	Mean solar radiation
Poaceae	*Dactylis glomerata*	**-0.55***	**-0.54***	-0.47	**-0.60***	**-0.66****
	Lolium perenne	0.03	0	-0.05	0.1	0.29
	Festuca rubra	-0.11	-0.16	-0.04	-0.15	0.26
	Bromus erectus	-0.06	-0.07	-0.08	-0.05	0.17
	Poa pratensis	0.09	0.08	0.36	0.06	0.38
Fabaceae	*Trifolium repens*	**0.59***	**0.67****	0.17	**0.57***	0.17
	Trifolium pratense	0.11	0.1	0.37	0.08	0.41
	Lotus corniculatus	**0.76*****	**0.75****	0.48	**0.76*****	**0.76****
	Onobrychis viciifolia	0.13	0.1	0.3	0.15	0.51
	Medicago sativa	0.04	0.06	-0.35	0.04	0.21
Rosaceae	*Sanguisorba minor*	0.09	0.16	0.04	0.06	-0.05
Plantaginaceae	*Plantago lanceolata*	-0.05	-0.06	-0.06	-0.03	0.19

Table S2: Pearson's correlation coefficients (r) showing relationships between cumulative root length production and climatic variables.

Variables tested include: mean daily soil temperature, mean daily air temperature, minimum daily air temperature, maximum daily air temperature, mean daily solar radiation and a) RLP_{TOT}: cumulative root length production of the 30 roots that were analysed, b) RLP_{OLD}: cumulative root length production of roots that were already present at the previous sampling date (i.e. that were older than 14 days), c) RLP_{NEW}: cumulative root length production of newly initiated roots (i.e. that were aged 1 to 14 days). The correlations were performed for data from each RLP sampling date, i.e. every 2 weeks for each species over the 10 month period.

a - Correlation between climate factors and RLP_{TOT} of the **total root system**

		Soil temperature	Mean air temperature	Minimum air temperature	Maximum air teperature	Average solar radiation
Poaceae	*Dactylis glomerata*	-0.4	-0.41	-0.46	-0.45	-0.38
	Lolium perenne	-0.02	-0.05	-0.08	0.04	0.29
	Festuca rubra	-0.19	-0.25	-0.32	-0.18	0.18
	Bromus erectus	-0.01	-0.05	0.01	0.03	0.31
	Poa pratensis	0.12	0.09	0.35	0.12	0.46
Fabaceae	*Trifolium repens*	0.36	0.37	-0.01	0.33	0.38
	Trifolium pratense	0.03	0	0.03	0.07	0.34
	Lotus corniculatus	**0.72****	**0.72****	0.46	**0.70****	**0.74****
	Onobrychis viciifolia	0.13	0.09	0.3	0.17	**0.54***
	Medicago sativa	0.01	-0.01	-0.04	0.02	0.24
Rosaceae	*Sanguisorba minor*	-0.06	-0.07	-0.05	-0.04	0.18
Plantaginaceae	*Plantago lanceolata*	0	-0.04	0	0.04	0.31

b - Correlation between climate factors and RLP_{OLD} of the **old roots**

		Soil temperature	Mean air temperature	Minimum air temperature	Maximum air teperature	Mean solar radiation
Poaceae	*Dactylis glomerata*	-0.29	-0.34	-0.38	-0.28	0
	Lolium perenne	0	-0.07	-0.02	-0.08	0.24
	Festuca rubra	-0.16	-0.22	-0.31	-0.15	0.19
	Bromus erectus	0.18	0.25	0	0.23	0.38
	Poa pratensis	0.02	0.03	0.4	0.1	0.31
Fabaceae	*Trifolium repens*	0.28	0.26	0.2	0.41	**0.51***
	Trifolium pratense	0.22	0.26	0.02	0.29	0.47
	Lotus corniculatus	**0.72****	**0.69****	0.52	**0.75*****	**0.90*****
	Onobrychis viciifolia	0.06	0.03	0.28	0.1	0.42
	Medicago sativa	0.04	0	0.02	0.08	0.33
Rosaceae	*Sanguisorba minor*	-0.04	-0.05	-0.06	-0.02	0.18
Plantaginaceae	*Plantago lanceolata*	0	-0.04	0.02	0.05	0.33

c - Correlation between climate factors and RLP_{NEW} of **new roots**

		Soil temperature	Mean air temperature	Minimum air temperature	Maximum air teperature	Mean solar radiation
Poaceae	*Dactylis glomerata*	**-0.52***	**-0.51***	-0.51	**-0.54***	-0.48
	Lolium perenne	-0.25	-0.3	-0.43	-0.27	0.04
	Festuca rubra	-0.08	-0.14	-0.03	-0.1	0.33
	Bromus erectus	0.01	-0.03	0.01	0.05	0.33
	Poa pratensis	0.13	0.1	0.34	0.12	0.46
Fabaceae	*Trifolium repens*	**0.51***	**0.57***	0.11	0.49	0.26
	Trifolium pratense	0.17	0.14	0.36	0.17	**0.51***
	Lotus corniculatus	**0.69****	**0.69****	0.41	**0.70****	**0.77*****
	Onobrychis viciifolia	0.06	0.01	0.02	0.13	0.43
	Medicago sativa	0.03	0.02	-0.03	0.05	0.27
Rosaceae	*Sanguisorba minor*	0.17	0.14	0.19	0.22	0.46
Plantaginaceae	*Plantago lanceolata*	0.01	-0.03	0.01	0.05	0.32

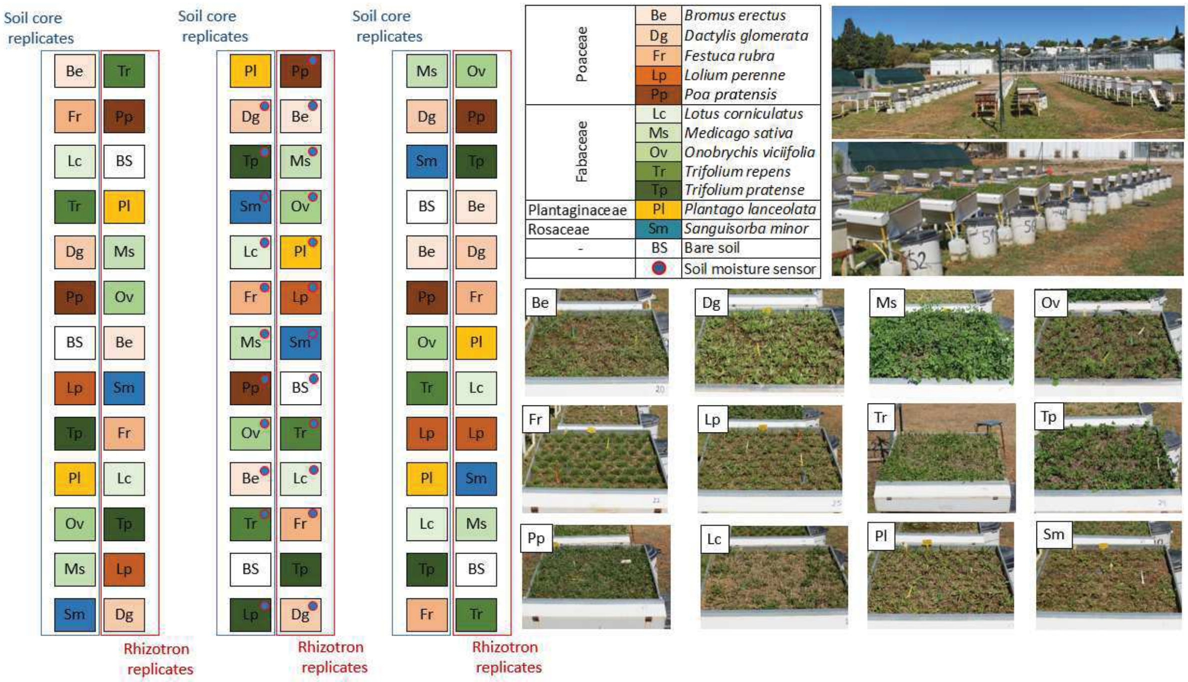
Table S3: Pearson's correlation coefficients (*r*) showing relationships between root variables and soil variables. Table S3a) shows the significant correlations obtained with the means of the three replicates for every soil and root characteristic for a total of n=12. Table S3b) shows the significant Pearson's correlations obtained using all data where n = 34. Abbreviations: RER$_{\mathrm{TOT}}$ – root elongation rate of the entire root system; RER$_{\mathrm{OLD}}$ – of roots older than 14 days; RER$_{\mathrm{NEW}}$ – of new roots aged 1 – 14 days; RLP$_{\mathrm{TOT}}$ – root length production of the entire root system; RLP$_{\mathrm{OLD}}$ – of old roots; RLP$_{\mathrm{NEW}}$ – of new roots; Root biomass – total root biomass of a core sampled at 37 weeks; absorptive root diameter– mean diameter of absorptive roots at 37 weeks; hemicellulose + water soluble compounds – concentration of hemicellulose and water soluble compounds in absorptive roots; cellulose, lignin – concentrations of cellulose and lignin in absorptive roots; C:N – ratio of carbon to nitrogen in absorptive roots; SIR – microbial substrate induced respiration.

		a) Pearson's correlations at species level (n = 12, mean of three replicates)						**b) Pearson's correlations at individual level (n = 34)**					
		ΔC_{SUM}	ΔC_{POM}	$\Delta C_{finePOM}$	ΔC_{silt}	$\Delta C_{silt+clay}$	SIR	ΔC_{SUM}	ΔC_{POM}	$\Delta C_{finePOM}$	ΔC_{silt}	$\Delta C_{silt+clay}$	SIR
Root growth dynamics	RER$_{\mathrm{TOT}}$	-0.32	-0.2	-0.06	-0.1	-0.17	0.45	-0.32	-0.2	-0.06	-0.1	-0.17	0.45
	RER$_{\mathrm{OLD}}$	-0.17	-0.25	-0.04	**0.72****	0.37	**0.74****	-0.17	-0.25	-0.04	**0.72****	0.37	**0.74****
	RER$_{\mathrm{NEW}}$	-0.24	-0.13	-0.2	-0.12	-0.55	0.18	-0.24	-0.13	-0.2	-0.12	-0.55	0.18
	RLP$_{\mathrm{TOT}}$	-0.34	-0.33	-0.18	0.37	0.34	0.51	-0.34	-0.33	-0.18	0.37	0.34	0.51
	RLP$_{\mathrm{NEW}}$	-0.3	-0.3	-0.15	0.33	0.17	0.43	-0.3	-0.3	-0.15	0.33	0.17	0.43
	RLP$_{\mathrm{OLD}}$	-0.32	-0.25	-0.22	**0.66***	0.34	**0.70***	-0.32	-0.25	-0.22	**0.66***	0.34	**0.70***
Root morphological traits	Root biomass	-0.2	-0.46	-0.37	**0.78****	0.3	**0.80****	0.18	-0.08	0.02	0.3	-0.07	**0.69*****
	Absorptive roots diameter	-0.2	**-0.72****	-0.17	**0.79****	0.14	0.56	0.08	**-0.44***	0.21	0.14	0.36	**0.50****
Root chemical traits	Hemicelluloses +H_2O soluble compounds	-0.06	**-0.61***	-0.13	**0.82****	-0.3	**0.68***	0.26	-0.3	0.25	0.22	0.23	**0.60*****
	Cellulose	-0.18	0.47	-0.14	-0.22	0.19	-0.53	-0.24	0.13	-0.16	-0.03	0.18	-0.31
	Lignin	0.15	0.56	0.26	**-0.84*****	0.49	**-0.60***	-0.19	0.18	-0.17	-0.21	-0.26	**-0.46****
	Root C:N ratio	0.26	0.37	0.09	**-0.68***	0.29	**-0.86*****	-0.06	0.28	-0.07	-0.3	-0.17	**-0.79*****
	SIR	-0.16	-0.19	-0.11	**0.65***	-0.57		0.27	-0.15	0.18	**0.48****	0.12	

In bold, significant *r* values: * *p* <0.05, ***p* < 0.01, *** *p* < 0 .001

Fig. S1: Spatial disposition of growth boxes.

In left panel, replicates are depicted in different colours according to their family (green: N_2-fixing Fabaceae, brown: non N_2-fixing Poaceae, yellow: non N_2 fixing *P. lanceolata*, blue: non N_2-fixing *S. minor*). The different shades of colour representing different species are shown in the legend. The blue dot on the upper right-hand corner of the different colored squares shows the boxes that were equipped with air/soil temperature and soil moisture sensors. Soil cores were removed from each row of 'soil core replicates' growth boxes. Half the boxes were fitted with rhizotrons ('rhizotron replicates'). Photographs of each species can be seen in the bottom right panel of the figure.



Soil core replicates	Rhizotron replicates	Soil core replicates	Rhizotron replicates	Soil core replicates	Rhizotron replicates
Be	Tr	Pl	Pp	Ms	Ov
Fr	Pp	Dg	Be	Dg	Pp
Lc	BS	Tp	Ms	Sm	Tp
Tr	Pl	Sm	Ov	BS	Be
Dg	Ms	Lc	Pl	Be	Dg
Pp	Ov	Fr	Lp	Pp	Fr
BS	Be	Ms	Sm	Ov	Pl
Lp	Sm	Pp	BS	Tr	Lc
Tp	Fr	Ov	Tr	Lp	Lp
Pl	Lc	Be	Lc	Pl	Sm
Ov	Tp	Tr	Fr	Lc	Ms
Ms	Lp	BS	Tp	Tp	BS
Sm	Dg	Lp	Dg	Fr	Tr

Poaceae	Be	*Bromus erectus*
	Dg	*Dactylis glomerata*
	Fr	*Festuca rubra*
	Lp	*Lolium perenne*
	Pp	*Poa pratensis*
Fabaceae	Lc	*Lotus corniculatus*
	Ms	*Medicago sativa*
	Ov	*Onobrychis viciifolia*
	Tr	*Trifolium repens*
	Tp	*Trifolium pratense*
Plantaginaceae	Pl	*Plantago lanceolata*
Rosaceae	Sm	*Sanguisorba minor*
-	BS	Bare soil
	●	Soil moisture sensor

Fig. S2: Growth boxes used in the experiment.

Above a gravel layer, soil was homogeneously compacted into growth boxes. Seeds were sown at a density of 155 plants m^{-2}. Panes of plexiglass on the front of the box allowed root elongation to be observed over the 37 week long experiment.

Fig. S3: Climatic conditions over the 37 weeks of experimentation.

a) Mean daily air temperature (solid black line), minimum air temperature (segmented blue line), maximum air temperature (segmented red line), mean daily soil temperature (dotted black line) and mean daily air humidity (segmented black, dotted line). In b), mean daily solar irradiation (solid black line) and evapotranspiration (segmented red line).

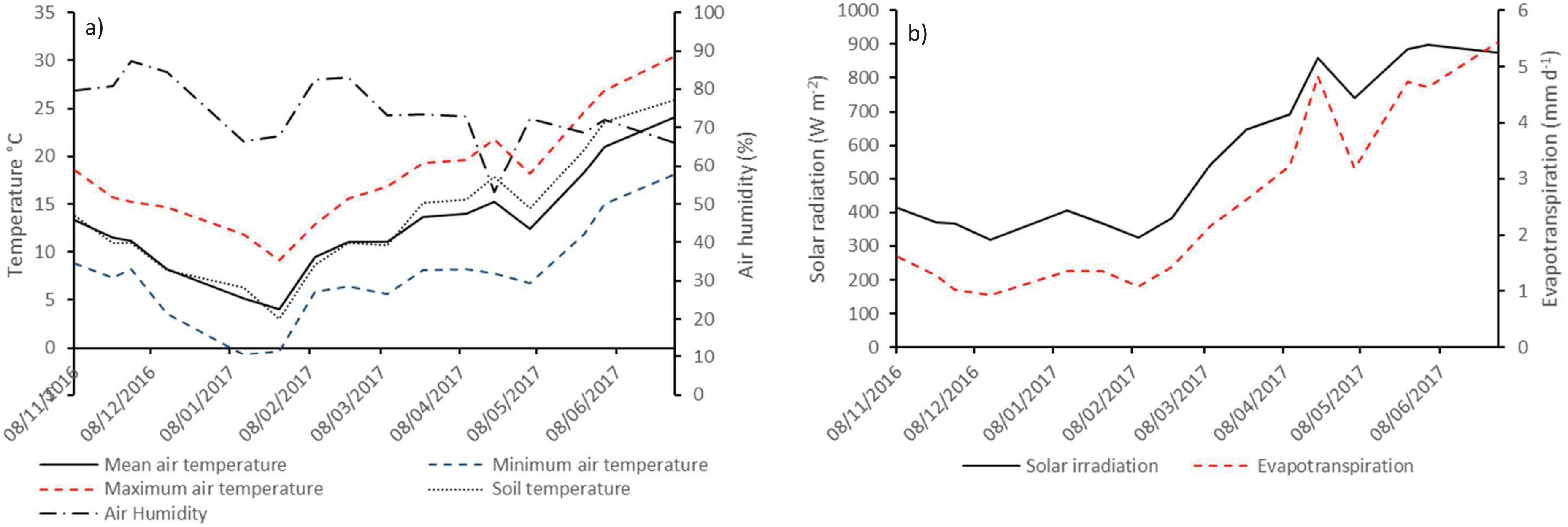


Fig. S4: Example of an image analyzed using SmartRoot software.

a) primary roots (in orange) from which lateral roots are initiated (in green). Each orange circle along the root axis represents a single ‘mouse click’ for root selection. b) A bundle of roots growing close together, where the number and diameter of roots cannot be recognized by the SmartRoot software.

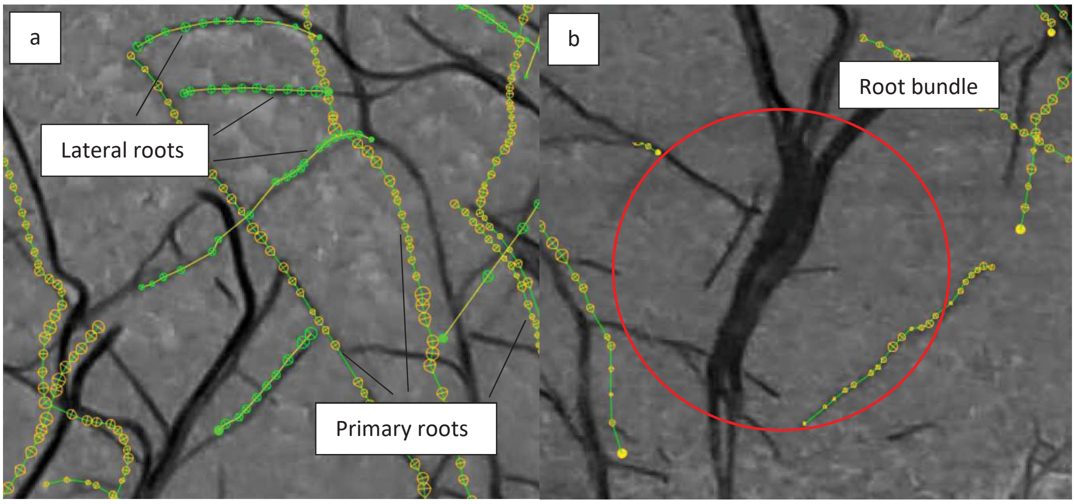


Fig.S5: a) Example of two subsequent images of roots of *Bromus erectus* taken on 23/02/2019 and 10/03/2019 and b) conceptual scheme to explain root selection procedure.. a) The figure on the left shows newly initiated roots that will be analyzed to calculate the RER_{NEW} and RLP_{NEW} on 23/02/2019. At the next date for image analysis (10/03/2019), some of the previously analyzed roots were the same length (RER=0, middle of the rhizotron), whereas other roots elongated (RER>0, bottom of the rhizotron), and were used to calculate RER_{OLD} and RLP_{OLD}. On the top part of the rhizotron, some new roots were initiated, and analyzed to calculate the RER_{NEW} and RLP_{NEW} on 10/03/2019. b) Conceptual scheme showing the procedure to select 'old roots' at each sampling: at Time x 30 new roots and 30 old roots have been analyzed. Of these 30 roots, 20 are primary axis roots while 10 second order lateral (Fig. S4a). 10 roots from Time x primary old roots and 10 from Time x primary new roots are randomly selected to be analyzed and constitute the Time x+1 old primary roots. 5 roots from Time x secondary old roots and 5 from Time x new secondary roots are selected and analyzed and constitute the Time x+1 secondary old roots.

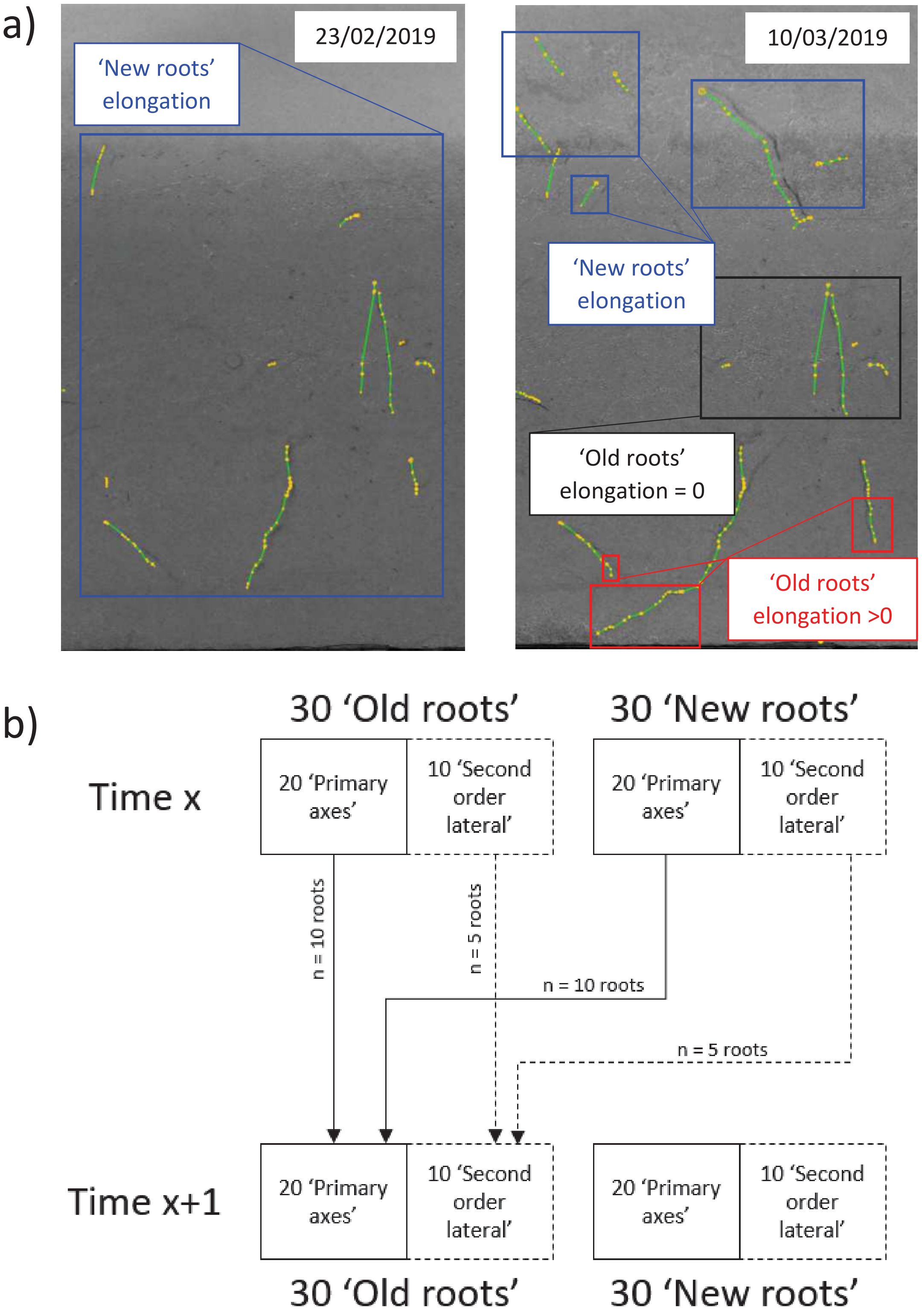

a)
23/02/2019
'New roots' elongation
10/03/2019
'New roots' elongation
'Old roots' elongation = 0
'Old roots' elongation >0
b)
30 'Old roots'
30 'New roots'
Time x
20 'Primary axes'
10 'Second order lateral'
20 'Primary axes'
10 'Second order lateral'
n = 10 roots
n = 5 roots
n = 10 roots
n = 5 roots
Time x+1
20 'Primary axes'
10 'Second order lateral'
20 'Primary axes'
10 'Second order lateral'
30 'Old roots'
30 'New roots'

Fig. S6: Carbon (C) content in each soil C pool beneath the 12 species and in the control bare soil, 37 weeks after sowing.

The C pools analyzed are C_{POM} in the coarse particulate organic matter >200µm), $C_{finePOM}$ (C in the fine particulate organic matter 200-50µm), C_{SILT} (in the coarse silt fraction 50-20µm) and $C_{SILT+CLAY}$ (C in the fine silt + clay fraction <20µm). The letters on the left hand side of the fraction bars indicate significant differences (Tukey HSD, *p* < 0.05) between C pools and within species.

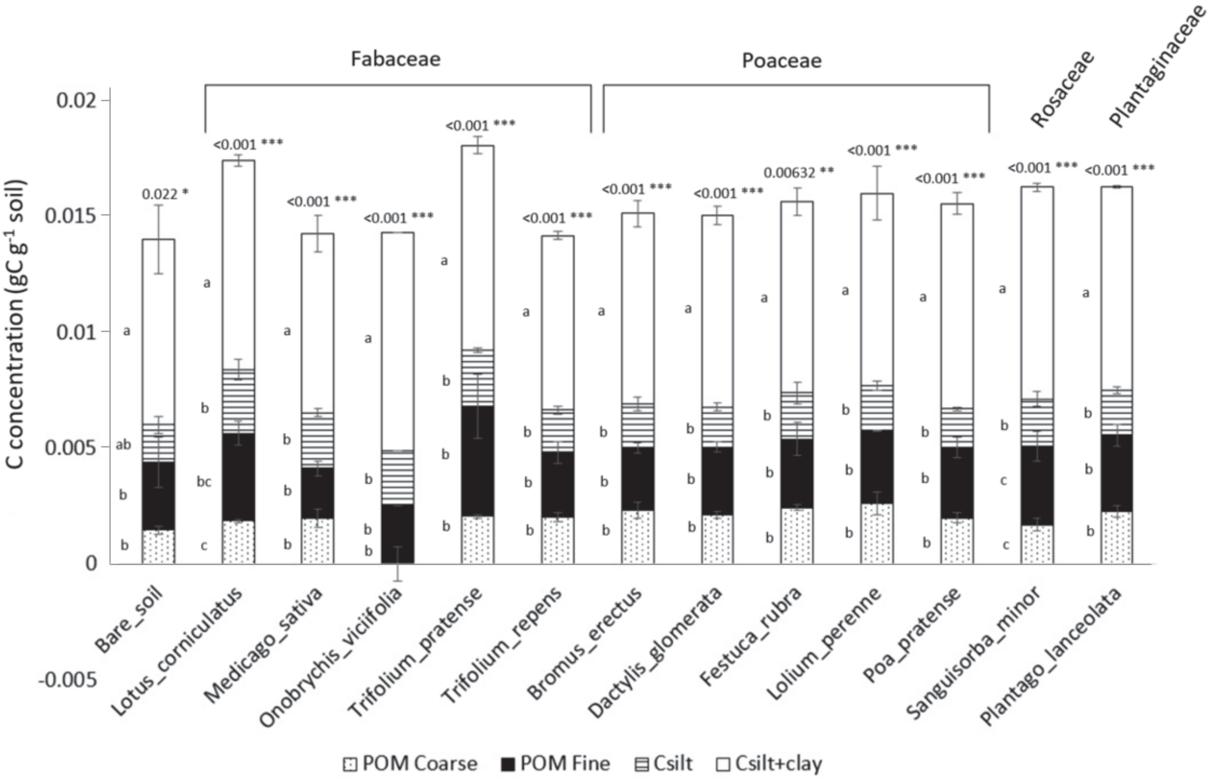


Fig. S7: Mean daily root elongation rate (RER_{TOT}) for all the roots analyzed in the rhizotrons (without distinguishing between old and new roots) in Fabaceae (N_2-fixing, black circles) and Poaceae (non N_2-fixing, white squares) family.

Mean daily RER_{TOT} in Fabaceae peaked in May - June, whereas in Poaceae, mean daily RER_{TOT} was fairly constant between February and June, with no marked peaks. Data are means ± standard error of the RER data in the 2 weeks prior to the measurement of root elongation.

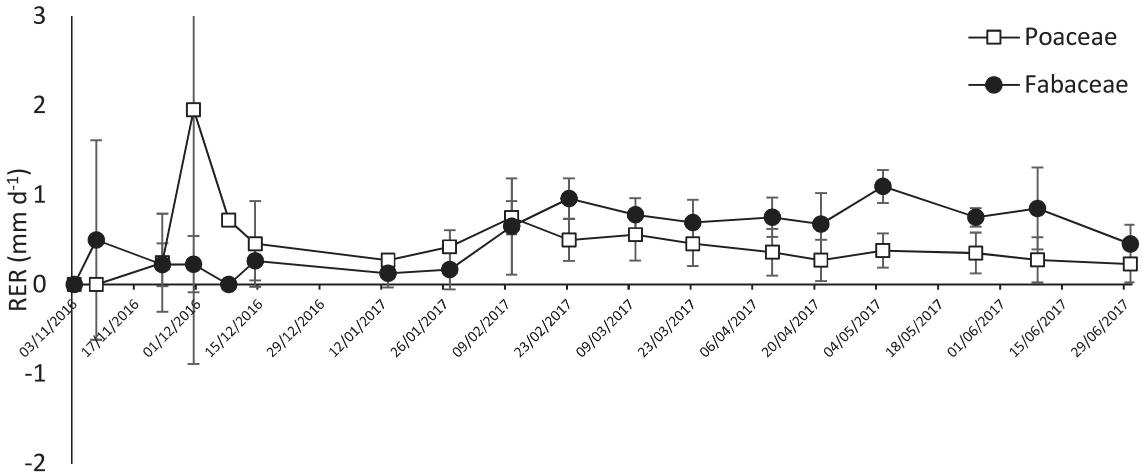


Fig.S8: Mean root elongation rate of individual roots (RER, measured every 2 weeks) for each of the 12 species.

The solid black line is the RER_{NEW} of the roots that were newly initiated (aged 1 to 14 days), and were not present at the previous sampling date. The dotted black line represents the RER_{OLD} of the roots that were already present at the previous sampling date, and so were older than 14 days.

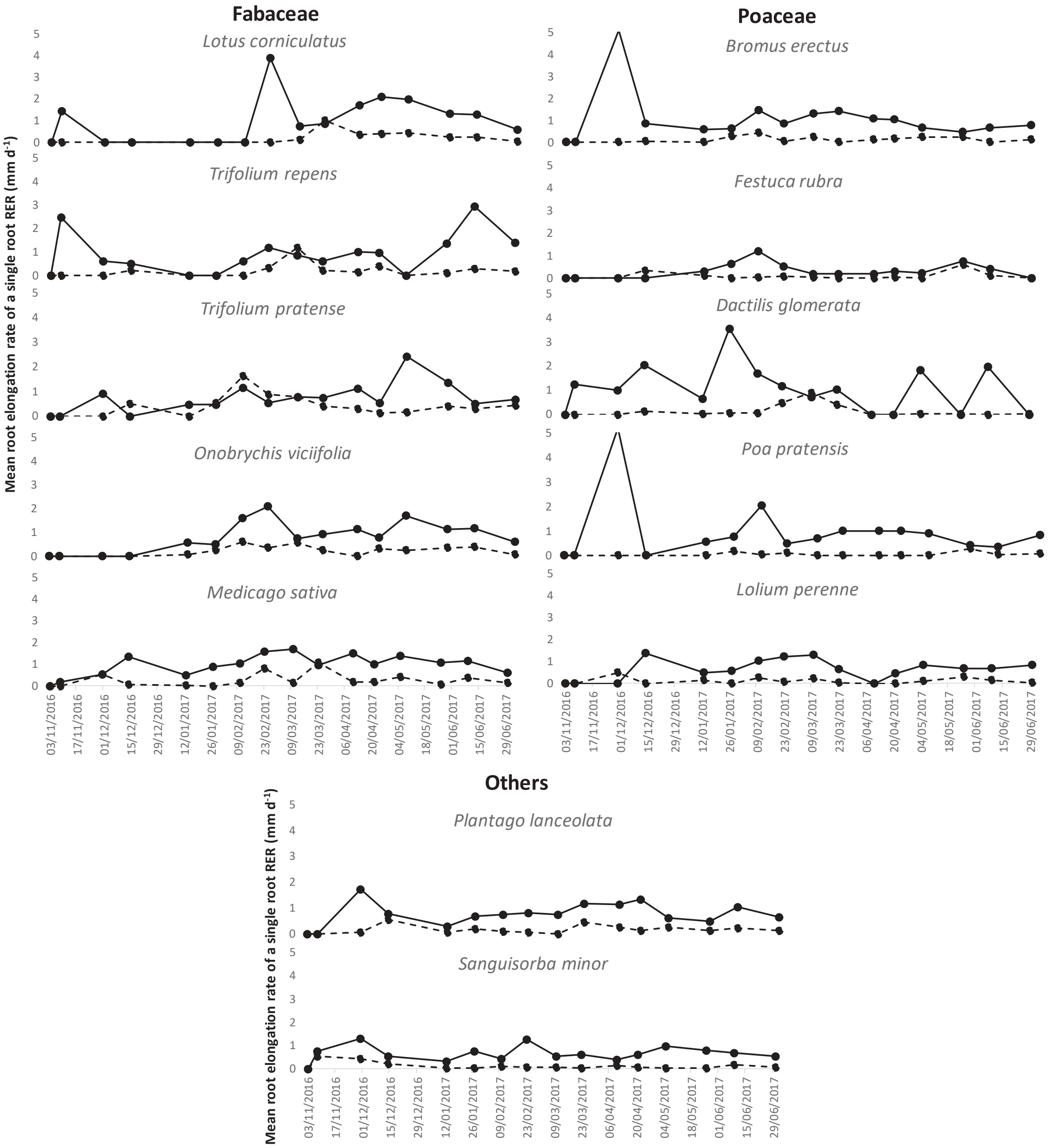


Fig. S9: Linear regression at the individual level (n = 34 samples), between ΔC_{POM} (as the difference in carbon (C) in the coarse POM C pool, $\Delta C = C_{t37} - C_{t0}$, after 37 weeks), and a) diameter of absorptive roots and b) hemicelluloses + water soluble compounds.

The black symbols are the N_2-fixing Fabaceae species, the white symbols the non N_2-fixing Poaceae species, the red dots are *S. minor* and the green dots are *P. lanceolata*. The red line is the linear model function of the variables and R^2, F and p of the linear model are shown.

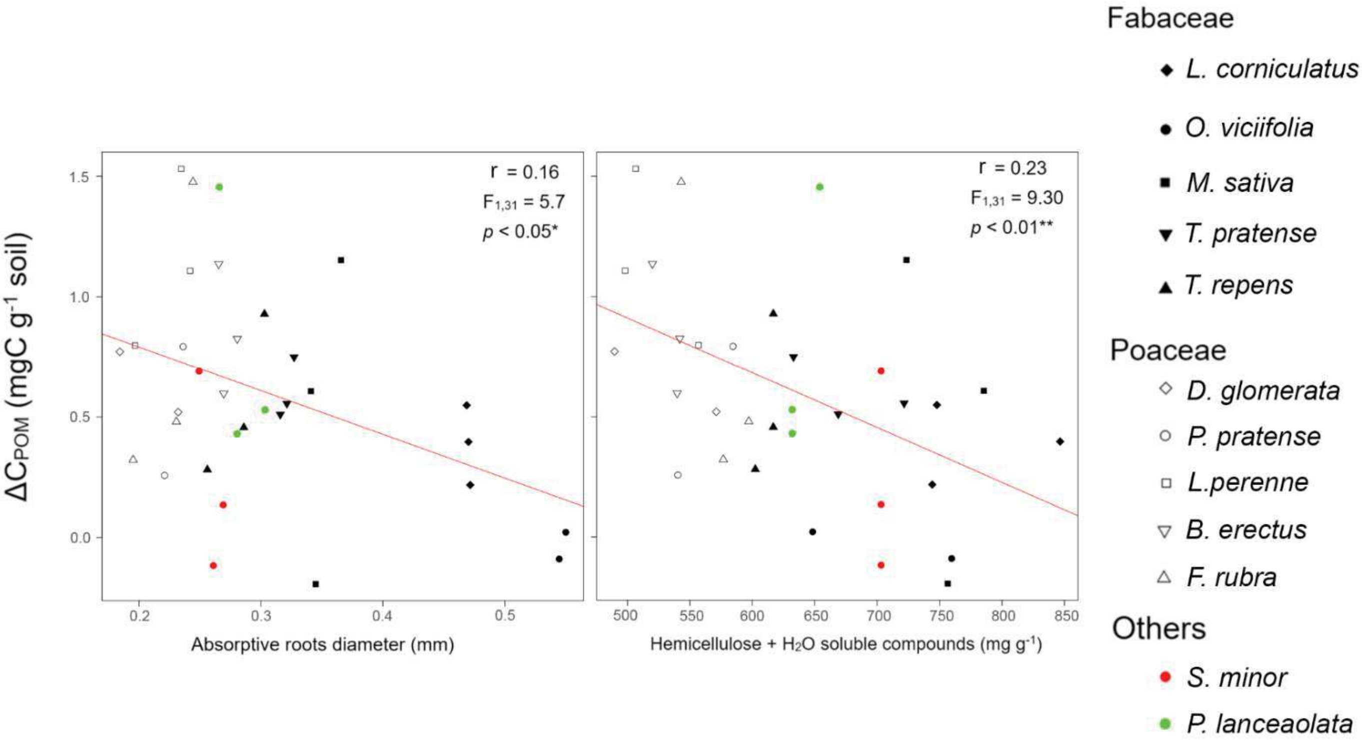


Fig. S10: Linear regression at individual level (n = 34 samples) between ΔC_{silt} (as the difference in carbon (C) in the coarse silt C pool, $\Delta C = C_{t37} - C_{t0}$, after 37 weeks), and a) root biomass, b) diameter of absorptive roots, c) lignin content, d) hemicelluloses + water soluble compounds, e) C:N ratio and f) substrate induced respiration rate (SIR).

The black symbols are the N_2-fixing Fabaceae species, the white symbols the non N_2-fixing Poaceae species, the red dots are *S. minor* and the green dots are *P. lanceolata*. The red line is the linear model function of the variables and R^2, F and *p* of the linear model are shown. For the legend refer to figure S9.

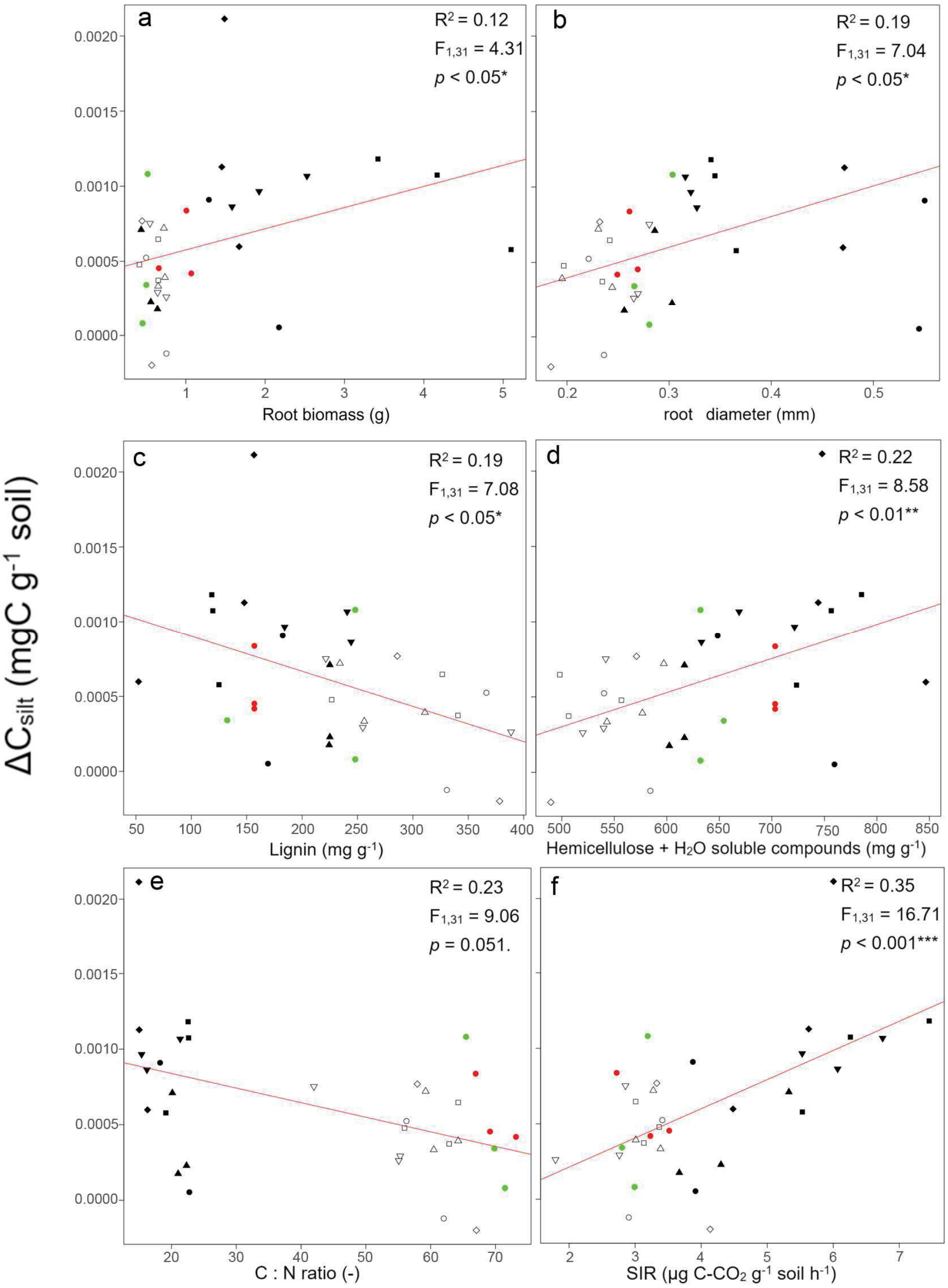
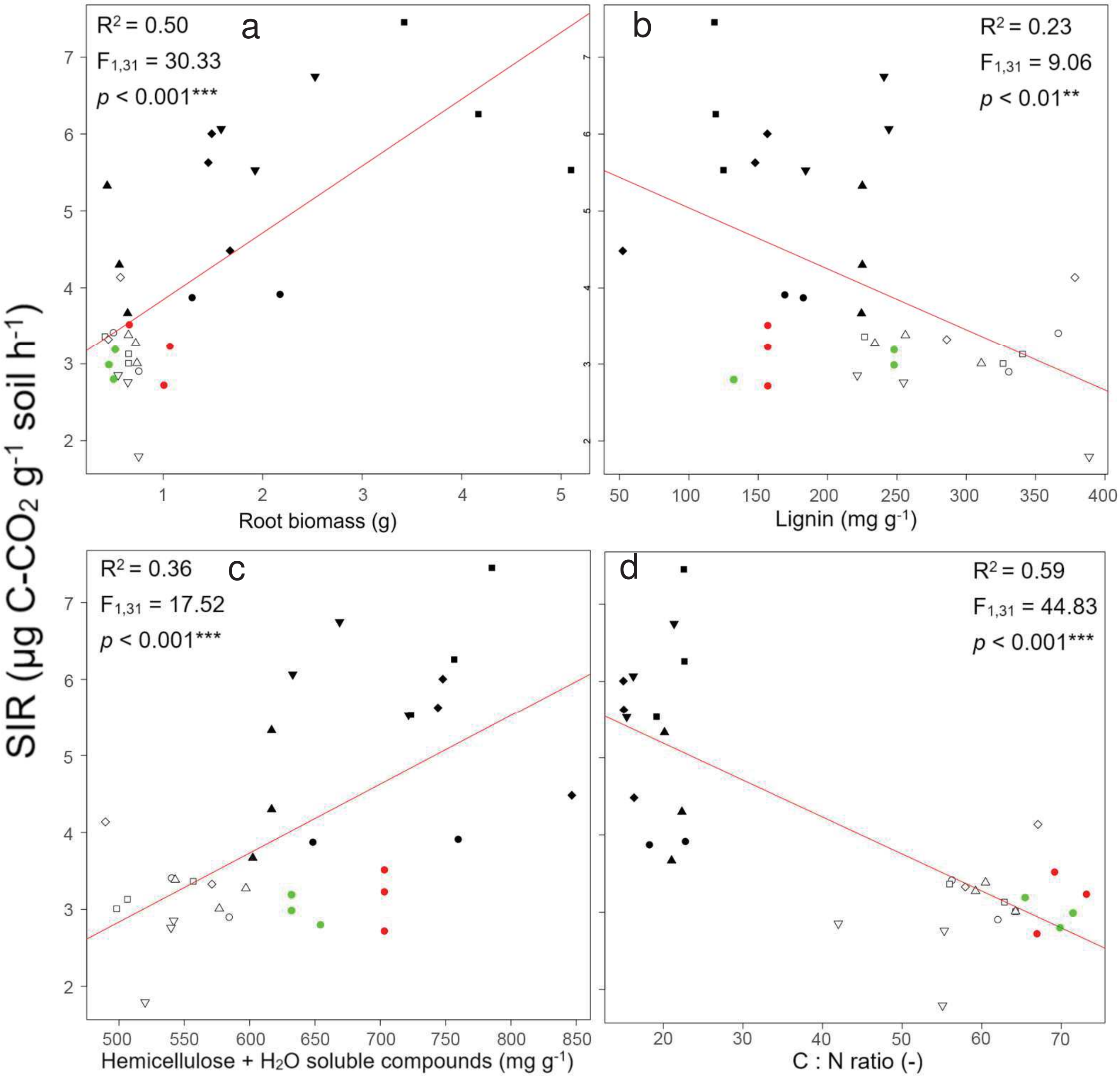
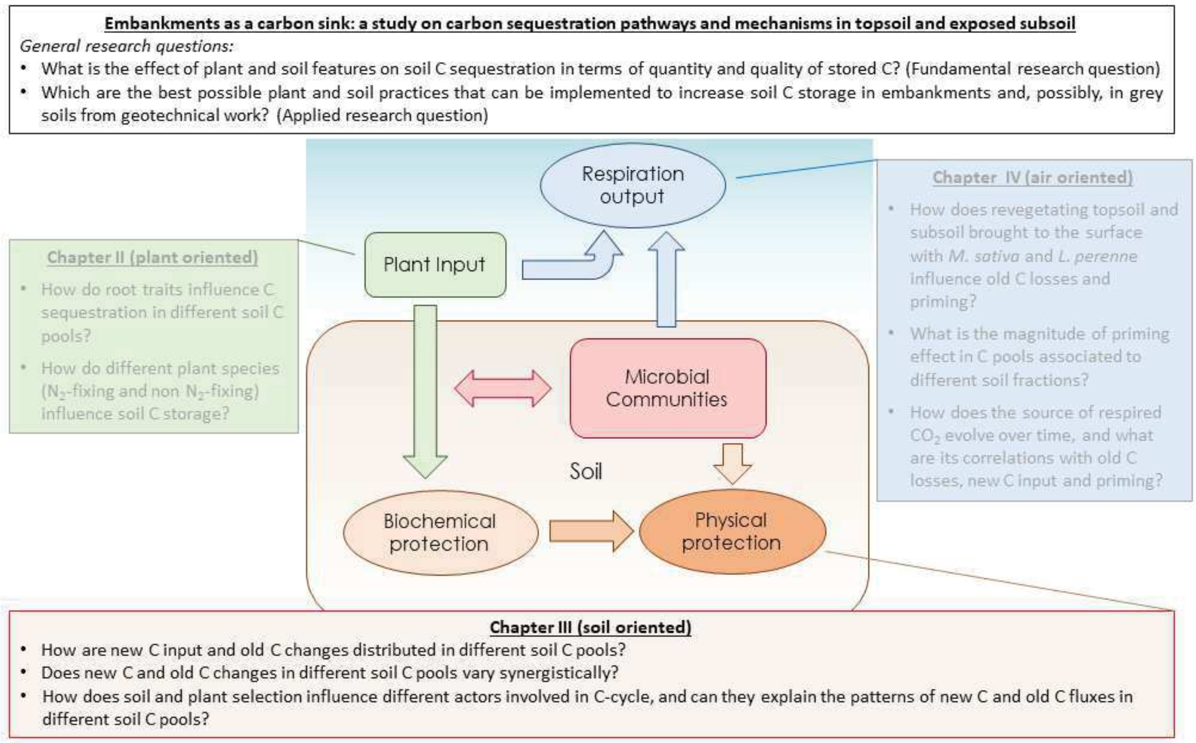


Fig. S11: Linear regression at individual level (n = 34 samples) between substrate induced respiration rate (SIR) and a) root biomass, b) lignin content, c) hemicelluloses + water soluble compounds, d) C:N ratio.

The black symbols are the N_2-fixing Fabaceae species, the white symbols the non N_2-fixing Poaceae species, the red dots *S. minor* and the green dots *P. lanceolata*. The red line is the linear model function of the variables and R^2, F and *p* of the linear model are shown. For the legend refer to figure S9.



Chapter III: The fates of fresh new carbon and old soil carbon differ in topsoil and newly exposed subsoil and are explained by root, microbial, and soil particle size



Embankments as a carbon sink: a study on carbon sequestration pathways and mechanisms in topsoil and exposed subsoil

General research questions:

- What is the effect of plant and soil features on soil C sequestration in terms of quantity and quality of stored C? (Fundamental research question)
- Which are the best possible plant and soil practices that can be implemented to increase soil C storage in embankments and, possibly, in grey soils from geotechnical work? (Applied research question)

Chapter II (plant oriented)

- How do root traits influence C sequestration in different soil C pools?
- How do different plant species (N_2-fixing and non N_2-fixing) influence soil C storage?

Chapter IV (air oriented)

- How does revegetating topsoil and subsoil brought to the surface with *M. sativa* and *L. perenne* influence old C losses and priming?
- What is the magnitude of priming effect in C pools associated to different soil fractions?
- How does the source of respired CO_2 evolve over time, and what are its correlations with old C losses, new C input and priming?

Chapter III (soil oriented)

- How are new C input and old C changes distributed in different soil C pools?
- Does new C and old C changes in different soil C pools vary synergistically?
- How does soil and plant selection influence different actors involved in C-cycle, and can they explain the patterns of new C and old C fluxes in different soil C pools?

In Chapter II we studied the effect of revegetation on C storage in different soil C pools. In Chapter III we aim to refine the understanding of C pathways in soil by selecting two species with contrasting root characteristics (among the species in Ch. II) and sowing them on two soils showing contrasting characteristics (fertile topsoil and poor subsoil) in a ^{13}C constantly enriched environment. We will differentiate the input of fresh new C and changes in preexistent old C in the different soil C pools and study their relationships with root traits, microbiological and soil characteristics.

Abbreviation	**Definition**	**Corresponding symbol**
C	Carbon; soil carbon refers to soil organic carbon in this study	
C pool	Soil carbon contents associated with different soil particle fraction sizes: particulate organic matter fraction (50-200 µm), fine particulate organic matter fraction (20-50 µm), coarse silt fraction (20-50 µm) and fine silt and clay fraction (<20 µm)	*X = POM*, *finePOM*, *SILT* and *SILT+CLAY*
C content	Soil carbon concentration (in mgC g^{-1}soil) per unit weight of soil for each C pool or pool summed	C_{POM} , $C_{finePOM}$, C_{SILT}, $C_{SILT+CLAY}$, C_{SUM}
C change	Difference in soil carbon contents (in mgC g^{-1}soil) between the end and beginning of the experiment	ΔC_{POM} , $\Delta C_{finePOM}$, ΔC_{SILT}, $\Delta C_{\mathrm{SILT+CLAY}}$, ΔC_{SUM}
C quality	Proportion (in %) of soil carbon content belonging to each carbon pool	$\%C_{POM}$, $\%C_{finePOM}$, $\%C_{\mathrm{SILT}}$, $\%C_{\mathrm{SILT+CLAY}}$
new C	Fresh soil carbon due to plant inputs	$\Delta New\ C_X$ (new C gain for the pool *X*)
old C	Existing soil carbon before plant growth	$\Delta Old\ C_x$ (old C change for the pool *X*)
t0	Time zero, beginning of the experiment	
t6	Time after 6 months, end of the experiment	
POM	Particulate organic matter	
C:N	Carbon – nitrogen ratio in plant tissue	
GMA	Global Metabolic Activity of microbial communties	
SIR	Substrate induced respiration	
ANOVA	Analysis of variance	
PCA	Principal component analysis	
H	Shannon metabolic diversity index	

3.1. INTRODUCTION

3.1.1. General context in soil organic carbon sequestration on embankments: can subsoil brought to the surface be used as a C sink?

Soil is the largest terrestrial carbon (C) reservoir and soil organic C (SOC) exchanges rapidly with C in the atmosphere and biosphere (Torn et al. 2009). In the context of global warming, knowing the fate of SOC is essential for greenhouse gasses mitigation. So far, national and supranational programs have been developed to maintain soil organic C stability and promote C sequestration in soil (e.g. 4p1000). Appropriate soil and vegetation management that favors C transfer from air to soil via plants has been shown to be a promising way to increase the soil C sink (Rees et al., 2005; Minasny et al., 2017). Most of the research has been carried out on agricultural and 'natural' soils, while heavily disturbed antropized soils, i.e.. soils related to geotechnical operations. In this work we focus on the revegetation of geotechnical road and railroad embankments, and their potential for soil C storage. Topsoil has often been used for the revegetation of embankments, however, subsoil can be brought to the surface and revegetated directly.We argue that revegetating subsoil brought to the surface has a high influence on the C-cycle due to the different characteristics of subsoil compared to topsoil: lower fertility levels, different aggregate characteristics, microbiological communities and their evolution and dynamics (Taylor et al. 2002, Murray et al. 2004, Chabbi et al. 2009, Jones et al. 2018) and, most notably, C saturation (Lorenz and Lal 2005; Rumpel and Kögel-Knabner 2011, Beare et al. 2016). It has been hypothesized that soil has a C saturation level associated to its fine particle size partition (i.e., clay content) and the initial old C content (Six et al. 2002; Stewart et al. 2007). Protection via organomineral interactions relays on surface area of soil particles, and after the available areas and reactive surfaces are occupied by C, further C input will not be adsorbed anymore and therefore will not be protected (Six et al. 2002). The potential amount of C protected via organomineral complexation depends on the amount of the <20µm fraction and the initial amount of C in the associated soil C pool. We argue that subsoils have a lower C saturation compared to topsoil due to higher clay content and lower C content (Lorenz and Lal 2005; Rumpel and Kögel-Knabner 2011, Lawrence-Smith et al. 2015) and, therefore, could store stable C more efficiently via organomineral interactions in the finer silt and silt+clay soil fractions (<20µm). For this reason, subsoil brought to the surface could be an interesting feature for C storage, and we aim to investigate the influence of excavating and revegetating subsoil on the main actors involved in C-cycle and their influence on C storage in different soil C pools associated to granulometry of soil fractions.

3.1.2. New and old carbon in soil

The soil C stock within a defined time frame is the balance between input and transformation of newly photosynthesized C from plants to soil (new C) and losses of existing soil organic C (old C) through microbial and plant respiration (Kuzyakov and Domansky, 2000; Fontaine et al., 2004). Although total soil C sequestration is increasingly measured as an important ecosystem service, few studies have quantified the proportions of new C input from plants and the losses of old soil C during respiration. It is unclear how the input of new C and the losses of old C participate to the final soil C sequestration and if trade off or synergetic patterns exist between new C input in soil and old C losses. The underlying mechanisms behind these processes are poorly understood, but are crucial if we wish to improve soil C sequestration.

3.1.3. Soil organic carbon quality: carbon pools are associated to different soil granular fractions

More and more studies have highlighted the importance of C quality in soil (Chapter II, this thesis; Cardinael et al.,2015). High quality soil C refers to organic C compounds that have long mean residence time and good stability against mineralization because of their physical or physiochemical associations with soil particles. Characterizing absolute and relative sizes of soil C pools associated to soil particle size fractions is a powerful instrument to evaluate soil C quality. The commonly used classification of soil pools in literature refers to (i) C in particulate organic matter (POM) (C_{POM}, 2000-200µm), (ii) C in fine POM ($C_{finePOM}$, 200-50µm), (iii) C in coarse silt (C_{SILT}, 50-20 µm) and (vi) C in fine silt+clay ($C_{SILT+CLAY}$, <20µm). In the POM and finePOM pools, C is usually supposed to originate from plant litter debris at different levels of degradation, and is more exposed to decomposers (Kögel-Knabner, 2002), wheras C in the SILT and SILT+CLAY pools is considered more stable due to the organomineral binding with fine soil particles (Sollins et al., 1996; von Lützow et al., 2006; Cotrufo et al. 2013). Although recent studies have quantified soil C in different pools instead of that in total soil C (Cardinael et al., 2015; Chapter II, this thesis), no study to our knowledge has bridged the link between C pools and the fates of new C and old C. Speculating such an association is reasonable, as fates of new C and old C should have different sensitivities to fresh plant C inputs, that has been shown to have a significant impact on the relative size of soil C pools, i.e., soil C quality (Cardinael et al., 2015). To differentiate the inputs of new C and the changes of old C in different C pools, stable isotopic labelling has proven to be a powerful methodology. Growing plants in an atmosphere with increased % of ^{13}C in the CO_2 allows to differentiate the new C inputted in soil from the preexistent old C (Staddon 2004). Being able to differentiate old C is very interesting, since it allow to quantify even the changes in the old C pool in a determined timeframe, other than the input of new C.

3.1.4. New old carbon distribution in different soil pools: drivers and mechanisms

Besides understanding the fates of new and old soil C in different soil fractions, we also need to determine how plant and soil characteristics affect the trajectory of new and old C. The dynamics of new C in soil is assumed to be jointly determined by plant performance and soil C storage capacity. Plants transform atmospheric C via litter decomposition or root exudation, therefore, traits related to decomposition and exudation should be examined in soil C sequestration studies (De Deyn et al., 2008; Roumet et al. 2016; Henneron et al., 2019). Roumet et al. (2016) suggested that species with contrasting growth strategies and tissue quality, i.e., N_2-fixing fast-growing species with a low tissue carbon:nitrogen (C:N) ratio versus non N_2-fixing slow-growing species with a high tissue C:N ratio, result in contrasting soil C sequestration. However, the relative importance of such traits on soil C sequestration is not yet known. In this regard, studying the effects of N_2-fixing and non N_2-fixing species is of particular interest, since they are placed at the two opposite ends of the root economic spectrum (Roumet et al. 2016, Rossi et al., submitted, Chapter II) and expected to significantly influence the C sequestration quantity and quality in different soil fractions. The capacity of soil to influence new C storage is related, as already mentioned, to its C saturation levels. New C input increase soil aggregation that, with a double feedback effect, in turn protect the C from microbial mineralization via physical protection in the aggregate structure (Tisdall and Oades 1979,1982; Chevallier et al., 2004). Aggregate stability, as a proxy for aggregation resistance to disruption, might very well be associated with C protection in soil. Furthermore, N levels in soil will affect the soil fertility, and therefore, plant development and microbial biomass and activity (Sarker et al., 2017).

Studies over the last 20 years have greatly focused on the priming effect, i.e., the phenomenon that fresh biomass may, in most cases, stimulate microbial activities and thus accelerate the loss of old C existing in soil (i.e., positive priming) (Kuzyakovet al. 2000; Blagodatskaya & Kuzyakov, 2008). Positive priming can offset the gain of new C in soil and result in a net negative C balance (Cheng et al., 2003, Fontaine et al., 2004). Many factors, from fresh tissue recalcitrance to soil physical properties are found to influence the loss of old C. In particular, fresh tissue recalcitrance greatly affects the proliferation rate of the microbial population and subsequent soil respiration rate. However, soil aggregate and particle size determine the ability of soil to protect old C from microbial mineralization (Six et al., 2002). For example, aggregates act as a physical barrier that separate occluded C from microbes and enzymes (Besnard *et al*., 1996; Rasse *et al*., 2005; Bardgett *et al*., 2014; Sokol *et al*., 2019

I ask therefore, if plant traits, and soil characteristics (as aggregate stability, N content, and particle size fractions) alter new C and old C dynamics in different soil fractions through their direct effect and indirect influence on microbial communities. Microbial abundance (in terms of biomass that can be calculated as concentration of DNA in soil), the global metabolic activity as the amount of respired CO_2

per g of soil in a specific timeframe (GMA), and the diversity in metabolic substrate consumption, often represented by the Shannon index (H), will be deeply influenced by soil (Liang et al. 2017) and plant species (Cotrufo et al. 2013). Microbial communities have a pivotal role determining the mineralization and losses of old and new C, its subsequent transformation in degraded POM or the C input in the protected silt and silt+clay fractions. The theory of the 'microbial carbon pump' (Liang et al., 2017) put microbial communities at the center of C sequestration mechanisms. It state that microbial communities, consume and input C in protected structure (aggregates) or transform it in recalcitrant structure that are stabilized in soil as microbial necromass, the so called 'entombing effect' (Liang et al., 2017).

3.1.5. Research hypothesis

Using a microcosm experiment, coupled with stable isotopic (^{13}C) labelling, we aim at characterizing the fates of new C from plant roots (root debris and exudations) and old C (pre-existing C in soil), as well as their interdependence, across different soil fractions under a fully crossed soil and vegetation treatment: two types of natural soils (subsoil and topsoil) × three vegetation treatments (bare soil, *Medicago sativa* and *Lolium perenne*). Soil and microbial community characteristics and plant root traits were measured to disentangle the effects of different drivers on changes in new and old C. I hypothesize that:

i) Soil particle size can regulate the fates of old and new C within fractions. We hypothesize that the input of new C will be higher in the particulate organic matter and in the SILT+CLAY fraction due to exudation and microbial in vivo transformation of C. Old C is expected to be depleted from coarser fractions (POM and finePOM) via microbial mineralization, and transferred to finer fractions, increasing the old C in the SILT and SILT+CLAY fractions.

ii) The fates of new and old C show independent patterns: old C losses are expected to be more related to microbial characteristics than to input of new C, however the influence of new C on microbial activity might show an indirect effect decreasing old C concentration due ti priming effect.

iii) the patterns of new C and old C fluxes in different soil C pools could be explained by plant, micro-organism and soil characteristics. More specifically, root traits connected to high root biomass and labile input (i.e. acquisitive resource strategies N2-fixing species) are expected to increase new C in the soil, especially in the SILT and SILT+CLAY pools. We suppose subsoil to have a higher new C accumulation in the SILT+CLAY fraction due to higher fine fraction and lower initial C content, decreasing soil C saturation. In this respect, we think the percentage of the fine soil fraction (FF) will be positively correlated with new C in the SILT+CLAY fraction. Aggregate stability (measured as mean weight diameter, MWD) is expected to be positively correlated with new and old C accumulation in silt and silt+clay fractions due to C protection. We believe soil N content will increase the overall input of new C in all the fractions due to its connection with soil fertility and iomass production. Microbial characteristics (GMA, H and DNA concentration) are expected to be positively correlated with the accumulation of new C in the SILT and SILT+CLAY fraction and and decrease the old C content due to metabolism and respiration of C.

3.2. MATERIALS AND METHODS

3.2.1. *Soil and plant preparation, experimental design and set-up*

The soil used in the experiment was excavated from a grassland in Pisciotta (Italy, 40°07’N, 15°14’E, 178 m a.s.l.) at two depths of the same soil profile: topsoil (0.0 – 0.3 m depth) and subsoil (1.1 – 1.4 m depth). The soil was a clay loam soil (USDA) with a comparable granulometric texture between topsoil and subsoil (topsoil: 27.3% clay, 31.1% silt, 41.6% sand; subsoil: 34.8% clay, 36.8% silt, 28.4% sand). Topsoil (7.0) had a lower pH than subsoil (8.4).

Both top- and subsoil were sieved to 5 mm prior and then placed in containers (20 cm x 20 cm x 20 cm), where it was packed manually to a depth to 10 cm. Pots were weighed to ensure that they contained the same amount of soil (+/- 2.5 %). N_2-fixing *Medicago sativa* L. and non N_2-fixing *Lolium* *perenne* L. were planted as monocultures with exactly the same pattern. In each pot, three seeds were put at six equidistant spots. After germination, one seedling was removed with scissors at ground level, at each spot. For each soil type (i.e. top- and subsoil) and species, six replicate containers were prepared and six bare containers per soil type were used as controls (n = 36 in total).

Containers were placed into three identical microcosms at the Ecotron growth facility at Montpellier, France (http://www.ecotron.cnrs.fr/). In each microcosm, two replicates of all treatments, i.e., 12 pots, were placed randomly to avoid any effect of microcosm on plant growth and soil processes. Plants were grown at a constant air temperature of 21°C and at 80% humidity (to reduce the soil water loss by evapotranspiration). Artificial light was provided by three lamps (Gavita PRO 300 LEP 02, Netherlands) in each microcosm with a 12h day/night cycle, shifted to allow air sampling at the same moment of the plant's circadian rhythm (data not shown in this study, see Chapter 4). A shade was placed on the lamps and the distance of the lamps from the plants was adjusted to achieve the most possible homogenous light intensity on the foliage (300 µmol m^{-2} s^{-1}). Soil moisture was kept at 45 ± 10% of the soil water holding capacity for the entire duration of the experiment (that was previously calculated for topsoil and subsoil separately, data not shown). To minimize disturbances to the ^{13}C concentration due to the opening of microcosm, a system of plastic pipes was installed into the microcosm for watering. Every two weeks, pots were taken out to assess their evapotranspiration rate and weight (data not shown). Each time pot position was randomized when they were put back to the microcosm. Air enriched with enriched ^{13}C (with a concentration of 2%, approximately two times higher than the natural ^{13}C abundance of 1.1%, in other words $\delta^{13}C$ of CO_2 in the chamber was roughly +760%, as compared to the ambient -8) was supplied into the microcosms once the first emergence of seedlings was observed in any microcosm (approximately three weeks). The air enrichment with ^{13}C was supplied only during the 12h day cycle and the ^{13}C infusion was stopped during the night period. The whole experiment lasted for 183 days, or six months from 29 September 2017, (*t0*) to 31 March 2018 (*t6*). Experiment length enabled us to (i) track the changes in soil C immediately after seedling emergence (ii) avoid the effect of plant leaf and flower litter on soil C, which was not our study objective. Any plant litter was removed manually every 2 weeks from the soil surface.

3.2.2. Soil fractionation and assessment of soil carbon and $\delta^{13}C$

Before the experiment, three soil samples per soil type were sampled for the measurement of carbon content in different fractions, mean weight diameter of aggregates, nitrogen content, microbial activity, DNA concentration and Shannon metabolic diversity at *t0*. Each sample was mixed and divided into four parts, and an equal amount of soil from each part was collected and mixed to obtain a homogenized sample of soil.

At *t6*, soil was removed from each pot, weighed and then cut into two equal-size half blocks with a saw and a ruler: one half was air dried and used for soil analyses and the other half was used for plant trait and microbiological measurements. A mixed sample from each pot was collected from a depth of 3.5-10 cm depth. The soil samples at t0 and t6 were then sieved at 2 mm and 40g were sub-sampled and fractioned using the Gavinelli et al. (1995) method (Supplementary material, Method S1, Fig. S1). The resulting four fractions (POM: >200µm, finePOM: 200-50µm, SILT: 50-20 µm, SILT+CLAY: <20µm) were analyzed for both C content and $\delta^{13}C$ using an elemental analyzer Isoprime100 coupled with an Elementar Varo Isotope Cube (machine reference no). The sum of C in different fractions represents the total C in the sample. A subsample of 0.1 g was taken from each 40 g sample and analyzed without fractioning to determine the total C in the bulk sample. We checked the accordance between the mean difference between total C in bulk soil and the sum of C in the different soil fractions and the results were satisfactory (mean 93.3% of recovery).

To assess the changes in total C in each fraction, the differences between C content at t0 and t6 were assessed:

$$\Delta C_x = \Delta C_{x,t6} - \Delta C_{x,t0} \qquad [1]$$

Where ΔC_X is the change in C content (mg C g^{-1} soil) in a given soil C pool. All the raw data can be found in the Harvard Dataverse 'Embankment as a carbon sink: a study on carbon sequestration pathways and mechanisms in topsoil and exposed subsoil', DOI: 10.7910/DVN/QTFLVE.

Every Fractionation method present some advantages and drawbacks. We chose the Gavinelli et al. (1995) methodology for several reasons: it allows to reducing the shaking time of the sample to avoid transfer of C from the sand to the clay fraction, an important factor given the sandy texture of the soil used in this experiment (Gavinelli et al. 1995). The soil sieving at 2mm prior to fractionation decrease the amount of unprotected POM, up to 50% (Duddigan et al., 2019). However, this problem is particularly relevant for soils having a high C content and high organic fertilization; the soil used in this experiment have low C content and no fertilization, therefore the issue is sensibly reduced (Duddigan et al., 2019). The use of an aliquot to assess the silt-clay fraction allowed to less time consuming and more economical procedure, a key element when a high number of samples need to be fractionated. It is possible, however, using a wet sieving fractionation method, that part of the OM was transferred in the SILT+CLAY fraction, overestimating its C content (Duddigan et al., 2019). However, the fractionation has been performed by the same operator using the same protocol at time 0 and time 37, therefore the overestimation should be considered a standard error that does not affect the difference in C between the two sampling times. Since the separation of POM in the Gavinelli et al. (2005) methodology via density fractionation happens after soaking soil with exametaphosfate and 2 hours shaking with agate balls, the POM fraction includes also the POM occluded in macroaggregates. However, this methodology allows assessing the amount of C protected in microaggregates after their breaking via sonication, and this fraction is considered the most important for physically protected C (Kong et al., 2005; Six and Paustian, 2014).

3.2.3. Estimation of new and old carbon in soil fractions

The increased atmospheric $\delta^{13}C$ signature in the microcosm allowed a calculation of the proportions of new C in the different soil fractions. We used an isotope mixing model (Balesdent and Mariotti, 1996):

$$\%NewC = \frac{\delta(t6)-\delta(t0)}{\delta B-\delta(t0)} \qquad [2]$$

Where *%New C* is the percentage of new C in a specific fraction, $\delta(t6)$ is the $\delta^{13}C$ signature of C measured in a specific fraction at *t6*, δ(t0) is the $\delta^{13}C$ signature of C of a specific soil fraction t0, δB is the $\delta^{13}C$ signature of the new C input in the system (in our case the signature of the absorptive and transport root biomass). The δB was specific for each pot based on the analysis of the root biomass, and the mean was 615±38. The choice of root biomass as the $\delta^{13}C$ reference for C input was made because root material was considered to be the main input of C, given that shoot litter was negligible, and comparable with exudates signature.

The new C at t0 was zero. To calculate the gain of new C (mgC g^{-1} soil) in a specific soil C pool X, we multiplied %Cnew by the total amount of C at *t6* (C_X) of the pool *X*:

$$\Delta NewC_X = C_X(t6) \times \%NewC \qquad [3]$$

To assess the changes in the old C in different soil C pools, we subtracted the new C from the $\Delta C$of each soil C pool.

$$\Delta OldC_X = \Delta C_X - \Delta NewC_X \qquad [4]$$

The isotope labeling approach was chosen since it allows following the path of C when the desired C input is ‘traced’ with an increased (or decreased) C isotope ratio (Staddon, 2004; Hungate, 1996). The different 13C levels, compared with the natural abundance and input 13C signal, allow following the carbon from the atmosphere to the different plant systems, the trophic transfer via microorganisms, to end up in soil or in respired carbon (Leake et al., 2006; Bradford et al., 2007; Högberg et al., 2008). We chose stable labeling methodology since pulse labeling, even if more economically convenient and easier to implement than stable labeling, cannot achieve a uniform labeling distribution in the system as stable labeling does, and this can result in a bias results analysis (Meharg, 1994). Stable isotope labeling has been extensively used to trace respired $CO_2$and understand its origins, separating root respiration from microbiological respiration of old or new carbon (Rygiewicz and Andersen, 1994; Kuzyakov et al., 2001; Trubore, 2006; McDowell et al., 2004; Subke et al., 2004; review from Hanson et al. 2004). However, different challenges can be encountered when using stable ^{13}C labelling in CO_2, more specifically the stability of the enrichment, possible leaking, and different photosynthetic efficiency and ^{13}C adsorption from plants. For this reason, the chambers have been open as fewer times as possible and only when the ^{13}C enrichment was not in place (in chambers 'night hours').

3.2.4. Microbial global metabolic activity (GAM) and Shannon metabolic diversity index (H)

To analyze functional diversity, precisely GAM and H from microbial communities, 20 g of soil were collected immediately after sampling from each container at t6 and t0 from the half of soil collected for chemical analyses. We used a Microresp system that comprises a Deepwell plate (Fisher Scientific E39199) holding soil subsamples saturated with a solution with different substrates, a detection plate containing the detection gel, a rubber seal to connect the deepwell and the detection plate and metal clamp to keep the two parts tightly together (MicroResp™, Aberdeen, UK) (Fig. S2). The output of Microresp is to assess the respiration rate of soil saturated (at 80% of field capacity) with different substrates presenting different levels of recalcitrance and biological properites. Detailed methodology is provided in supplementary materials, Method S2. Substrates utilized for MicroResp are shown in Table S1.

To have a proxy of the global metabolic activity (GAM) of the microbial communities, the respiration rates from the different 15 C substrates were summed (mg $C\text{-}CO_2\ g^{-1}$ soil h^{-1}; Frac et al. 2012, Ammar et al. 2017). For each replicate, a Shannon metabolic diversity index was calculated as:

$$H = -\sum_{i=1}^{15} \pi \times \log(\pi) \qquad [5]$$

Where π is the standardized respiration rate for substrate (i) (Shinan et al. 2017).

MicroResp was chosen since is a method that allows a convenient, sensitive, rapid methodology to determine microbial activity and functional diversity, assessing their substrate utilization (Campbell et al. 2008). SIR, instead, allow only to assessing the potential activity with glucose. The method has been utilized in a wide range of soils and land uses, and always demonstrated its value (Chapman et al. 2007; Creamer et al. 2016; Shinnan at al. 2017).

However, MicroResp suffers from three main limitations according to Renault et al. (2013) that needs to be taken into account: first, the method consider the CO_2 in the well air space to be only of microbial origin (Campbell et al., 2003). The volume of the deep well that is not filled with soil is neglected and given the low volume of soil solution this can lead to overestimation of respired CO_2. Second, the increasing CO_2 in the air decrease the pH of the solution (Stumm and Morgan, 1996) and, again, could lead to overestimate the CO_2 in solution. Third, there was no check done to see if the transfer between calcite, soil solution, air and gel is in equilibrium, otherwise calibration would be impossible (Renault et al. 2013).

3.2.5. Microbial DNA concentration as proxy for microbial biomass

To examin microbial biomass in different communities, 10g of soil was immediately frozen at -20°C after sampling, and kept until samples were processed for DNA extraction. Total DNA was extracted from soil (0.5 g). DNA extraction was performed using FastDNA® SPIN Kit for Soil Isolation Kit according to manufacturer’s instructions (MP Biomedicals, USA). An additional step to wash the DNA binding matrix with 500 µl of guanidine thiocyanate 5.5M was added following Tournier et al. (2015). The concentration of extracted DNA in solution (ng/µL) were measured using a Quant-iT™ PicoGreen™ dsDNA Assay Kit for DNA quantification and used as a proxy for microbial biomass (Bohórquez et al. 2017).

The main drawback of using DNA concentration as a proxy of microbial biomass is that this methodology does not make the distinction between intracellular DNA of live microbial cells, dead microbial cells, and extracellular DNA released via cellular lysis (Torti et al., 2015). This bias can result in an overestimation of microbial biomass. However, the aim of this analysis is not to precisely estimate microbial biomass (for which other methodologies are more suitable) but to compare the changes in biomass. As a general comparative biomass growth indicator, this methodology has proven to be valid and easy to implement (Bohórquez et al., 2017).

3.2.6. Percentage of fine fraction in soil, soil nitrogen and aggregate stability

After the wet sieving and weighing of the different soil fractions, the percentage of fine fraction (FF, in %) was determined as the ratio of the SILT+CLAY soil fraction weight (<20 µm) and the total mass of the fractioned soil sample (in average 40g). When analyzing C content and δ^{13}C for each bulk soil fraction, the amount of nitrogen in soil (N; mg g^{-1} soil) was also determined.

As a proxy for aggregate stability, mean weight diameter (MWD) of aggregates was assessed following the conventional methodology according to Le Bissonnais et al. (2006). 20g of aggregates were collected from the half pot for soil analysis, air dried and sieved first at 5 mm and then at 3 mm, to isolate the 3 - 5mm aggregates fractions. This aggregate fraction were put in the oven for 24h to reach the same water matrix potential. First, 5g of 3-5mm fraction are weighed and gently immerse in a 250 cm^3 beaker filled with 50 cm^3 of ethanol for 10 minutes. After ethanol was carefully sucked off with a pipette and the sample transferred in a 250 cm^3 Erlenmayer flask containing 50 cm^3 of deionized water, and brought to 200 cm^3. The flask was agitated 20 times and left 30 minutes for sedimentation of coarse particles. Water was sucked off with a pipette and the mixture of soil and water transferred to a 50 µm sieve previously immersed in ethanol. The disaggregated soil was sieved gently by moving 5 times the sieve in the ethanol to separate the <50 µm soil fraction from the >50 µm. The >50 µm aggregate fraction was collected from the 50-µm sieve, oven-dried and gently dry-sieved by hand on a column of six sieves: 2000, 1000, 500, 200, 100 and 50 µm. The mass percentage of each aggregate fraction was calculated, and by subtracting the mass of soil collected on sieves from the initial mass analyzed mass we calculated even the <50µm fraction. MWD is calculated as the sum of the mass fraction of soil remaining on each sieve after sieving multiplied by the mean aperture of the adjacent mesh:

$$MWD = \frac{\sum_{f>2mm}^{f0.05-1mm}(Af*df)}{100} \qquad [6]$$

Where *Af* is the aggregate fraction abundance in % of the total weight of the analyzed sample remaining in a specific *f* aggregate fraction (f = >2mm, 1-2mm, 0.5-1mm, 0.2-0.5mm, 0.1-0.2mm, and 0.05-0.01mm), and *df* the diameter in mm of the smaller sieve characterizing the lower boundary of the *f* aggregate fraction.

To assess aggregate stability we chose Le Bissonais (1996) methodology since it unifies different previously utilized methodologies that sum up the main aggregate disruption processes and it is applicable to a wide range of soils (le Bissonais et al., 1996). This methods allow to simulate the main mechanisms for aggregate disruption: slacking due to compression of entrapped air during wetting, 2) micro cracking originating from swelling, 3) breaking due to mechanical action and 4) dispersion due to osmotic stress (Rohošková and Valla, 2004). Being one of the most complete and comprehensive tests for aggregate stability assessment it it is widely used, however it present a major drawback: it might overestimate MWD due to the fact that sand particles are not removed from its calculation (Rohošková and Valla, 2004)

3.2.7. Root traits

For each species, three out of the six plants in each pot were sampled and their root system carefully washed and separated from the aboveground part. Roots were water-rinsed in a plate container. A 0.1 g composite subsample of roots was cut off from several parts of the root systems with scissors. After being carefully washed, they were stained with a solution of methyl violet (0.5 g L^{-1}). Following

McCormack *et al.* (2015), we visually separated transporting (long, thick, high-order roots (>3) and absorptive roots (short, thin, low-order roots 1 – 3). Both types of roots were separately extended over a transparent water filled tray and scanned at 800 dpi (Epson® perfection V700 PHOTO, Canada). The software Winrhizo Pro® (Regent Instruments, Quebec, Canada) was used to determine root length (L, in mm) per diamet”er class stretching from 0 to 2 mm with a 0.1 mm interval. . Winrhizo Pro® also provides the mean diameter of the analyzed root sample (Prieto et al., 2016). We then calculated the mean diameter of adsorptive roots (D_{ad}), for in each soil and species type.

Specific root length (SRL, m g^{-1}) was calculated as the ratio between root length and dry root mass (Esseinstat, 1992). Only absorptive root data were then used in the data analysis as they are most short-lived and active in exudation, thus should be the main contributor of new C deposition to soil.

Following the same sampling and sorting manner, another sample of 0.1 g absorptive roots from the same plants was obtained and then finely ground. The ground samples were analyzed with an elemental analyzer Isoprime100 coupled with an Elementar Varo Isotope Cube to determine root C and N contents and root δ^{13}C.

The remaining roots in the half pot were then oven dried at 60°C for three days and weighed to determine the total root dry mass (in g) for each pot

Finally the amount of new C moved into the SILT+CLAY pool by g of root was calculated by standardizing the $\Delta New\ C_{SILT+CLAY}$ for the g of dry root weight per gram of soil (DRW; g dry roots g^{-1} soil). Also the amount of new C moved into the SILT+CLAY pool by cm of root was calculated by standardizing the $\Delta New\ C_{SILT+CLAY}$ for the length of the root (L, cm of roots g^{-1} soil) per gram of soil. To calculate the root L, we multiplied SRL per the DRW.

3.2.8. Statistical analysis

One way analysis of variance (ANOVA) was performed to test the effects of plant and soil treatments on

1) C content: ΔC_X, $\Delta New\ C_X$ and $\Delta Old\ C_X$ for the C pool *X* and all pool summed (*SUM*)
2) C quality: $\%C_X$ each C pool *X*
3) Soil, root and microorganism feature indicators, including initial SOC stock, FF, soil N content, MWD, total root biomass, mean absorptive diameter, C:N ratio of absorptive roots, SRL of absorptive roots, concentration of extracted DNA in ml^{-1} of soil solution as proxy for microbial biomass, microbial activity (GMA) and metabolic community diversity (H) .

The normality of distribution of residues was verified using a Shapiro-Wilk test ($\alpha p = 0.05$). Principal component analysis (PCA) and Pearson's correlations factors were used to study the relationships between C sequestration indicators and soil type, root and microbial indicators. All the statistical analyses were performed using the open-source statistical environment 'R', version 3.4.3 (R Development Core Team, 2017) using the packages *vegan* and *Hmisc*. (Oksanen et al. 2019, Harrel 2007).

3.3. RESULTS

3.3.1. Changes in total soil carbon

In general, a decrease in mean total soil C content occurred after 6 months in subsoils (Fig. 1a,b). The mean negative ΔC in bare subsoil (-0.37±0.18 mgC g^{-1} soil) was not significantly different to that with either *L. perenne* (-0.38±0.11 mgC g^{-1} soil) or *M. sativa* (-0.17±0.25 mgC g^{-1} soil) present. Although the presence of vegetation did not significantly affect total C in subsoil (ANOVA, $p \geq 0.05$) (Figure 1b), mean ΔC had a significant increase in topsoil (ANOVA, $p < 0.05$), with the highest increase in C content under *M. sativa* (+0.68±0.36 mgC g^{-1} soil), followed by *L. perenne* (+0.1±0.51 mgC g^{-1} soil). In bare soil, ΔC was negative (-0.47±0.28 mgC g^{-1} soil) (Figure 4.1a,b). ΔC_{SUM} was significant lower under *M. sativa* subsoil compared to *M.* sativa topsoil (ANOVA, *p* <0.05), but no difference between soil types where observed under *L. perenne* (ANOVA, *p* ≥0.05) due to the high variability in data. There was no effect of soil type on ΔC_{SUM} in bare soil (ANOVA, *p* ≥0.05) (Fig. 1b).

Mean old C decreased in all treatments (Fig. 1a), although a slight positive ΔOld C could be occasionally found in some pots (Fig. 2a). In topsoil, the increase in new C was sufficient to compensate for the loss of old C, but it was not the case in subsoil, where ΔNew C was less than the ΔOld C (Figure 1a). As expected, bare soil had a negligible input of new C, while topsoil under *M. sativa* had both the smallest lower negative ΔOld C and the highest positive ΔNew C. Data were highly variable with regard to negative ΔOld C in topsoil under *L. perenne* (Fig. 2a). The effect of species, ΔOld C and ΔNew C were less pronounced in subsoil than in topsoil. In vegetated soils, positive ΔNew C was accompanied by a smaller loss of old C (lower ΔOld C) (Fig. 2a). However, there were no consistent relationships between changes in old C and in new C in either soil type or plant species (Fig. 2a).

Over 6 months, the amount of active C (i.e., $|\Delta C_{New}| + |\Delta C_{Old}|$) represented 9.1% and 6.1% of the total C contents for subsoil and topsoil, respectively. The amount of active C in topsoil was 1.5 times higher than that in subsoil.

3.3.2. Changes in soil carbon in different soil C pools associated to soil fractions

In subsoil, $\Delta C_{finePOM}$ was significantly lower than in the other pools, (ANOVA, *p*=<0.05). The ΔC between pools was not significant for *M. sativa* and bare soil due to the high variability in data (Figure 3a). In topsoil, mean $\Delta C_{finePOM}$ was not significantly different with that in the ΔC_{POM} or ΔC_{SILT}. $\Delta C_{SILT+CLAY}$ was usually the highest among all the four C pools (Fig. 3a,b).

Regardless of soil type, plant species had a limited effect on $\Delta C_{finePOM}$ and ΔC_{SILT} (Figure 3a, b). In subsoil, plants increased ΔC_{POM} (Fig. 3a), but not in topsoil. However, in topsoil, plants increased $\Delta C_{SILT+CLAY}$

(1.28±0.63 mgC g^{-1} soil for *M. sativa*, 1.00 ± 0.44 mgC g^{-1} soil for *L. perenne*), compared to bare soil (0.90±0.29 mgC g^{-1} soil). In the remaining C pools, *ΔC* significantly decreased (Figure 3b).

The only significant difference in soil planted with either species, was the increase in $\Delta C_{SILT+CLAY}$ in the topsoil compared to subsoil (ANOVA, $p < 0.01$).

*Δ*New $C_{finePOM}$ and *Δ*New C_{SILT} changed negligibly with regard to soil type and plant species (Fig. 4a,c). Compared to bare soil, new C gain in planted subsoil soil was mainly located in the POM pool, while new C gain in planted topsoil was located in both POM and CLAY+SILT C pools (Fig. 4a,c). Soil under *M. sativa* had significantly higher *Δ*New C_{POM} and *Δ*New $C_{SILT+CLAY}$ pools compared to *L. perenne* (Fig. 4a,c).

The effect of soil type on old C was more accentuated compared to New C. In subsoil, neither C pool and plant species had any effect on old C loss, which was always negative (C depletion). In topsoil, instead, here was a positive accumulation for *Δ*Old $C_{SILT+CLAY}$, while this decreased in all the other pools which did not differ among each other (Fig. 4b,d). Plant species had no effect on old C loss in any C pool (ANOVA, $p \geq 0.05$).

3.3.3. Changes in carbon quality

M. sativa increases the % of C stored in the POM C pool over the total amount of C in the soil thanks to high input of new C, while *L. perenne* and bare soil decreased the % of C in this pool (Fig. 5a). The % of C stored in the finePOM and SILT pools decreased over at t6, with negligible inputs of new C (Fig. 5b,c). Finally, every treatment increased the % of C stored in the SILT+CLAY pool over the 6 months compared to t0 (Fig. 5d).

3.3.4. Root, soil and microbial characteristics

Root biomass of both plant species was significantly higher in topsoil (*M. sativa* 17.53±2.03 g pot^{-1}, *L. perenne* 4.09±1.43 g pot^{-1}) than in subsoil (*M. sativa* 5.13±1.36 g pot^{-1}, *L. perenne* 1.05±1.36 g pot^{-1})

(Tables 1, 2). In *M. sativa* soil type did not influence mean SRL, diameter or C:N ratio of absorptive roots. In *L. perenne*, greater SRL (topsoil +11.97 m g^{-1}) and C:N ratio (+39.30) in subsoil was found compared to topsoil (Tables 1, 2). In *L. perenne*, absorptive roots were thinner in subsoil compared to topsoil (-0.01 mm; Tables 1, 2). *M. sativa* had greater root biomass (topsoil +13.44 g pot-1, subsoil +4.08 g pot-1) and mean diameter, but lower SRL and C:N ratio than *L. perenne,* in both soils (Tables 1, 2). In topsoil, GMA, H and DNA mass were all significantly greater compared to subsoil once plants had grown. In bare soil, GMA did not significantly differ among topsoil and subsoil (Tables 1, 2). In topsoil, the presence of both species significantly increased GMA (*M. sativa* +10.39±4.63 µgC-CO_2 g^{-1} soil h^{-1}, *L. perenne* +3.01±2.19 µgC-CO_2 g^{-1} soil h^{-1}) and DNA concentration (*M. sativa* +5.33±5.29 µgC-CO_2 g^{-1} soil h^{-1}, *L. perenne* +4.73±6.37 µgC-CO_2 g^{-1} soil h^{-1}) that did not differ between treatments (Tables 1, 2). In subsoil, GMA decreased over the 6 months (*M. sativa* -1.59±1.53 µgC-CO_2 g^{-1} soil h^{-}1, *L. perenne* -0.16±2.73 µgC-CO_2 g^{-1} soil h^{-}1) while DNA concentration increased (*L. perenne* +2.00±1.41 ng μL^{-1}; *M. sativa* 4.20±1.64 ng μL^{-1}), and they did not differ between plant species (Table 2). In subsoil H was significantly higher in soil planted with either species compared to bare soil (Tables 1, 2).

Soil type significantly influenced the soil structural and characteristics and N content: topsoil had a higher MWD and N content regardless of plant species (Table 1, 2). The fine fraction (FF) was higher in subsoil compared to topsoil (Tables 1, 2). There was a significant increase in MWD with both the species in topsoil (*M. sativa* +0.52±0.29 mm, *L. perenne* +0.62±0.20 mm), while in subsoil MWD was not significantly different from t0 or between treatment (Table 1, 2). Soil N and FF were not significantly different among treatments (Tables 1 and 2) and soil N was depleted during the 6 months, with subsoil showing an homogeneous depletion among treatments (-0.1±0.8 mgN g^{-1} soil) while in subsoil *L. perenne* had higher decrease in soil N (-0.13±0.12 mgN g^{-1} soil) compared to bare soil and *M. sativa*. In subsoil, the presence of vegetation did not influence any of the soil characteristics.

3.3.5. *Relationship between changes in new C and old C and soil, microorganism and root variables*

The PCA conducted on the ΔNew C and ΔOld C in the different soil C pools, root traits, DNA mass, H and GMA, and soil structural characteristics explained 83.9% of the total variance (Fig. 6). The first PCA axis (horizontal) accounted for 63.3% of the variation. On the negative end, results were governed by the ΔNew C_{POM}, ΔNew $C_{finePOM}$ and ΔNew $C_{SILT+CLAY}$, and ΔOld $C_{finePOM}$. At the positive end, results were driven by total ΔOld C_{SUM} and ΔOld $C_{SILT+CLAY}$, while the remaining new C and old C pools were orthogonal and more related to the second PCA axis (vertical), that accounted for 20.6%. Microbial traits (GMA, DNA and H), MWD, soil N and root biomass, went all along the first axis (negative). Root traits linked with recalcitrance (C:N ratio and SRL) and fine fraction percentage FF went along the 1st axis (positive) together with ΔOld C_{SUM} and ΔOld $C_{SILT+CLAY}$.

The PCA strongly discriminated top- and subsoil at the two extremes of the first axis, with topsoil on the negative end of the first axis, characterized by high GAM, H and DNA concentration, high MWD, soil N and root biomass, correlated with ΔNew C and ΔOld $C_{finePOM}$. Subsoil was on the positive end of the first axis, with FF, root C:N and SRL, suggesting a loss of ΔOld C_{SUM} and ΔOld $C_{SILT+CLAY}$. Species were discriminated mostly by the second axis, with *L. perenne* on the positive end of the axis together with higher C:N ratio, SRL, and ΔOld C_{POM} and *M. sativa* on the negative end, with high root biomass and diameter of absorptive roots, illustrating a positive ΔNew C_{POM} pool and the ΔNew C_{SUM}, and negative ΔOld C_{SILT} and ΔOld $C_{SILT+CLAY.}$

The gain in new C, regardless of total new C, or within each soil C pool, was better related to every analyzed variable than the loss of old C (Table 3). The gain in ΔNew C in every C pool was positively correlated with microbial traits (GMA, H and DNA), except for ΔNew C_{POM} and *H* (Table 3). ΔNew $C_{POM,}$ ΔNew $C_{SILT+CLAY,}$ and ΔNew C_{SUM} were significantly and negatively correlated with SRL and C:N ratio of absorptive roots. Apart from ΔNew C_{POM}, the gain in new C in every soil fraction was positively correlated with MWD and soil N content (Table 3), but negatively correlated with FF. The negative ΔOld C was significantly and positively correlated with H in ΔOld C_{POM} and with GMA in ΔOld $C_{finePOM}$, but was negatively correlated with every microbial trait (GAM, H, DNA concentration) in ΔOld $C_{fSILT+CLAY}$ and ΔOld C_{SUM}. Root variables were poor predictors of total old C losses, except for root biomass, which was positively correlated with the loss in ΔOld $C_{finePOM}$ and negatively with that in ΔOld $C_{SILT+CLAY}$. Soil variables were all correlated with the losses in ΔOld $C_{SILT+CLAY}$ and ΔOldC_{SUM} demonstrating an opposing pattern compared with correlations with the gain in new C. The losses in ΔOld $C_{finePOM}$ and in ΔOld C_{SILT} were poorly correlated with most of the variables.

Finally, when standardized by dry root weight, ΔNew $C_{SILT+CLAY}$ in subsoil planted with either *M. sativa and L. perenne* was different than that found in topsoil, but the difference was not significant (Fig. S5a; ANOVA, $p > 0.05$). When the ΔNew $C_{SILT+CLAY}$ was standardized for every cm of root, no differences could be observed between top- and subsoil planted with either species (Fig. S5b; ANOVA, $p > 0.05$).

3.4. DISCUSSION

Soil, with its biotic and abiotic characteristics, had the highest influence on C sequestration. New C accumulation, old C and total C changes in terms of both absolute (C quantity) and relative (C quality) values significantly differ among soil C pools, thus validating our Hypothesis 1. The most reactive pools were POM and SILT+CLAY for new C accumulation. We examined the correlations between new C and old C for total soil C and each C pool and found synergetic patterns in a generally consistent manner, thus rejecting our Hypothesis 2. Finally, we showed that new C and old C changes could be partially explained by multiple soil, microorganism and root variables despite their disparities in drivers, validating our Hypothesis 3. In general, the main drivers for C storage were N content and microbial activity, which influenced soil quality. Root biomass development was the third driver showing high correlations with new C storage in soil fractions, but subdued to soil characteristics. We did not observe a positive effect of lower C saturation on C storage in SILT+CLAY in subsoil, due to the lower biomass development and microbial activity, resulting in a lower new C input. Regarding the applied aspect, we found topsoil had relatively higher new C gain and lower old C loss compared to subsoil and *M. sativa* had a better performance in gain of new C and limit of old C loss than *L. perenne*, although such an effect of species was moderated by soil type. Understanding and assessing the choice of plant and soil on C sequestration will help shape practical guidelines in revegetation and restoration programs of geotechnical systems, notably road embankments.

3.4.1. Importance of differentiating soil carbon origin and pools (Hypothesis 1)

Here, we clearly confirm the importance of disentangling the C fates of different origins and pools. Taking the vegetated topsoil as an example, we found that the increase in total C after six months was mainly attributed to a high input of new C in POM and to the high increase of new and old C in the most stable SILT+CLAY pool. This result is in line with previous studies on either C origins (De Deyn et al., 2008; Cotrufo et al., 2013; Liang et al., 2017; Vidal et al., 2018) or C quality by taking into account C pools (O'Brien and Jastrow, 2013; Cardinael et al., 2015; Saenger et al., 2015; Chapter II this thesis).

In topsoil, the total old C change was close to zero, but was actually an offset between an active and high gain in old C in the SILT+CLAY pool and an active and high loss in old C in the POM pool. As the fate of old C was not estimated in the different C pools, it is possible to wrongly diagnose that old C was little active during the whole revegetation process.

The correlations between total ΔC and diverse soil, root and microbial characteristics, did not reveal the relationships in most of the C pools. (e.g. Cardinale et al., 2015, Rossi et al., submitted). Being able to separate new C and old C fluxes thanks to isotopic enrichment have proved fundamental to investigate correlations that are hidden when considering the total ΔC as the sum of new and old C changes in the system. Jointly considering C origins and pools enabled us to better depict the pathways of C flux from plant roots to soil and among soil C pools. We found that, once soil was vegetated (either topsoil or subsoil), the increase in total soil new C was mainly due to the increase in the least stable POM pool. This result confirms the key role of plant roots in supply of C to the POM pool via root turnover, and is in line with results by e.g. De Deyn et al. (2008), Cotrufo et al. (2013) and Rees et al. (2005). Surprisingly, we found a high $\delta^{13}C$ signal in the SILT+CLAY pool for both soil types, which corresponds to a minor, but non-negligible amount of new C supply into the most stable pool. Given that this phenomenon is more pronounced for *M. sativa* (N_2-fixing species, lower tissue recalcitrance due to lower C:N ratio) than for *L. perenne* (non N_2-fixing species, higher tissue recalcitrance due to higher C:N ratio), we may partially attribute this phenomenon to the higher mineralization rate of the POM pool, that supplies the SILT+CLAY pool. However, in this case, we argue that POM is not the only cause of the new C increase in the SILT+CLAY pool, as POM, consisting of plant residues rich in cellulous and lignin, has a mean residence time much higher than six months, i.e., the experiment duration (Cotrufo et al., 2015). Instead, it would be more likely that such new C increase in the SILT+CLAY pool in the short term be a consequence of the higher microbial proliferation and activity induced by a higher root exudation / microbial symbiosis with *Rhizobium* in *M. sativa* (Cotrufo et al., 2015). Such a mechanism is incorporated as a part of the entombing effect in the recent “Soil Microbial Pump” hypothesis (Liang et al. 2017). As an alternative pathway to the routinely characterized ex vivo C flux from plant tissue to soil C pools via decomposition, the entombing effect refers to the in vivo C flux from triggered microbial necromass and metabolites to the very stable soil C pools (Liang et al. 2017). Although the estimation of microbial necromass was not available in this study and still remains a technical bottleneck (Liang et al. 2019), we may expect a higher level of microbial necromass due to the observed high GAM, H, DNA indicators in *M. sativa*, compared to *L. perenne* and bare soil. Accordingly, our observed new C enhancement in both POM and the very stable SILT+CLAY C pools in our experiment could be considered as novel data supporting the importance of the entombing effect.

3.4.2. Generally a strong synergy exists between new and old carbon (Hypothesis 2)

We found that soil that gained new C usually had a significantly smaller loss in old C. In topsoil sowed with *M. sativa*, that had more new C input, , more labile tissues (low C:N content) and higher microbial activity, old C loss in soil was much less than that in the vegetated subsoil treatment. This result is in accordance with substrate utilization hypothesis developed by Cheng and Kuzyakov (2005) and observed in an incubation experiment by De Graaf et al. (2010). However, to our knowledge, this is the first time we observed this mechanism in an *in vivo* experiment. According to this hypothesis, microorganisms prefer labile C to stable C, thus resulting in a limited consumption of old C, especially old C protected by fine soil particles. This mechanism is observed in soils with high fertility and mineral nutrients, and when the input of fresh new C is adequate, which is our case in the topsoil treatment. However, when mineral nutrients are low and fresh C input is low and insufficient to switch substrate utilization preference, the low input of C increases the activity of microbes, that augment the consumption of old C (Cheng and Kuzyakov 2005; De Graaf et al. 2010), as in our subsoil treatment. In addition to the preexisting hypothesis, due to the use of fractionation, we can argue that the entombing effect in the soil microbial pump hypothesis (Liang et al., 2017) can expand the comprehension of the synergetic pattern. We observed that the synergetic pattern between new C and old C changes was largely due to the same pattern existing in the SILT+CLAY C pool that received more than 50% of total soil C. Due to the entombing effect, the maintenance of old C content against old C loss in the SILT+CLAY pool may be a consequence of increased microbial biomass relying on the old C resource that consumes the old C in unprotected coarser fractions and transfers to the SILT+CLAY fraction via entombing of microbial exudates, exopolysaccharides and necromass (Cotrufo et al. 2013; Liang et al. 2017; Vidal et al., 2018). Accordingly, soil with a greater microbial biomass (in our study, topsoil) may have more advantages to maintain the size of the stable C pool via entombing effect (necromass, microbial exudates and exopolysaccharides). Such a kind of increase in C due to microbial necromass based old C should not be considered a part of old C. However, to what extent the increase in microbial necromass relies on old C and new C is unknown, hindering the validity of the speculation. Overall, understanding the role of microbial necromass and its underlying mechanism is an important scientific lacuna in soil ecology to explore in the future.

3.4.3. Root traits influence new carbon gain and old carbon changes, and are strongly mediated by soil variables (Hypothesis 3)

The two plant species that we examined had contrasting functional root traits, i.e., SRL, diameter of absorptive roots and C:N ratio that were negatively correlated with the gain of new C in the POM pool, and with new C in the SILT+CLAY C pool, however not with high significance. While this finding may be possibly due to the short-term experiment in which species impact is not yet fully exerted, it could also be attributed to the nature of these traits. Functional traits such as C:N ratio, diameter of absorptive roots, and SRL are classified as morpho-physio-phenological (MPP) traits according to Violle et al., (2007), and the impact of these functional traits can be compensated by the effect of biomass, i.e., a performance trait (Violle et al., 2007). In agreement with this hypothesis, we found root biomass a much better predictor of new C gain in every soil C pool compared to the C:N ratio and SRL.

We found that the effect of species on new C gain is much less pronounced in the subsoil than in the topsoil treatments, although the disparity of trait values between the two species in subsoil was still very clear. This result suggests that the effect of root traits on C sequestration is strongly mediated by soil characteristics. In the previous studies working on similar subjects soil treatment was usually excluded (Roumet et al., 2016; Henneron et al., 2019; Rossi et al. submitted, Chapter II, this thesis). In this study, we used two soil types that were similar in granulometric texture, but greatly differed in physical, chemical and biological qualities. Topsoil had greater initial C and N contents, aggregate stability and soil biodiversity than subsoil, suggesting that better soil quality is a primordial factor in influencing plant performance in C sequestration.

Compared to new C, Δold C were generally much less sensitive to plant traits, including MPP traits e.g., C:N ratio, SRL and mean diameter and biomass. This result suggests that Δold C does not share the same mechanism with Δnew C and was less dependent on ex vivo C flux from plants. Compared to the MPP traits, root biomass was a slightly better predictor of Δold C . This result can also be explained by the preferential substrate utilization hypothesis (Cheng and Kuzyakov, 2005) and the boosting effect of root biomass on microbial proliferation and activity (Fontaine and Barot 2005, De Deyn et al. 2008).

Microbial communities may prefer consuming new C to old C, resulting in better maintenance of old C.

M. sativa, as a N_2 fixing species increases microbial activity via symbiosis with *Rhizobium* bacteria (Poirer *et al.* 2018), augmenting microbial exudation and input of exopolysaccharides in the SILT+CLAY protected pool (Fehrmann and Weaver, 1978; Downie, 2010; Cotrufo et al., 2013). The increased biomass of *M. sativa* and its lower C:N ratio (due to its N fixing ability) increases the labile C input in soil (Warembourg *et al.* 2003; Roumet *et al.* 2005; Hernández *et al.* 2017) again increasing mineralization and deposition in the SILT+CLAY protected pool. Being able to differentiate fluxes of old C and new C in soil allowed to observe the increased input of new C from N_2-fixing *M. sativa*. This higher input, when analyzing the total ΔC, was hidden by the changes in old C that were soil dependent and not species dependent. This result helps to explain why different studies have discrepant results regarding the C storage from N_2 fixing and non N_2 fixing species, where not always N_2 fixing species significantly increased ΔC compared to non N_2-fixing species (e.g. Binkley, 2005; Fornara and Tilman, 2008; Chapter II, this thesis). The higher input of new C that N_2 fixing species provided due to a higher root biomass, lower C:N ratio and fastest growth, might have been hidden by soil dependent old C changes.

Overall, our finding highlights the necessity of studying the effect of functional traits on C sequestration in a more refined manner (i) differentiating soil C origins and pools for a given soil enables us to better identify soil C flux pathways that are more susceptible to vegetation; (ii) including the effect of soil type can allow us to determine the magnitude of influence of plant trait disparities so as to take into account more complex effects of interaction between soil features and root traits in future experimental design.

3.4.4. Microbiological activity can explain the disparity in new C and old C changes between topsoil and subsoil

The soil C saturation theory states that a soil with lower amount of C in the fine SILT+CLAY particle fraction has a higher potential for organomineral interactions and the derived C storage in the fine SILT+CLAY C pool (Six et al 2002). Given the lower initial C content in the SILT+CLAY pool at t0 and the significant slightly higher fine fraction ratio in subsoil, we expected a faster increase in new C in the SILT+CLAY pool given the same amount of C input from biomass. Our results support the C saturation theory to a certain extent, as the increase in new C in the SILT+CLAY per unit root biomass or length in subsoil was slightly higher than that in topsoil but not significant (Fig. S5). This difference was disproportionally less than the difference in initial C content between two soil types. We argue that the fine fraction abundance and soil C saturation can have a positive influence on C stored via organomineral interactions if other conditions, especially soil microbiological conditions are previously met. Recent studies have highlighted the importance of considering the robustness of soil microbial diversity as a soil quality indicator (Bouchez et al., 2016; Karimi et al., 2017), thus challenging the conventional use of only physical and chemical soil quality indicators. In this study, we have shown that microorganisms play a central role in the gain of new C and loss of old C in the SILT+CLAY pool. The higher microbial activity and diversity in topsoil from t0 to t6 may compensate the less favorable physical and chemical quality (lower fine fraction and higher initial C content) for C sequestration. Therefore, if microbial communities are not considered in the prediction of soil C sequestration, results will be flawed.

Along with greater microbial diversity, an increase in aggregate stability (MWD) and N content in topsoil could also promote a synergetic effect that augments C sequestration. The physical protective role of aggregates for C stock is widely documented (Hassink et al., 1992; Six et al., 2002; Chevallier et al. 2004; Rasse et al. 2005; O'Brien et al., 2013; King et al., 2019). A high soil N content in topsoil will also improve plant development and subsequent biomass, thus affecting C input and microbial diversity. We suggest therefore, that a comprehensive indicator of soil health for plant performance and C sequestration should incorporate physical, chemical and microbiological characteristics.

3.4.5. Practical applications

This study provides useful implications for future engineers to choose appropriate soil and species in road embankment revegetation to favor C sequestration. First, with the dominant effect over months of soil over species found in this study, choosing healthy and functional soil is of primary importance for C sequestration. Topsoil has shown a clearly better performance in C sequestration than subsoil. However, implementing topsoil over large scales is unrealistic when revegetating a site, because the amount of topsoil is relatively limited and over-exploitation of topsoil may further provoke environmental issues for the location where the topsoil is removed. Although subsoil has higher C sequestration potential due to its lower initial C content, attention should be paid to the microbial diversity and functioning in subsoil. Inoculation of soil with suitable microbial communities and fertilizer would therefore be necessary to favor both revegetation and soil C sequestration (Dou et al. 2016, Guo et al., 2019).

Once soil quality is ensured, choosing appropriate species will be a bonus for boosting new C input and protecting old C against priming. In our experiment that lasted 6 months, and so corresponds to the initial planting stage in the field, *M. sativa* had a better performance than *L. perenne*, and also enhanced soil aggregate stability, thus decreasing soil erodibility. However, the long-term effects of revegetation on long-term soil C fates should also be investigated.

3.5. CONCLUSIONS

We designed an experiment with fully crossed treatments between vegetation and soil in microcosms and used stable isotopic (^{13}C) labelling to assess new C input and old C changes in the soil system. We revealed the distinct fates of new C and old C in soil, in both absolute values and relative values, among different soil C pools related to soil fractions, highlighting:

- The major influence of soil, with the topsoil treatment having a higher C storage capacity compared to the subsoil treatment due to higher soil quality that increase biomass development and C input in POM C pool, and higher microbial biomass and activity that favors entombing of C in the stable SILT+CLAY pool.
- We evidence the necessity of considering both C fluxes in pools associated to soil fractions and origin of C (new and old C) when studying C dynamics in soil. An example being old C decreasing in the POM C pool and increasing in the SILT+CLAY C pool in the topsoil treatment. If only ΔC or Δold C in bulk soil was considered no changes would have been observed, and the old C would have been considered inactive, masking the real mechanisms behind soil C sequestration in the topsoil treatment.
- New C increased not only in the POM C pool, but also in the more stable SILT+CLAY pool. Given the short duration of the experiment, this flux is probably due to entombing of microbial necromass and microbial exudates and exopolysaccharides more than degradation of POM.
- New C and old C covaried similarly in the SILT+CLAY C pool. A higher increase of new C resulted in a lower decrease of old C due to a microbiological switch of substrate preference.
- Changes in new and old C differed depending on plant and soil characteristics. N_2 fixing *M. sativa* higher root biomass labile input in soil increased the amount of new C in soil. N_2 fixing *M. sativa* also increased microbial biomass and activity that favor the mineralization of C from exudates or POM and transport into the SILT+CLAY protected fraction. Root biomass was the trait better correlated with new C input in soil C pools.

- The lack of microbiological activity and the lower root biomass decreased the transfer of new C in the SILT+CLAY pool in the subsoil treatment. For this reason, the lower C saturation did not increase the total new C content in SILT+CLAY pool in the subsoil treatment as expected. When normalized for the root biomass, however, the system showed the opposite behavior, and the subsoil treatment had a higher amount of new C stored in SILT+CLAY for g of root. We argue that C saturation effect might be present but is subdued to soil fertility and microbiological activity.

Such a fundamental understanding of plant-soil interactions may help us to better optimize soil and vegetation management for road embankment revegetation. Long-term observations are now needed for a better assessment of the roles of plant and soil characteristics in soil C cycling and long-term sequestration.

REFERENCES

Balesdent, J. & Mariotti, A. 1996. Measurement of soil organic matter turnover using I3C natural abundances. In: Mass Spectrometry of Soils (eds T.W. Boutton & S. Yamasaki), pp. 83-1 11. Marcel Dekker Inc., New York.

Bardgett, R.D., Mommer, L., Vries, F.T. De., 2014. Going underground : root traits as drivers of ecosystem processes. Trends in Ecology & Evolution 29, 692–699.

Beare, M.H., Lawrence-Smith, E.J., Curtin, D., Kelliher, F.M., 2016. Potential use of full inversion tillage during pasture renewal to increase soil carbon storage in New Zealand. Ministry for Primary Industries - Soil C Workshop.

Besnard, E., Chenu, C., Balesdent, J., Puget, P., Arrouays, D., 1996 Fate of particulate organic matter in soil aggregates during cultivation. Eur. J. Soil Sci. 47, 495–503.

Binkley, D., 2005. How nitrogen-fixing trees change soil carbon. In: Binkley, D., Menyailo,O. (Eds.), Tree Species Effects on Soils: Implications for Global Change. NATO Science Series. Kluwer Academic Publishers, Dordrecht, pp. 155–164.

Blagodatskaya, E., Kuzyakov, Y., 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: Critical review. Biology and Fertility of Soils 45, 115–131. doi:10.1007/s00374-008-0334-y

Bohórquez, J., Mcgenity, T.J., Papaspyrou, S., García-robledo, E., 2017. Different types of diatom-derived extracellular polymeric substances drive changes in heterotrophic bacterial communities from intertidal sediments 8. Frontiers in Microbiology 2017; 8 - 245.

Bouchez, T., Blieux, A.L., Dequiedt, S., Domaizon, I., Dufresne, A., Ferreira, S., Godon, J.J., Hellal, J., Joulian, C., Quaiser, A. Martin-Laurent, F., (2016). Molecular microbiology methods for environmental diagnosis. Environmental Chemistry Letters, 14(4), pp.423-441.

Bradford M.A., Tordoff G.M., Black H.I.J, Cook R., Eggers T., Garnett M.H., Grayston S.J., Hutcheon K.A., Ineson P., Newington J.E. et al., 2007, ‘Carbon dynamics in a model grassland with functionally different soil communities’, Functional Ecology 21, 690–697.

Campbell, C.D., Chapman, S.J., Cameron, C.M., Davidson, M.S., Potts, J.M., 2003. A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. Applied and Environmental Microbiology 69, 3593–3599.

Campbell C.D., Cameron C.M., Bastias B.A., Chen C., Cairney J.W.G., 2008, Long term repeated burning in a wet sclerophyll forest reduces fun- gal and bacterial biomass and responses to carbon substrates. Soil Biol Biochem 40:2246–2252.

Cardinael, R., Chevallier, T., Barthès, B.G., Saby, N.P.A., Parent, T., Dupraz, C., Bernoux, M., Chenu, C., 2015. Geoderma Impact of alley cropping agroforestry on stocks , forms and spatial distribution of soil organic carbon — A case study in a Mediterranean context. Geoderma 259–260, 288–299. doi:10.1016/j.geoderma.2015.06.015

Chabbi, A., Kögel-Knabner, I., Rumpel, C., 2009 Stabilised carbon in subsoil horizons is located in spatially distinct parts of the soil profile. Soil Biology and Biochemistry41:256–271

Chapman S.J., Campbell C.D., Artz R.R.E., 2007, Assessing CLPPs using MicroReSp (TM)—a comparison with biology and multi-SIR. J Soils Sediments 7:406–410

Cheng, W., Kuzyakov, Y., 2005. Root effects on soil organic matter decomposition. In: S. Wright, S., Zobel, R. (Eds.), Roots and Soil Management: Interactions Between Roots and the Soil. Agronomy Monograph No. 48, American Society of Agronomy, Madison, Wisconsin, USA 119–143.

Cheng, W.X., Johnson, D.W., Fu, S.L., 2003. Rhizosphere effects on decomposition: controls of plant species, phenology, and fertilization. Soil Science Society of America Journal 67 (5), 1418–1427.

Chevallier, T., Blanchart, E., Albrecht, A., Feller, C., 2004. The physical protection of soil organic carbon in aggregates: A mechanism of carbon storage in a Vertisol under pasture and market gardening (Martinique, West Indies). Agriculture, Ecosystems and Environment 103, 375–387

Cotrufo, M.F., Soong, J.L., Horton, A.J., Campbell, E.E., Haddix, M.L., Wall, D.H., Parton, W.J., 2015. Formation of soil organic matter via biochemical and physical pathways of litter mass loss. Nature Geoscience 8, 776–779. doi:10.1038/ngeo2520

Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Denef, K., Paul, E., 2013. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: Do labile plant inputs form stable soil organic matter? Global Change Biology 19, 988–995.

Creamer R.E., Stone D., Berry P., Kuiper I., 2016, Measuring respiration profiles of soil microbial communities across Europe using MicroResp™ method. Appl Soil Ecol 97:36–43

De Deyn, G.B., Cornelissen, J.H.C., Bardgett, R.D., 2008. Plant functional traits and soil carbon sequestration in contrasting biomes. Ecology Letters 11, 516–531.

De Graaff, M.A., Classen, A.T., Castro, H.F., Schadt, C.W., 2010. Labile soil carbon inputs mediate the soil microbial community composition and plant residue decomposition rates. New Phytologist 188, 1055–1064

Dou, X., He, P., Cheng, X., Zhou, W., 2016. Long-term fertilization alters chemically-separated soil organic carbon pools: Based on stable C isotope analyses. Scientific Reports 6, 1–9. doi:10.1038/srep19061

Downie, J.A., 2010. The roles of extracellular proteins, polysaccharides and signals in the interactions of rhizobia with legume roots 34, 150–170. doi:10.1111/j.1574-6976.2009.00205.x

Duddigan S., Shaw L.J., Alexander P.D., Collins C.D., 2019, A Comparison of Physical Soil Organic Matter Fractionation Methods for Amended Soils, Applied and Environmental Soil Science

Esseinstat, D.M., 1991 On the relationship between root length and the rate of root proliferation: a field study using citrus rootstocks. New Phytol. 188, 63–68.

Fehrmann, R., Weaver, R., 1978. Scanning electron microscopy of Rhizobium spp. ad- hering to fine silt particles. Soil Science Society of America Journal 42, 279–281.

Fontaine, S., Bardoux, G., Abbadie, L., Mariotti, A., 2004. Carbon input to soil may decrease soil carbon content. Ecology Letters 7, 314–320

Fontaine, S., Barot, S., 2005. Size and functional diversity of microbe populations control plant persistence and long-term soil carbon accumulation. Ecology Letters 8, 1075e1087.

Fornara, D. A. and Tilman, D.: Plant functional composition influences rates of soil carbon and nitrogen accumulation, J. Ecol., 96, 314–322

Gavinelli, E., Feller, C., Larré‐Larrouy, M.C., Bacye, B., Djegui, Z., Nzila, J.D., 1995. Routine method to study soil organic matter by particle‐size fractionation: Examples for tropical soils.Soil Science Plant Analysis 26(11&12), 1749-1760

Guo, Z., Zhang, Z., Zhou, H., Wang, D., Peng, X., 2019. The effect of 34-year continuous fertilization on the SOC physical fractions and its chemical composition in a Vertisol. Scientific Reports 9, 1–10.

Harrell, F.E., 2007. Package ‘Hmisc’. Harrell Miscellaneous

Hanson P.J., Edwards N.T., Garten C.T., Andrews J.A., 2000, ‘Separating root and soil microbial contributions to soil respiration: A review of methods and observations’, Biogeochemisrty, 48, 115-146

Hassink, J., 1992. Effects of soil texture and structure on carbon and nitrogen mineralization in grassland soils 126–134.

Henneron, L., Picon-cochard, C., 2019. Plant economic strategies of grassland species control soil carbon dynamics through rhizodeposition.

Hernández, M.A., Romero, J., Jaime, C., León-pulido, J., 2017. Lignocellulosic Biomass from Fast-Growing Species in Colombia and their Use as Bioresources for Biofuel Production 58, 541–546.

Ho¨gberg P., Ho¨gberg M.N., Go¨ttlicher SG.., Betson N.R., Keel S.G., Metcalf D.B., Campbell C., Schindlbacher A., Hurry V., Lundmark T. et al., 2008, ‘High temporal resolution tracing of photosynthate carbon from the tree canopy to forest soil microorganisms’, New Phytologist, 220–228.

Hungate BA, et al. 1996‘Detecting changes in soil carbon in CO2 enrichment experiments.’ Plant Soil 187, 135–145

Jones, D.L., Magthab, E.A., Gleeson, D.B., Hill, P.W., Sánchez-rodríguez, A.R., Roberts, P., Ge, T., Murphy, D. V, 2018. Microbial competition for nitrogen and carbon is as intense in the subsoil as in the topsoil 117, 72–82.

Karimi, B., Maron, P.A., Boure, N.C.P., Bernard, N., Gilbert, D. Ranjard, L., (2017). Microbial diversity and ecological networks as indicators of environmental quality. Environmental Chemistry Letters, pp.1-17.

King, A.E., Congreves, K.A., Deen, B., Dun, K.E., Voroney, R.P., Wagner-riddle, C., 2019. Quantifying the relationships between soil fraction mass , fraction carbon , and total soil carbon to assess mechanisms of physical protection. Soil Biology and Biochemistry 135, 95–107.

Kong A. Y. Y., Six J., Bryant D. C., Denison R. F., van Kessel C., 2005, “The relationship between carbon input, ag- gregation, and soil organic carbon stabilization in sustainable cropping systems,” Soil Science Society of America Journal 69 (4), 1078–1085,

Kogel-Knabner, I., 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biology and Biochemistry 34, 139-162

Kuzyakov, Y., Domanski, G., 2000. Carbon input by plants into the soil. Review. Journal of Plant Nutrition and Soil Science 163, 421–431

Leake J.R., Ostle N.J., Rangel-Castro J.I., Johnson D., 2006, ‘Carbon fluxes from plants through soil organisms determined by field 13CO2 pulse labeling in an upland grassland’, Applied Soil Ecology 33, 152–175.

Le Bissonnais, Y., 2016. Aggregate stability and assessment of soil crustability and erodibility: I. Theory and methodology. European Journal of Soil Science 67, 11–21

Liang, C., Amelung, W., Lehmann, J., Kästner, M., 2019. Quantitative assessment of microbial necromass contribution to soil organic matter. Global Change Biology.

Liang, C., Schimel, J.P., Jastrow, J.D., 2017. The importance of anabolism in microbial control over soil carbon storage. Nature Microbiology 2, 1–6.

Lorenz, K., Lal, R., 2005 The depth distribution of soil organic carbon in relation to land use and management and the potential of carbon sequestration in subsoil horizons

McCormack, L.M., Dickie, I.A., Eissenstat, D.M., Fahey, T.J., Fernandez, C.W., Guo, D., Erik, A., Iversen, C.M., Jackson, RB., 2015. Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. New Phytologist 207, 505–518.

McDowell N.G., Bowling D.R., Bond B.J. et al., 2004, ‘Response of the carbon isotopic content of ecosystem, leaf, and soil respiration to meteorological and physiological driving factors in a Pinus ponderosa ecosystem.’ Global Biogeochemical Cycles, 18, GB1013.

Meharg A.A., 1994, ‘A critical review of labelling techniques used to quantify rhizosphere carbon-flow’, Plant and Soil 166, 55-62

Minasny, B., Malone, B.P., McBratney, A.B., Angers, D.A., Arrouays, D., Chambers, A., Chaplot, V., Chen, Z.S., Cheng, K., Das, B.S., Field, D.J., Gimona, A., Hedley, C.B., Hong, S.Y., Mandal, B., Marchant, B.P., Martin, M., McConkey, B.G., Mulder, V.L., O’Rourke, S., Richer-de-Forges, A.C., Odeh, I., Padarian, J., Paustian, K,. Pan, G., Poggio, L., Savin, I., 2017,Soil carbon 4 per mille. Geoderma 292, 59–86.

Murray, P.J., Hatch, D.J., Dixon, E.R., Stevens, R.J., Laughlin, R.J., Jarvis, S.C., 2004. Denitrification potential in a grassland subsoil : effect of carbon substrates 36, 545–547.

O’Brien, S.L., Jastrow, J.D., 2013. Physical and chemical protection in hierarchical soil aggregates regulates soil carbon and nitrogen recovery in restored perennial grasslands. Soil Biology and Biochemistry 61, 1–13

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., Szoecs, E., Wagner, H., 2019. Package 'vegan'. Community Ecology Package

Prieto, I., Stokes, A., Roumet, C., 2016. Root functional parameters predict fine root decomposability at the community level. Journal of Ecology 104, 725–733

Rasse, D.P., Rumpel, C., Dignac, M., 2005. Is soil carbon mostly root carbon ? Mechanisms for specific stabilization. Plant and Soil 269, 341–356

Rees, R.M., Bingham, I.J., Baddeley, J.A., Watson, C.A., 2005. The role of plants and land management in sequestering soil carbon in temperate arable and grassland ecosystems. Geoderma 128, 130–154

Renault, P., Ben-Sassi, M., Bérard, A., 2013, Improving the MicroResp™ substrate-induced respiration method by a more complete description of CO2 behavior in closed incubation wells. Geoderma, 207-208

Rohošková M., Valla M., 2004, 'Comparison of two methods for aggregate stability measurement - A review', Plant Soil and Environment 50(8):379-382

Roumet, C., Birouste, M., Picon-Cochard, C., Ghestem, M., Osman, N., Vrignon-Brenas, S., Cao, K. fang, Stokes, A., 2016. Root structure-function relationships in 74 species: Evidence of a root economics spectrum related to carbon economy. New Phytologist 210, 815–826.

Rumpel, C., Kögel-Knabner, I., 2011. Deep soil organic matter-a key but poorly understood component of terrestrial C cycle. Plant and Soil 338, 143–158.

Rygiewicz P.T., Andersen C.P., 1994, 'Mycorrhizae alter quality and quantity of carbon allocated below ground.', Nature 369, 58–60

Saenger, A., Cécillon, L., Poulenard, J., Bureau, F., De Daniéli, S., Gonzalez, J.M., Brun, J.J., 2015. Surveying the carbon pools of mountain soils: A comparison of physical fractionation and Rock-Eval pyrolysis. Geoderma 241–242, 279–288.

Sarker, J.R., Singh, B.P., He, X., Fang, Y., Li, G.D., Collins, D., Cowie, A.L. 2017. Tillage and nitrogen fertilization enhanced belowground carbon allocation and plant nitrogen uptake in a semi-arid canola crop–soil system. Nature 7, 1-13

Shihan, A., Hättenschwiler, S., Milcu, A., Joly, F., Santonja, M., Fromin, N., 2017. Changes in soil microbial substrate utilization in response to altered litter diversity and precipitation in a Mediterranean shrubland. Biology and Fertility of Soils 53, 171–185.

Six. J., Feller, C., Denef, K .,Ogle, S.M., Moraes Sa, J.C., Albrech, A. 2002. Soil organic matter, biota and aggregation in temperate and tropical soils effects of no-tillage. Agronomie 22, 755–775.

Six J. and Paustian K., 2014, "Aggregate-associated soil organic matter as an ecosystem property and a measurement tool," Soil Biol. Biochem, 68, A4–A9

Sokol, N.W., Kuebbing, S.E., Karlsen-ayala, E., Bradford, M.A., 2019. Evidence for the primacy of living root inputs, not root or shoot litter , in forming soil organic carbon. New Phytologist 221, 233–246.

Sollins, P., Hofmann, P., and Caldwell, B.A., 1996. Stabilization and destabilization of soil organic matter: mechanisms and controls. Geoderma 74, 65–105.

Staddon, P.L., 2004. Carbon isotopes in functional soil ecology. Trends in Ecology and Evolution 19, 148–154.

Stewart, C.E., Paustian, K., Conant, R.T., Plante, A.F., Six, J., 2007. Soil carbon saturation: Concept, evidence and evaluation. Biogeochemistry 86, 19–31

Stumm, W., Morgan, J.J., 1996. Dissolved carbon dioxide, In: Shnoor, J.L., Zehnder, A.J.B. (Eds.), Aquatic chemistry. Chemical Equilibra and Rates in Natural Waters, 3rd edition. John Wiley & Sons, New York, pp. 148–205

Subke J.A., Hahn V., Battipaglia G. et al. 2004, 'Feedback interactions between needle litter decomposition and rhizosphere activity'. Oecologia, 139, 551–559.

Taylor, J.P., Wilson., B., Mills, M.S., Burns, R.G., 2002. Comparison of microbial numbers and enzyrnatic activities in surface soils and subsoils using various techniques. Soil Biology and Biochemistry 34, 387–401.

Tisdall, J. M., Oades, J. M., 1979. Stabilization of soil aggre- gates by the root systems of ryegrass. Australian Journal of Soil Research 29, 729–743.

Tisdall, J.M., Oades, J.M., 1982. Organic matter and water-stable aggregates in soils. J. Soil Sci. 33, 141–163.

Torn, M.S., Swanston, C.W., Castanha, C., Trumbore, S.E., 2009. Storage and turnover ofor- ganic matter in soil. In: Senesi, N., Xing, B., Huang, P.M. (Eds.), Biophysico-Chemical Processes Involving Natural Nonliving Organic Matter in Environmental System. John Wiley & Sons, Inc., Hoboken, NJ, USA, pp. 215–269.

Trumbore S., 2006, 'Carbon respired by terrestrial ecosystems – recent progress and challenges', Global Change Biology, 12, 141-153

Vidal, A., Hirte, J., Bender, S.F., Mayer, J., Gattinger, A., Höschen, C., Schädler, S., Iqbal, T.M., Mueller, CW., 2018. Linking 3D Soil Structure and Plant-Microbe-Soil Carbon Transfer in the Rhizosphere. Frontiers in Environmental Science 6, 1–14.

Violle, C., Navas, M.L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., Garnier, E., 2007 Let the concept of trait be functional! Oikos, 116, 882–892.

von Lutzow, M., Kogel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B., Flessa, Hassink J 1997 The capacity of soils to preserve organic C and N by their association with clay and silt particles. Plant Soil 191, 77–87.

Warembourg, F.R., Roumet, C., Lafont, F., 2003. Differences in rhizosphere carbon-partitioning among plant species of different families 347–357.

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FIGURES AND TABLES

Table 1: Root, microbial and soil characteristics analyzed at time 0, after 183 days of experiment (time 6 months), and the difference between time 0 and time 6, for *M. sativa, L. perenne* sowed on tospoil and subsoil, plus bare soil controls. Root traits: Root biomass (g), specific root length (SRL, m g^{-1}), diameter of absorptive roots (mm), adsorptive roots C:N ratio; Microbial characteristics: global metabolic activity (GMA, μgC-CO_2 g^{-1} soil h^{-1}), Shannon metabolic diversity (H), concentration of DNA in solution as proxy for microbial biomass (DNA, ng μL^{-1}); Soil characteristics: mean weight diameter of aggregates (MWD, mm), soil nitrogen content (Soil N, mg N g^{-1} soil), percentage of fine fraction <20μm in soil (FF, %).

			SUBSOIL			**TOPSOIL**		
			Bare soil	*L. perenne*	*M. sativa*	Bare soil	*L. perenne*	*M. sativa*
Time 0	*Micorbial characteristics*	**GMA (μgC-CO_2 g^{-1} soil h^{-1})**	7.56±1.1	-	-	8.68±1.94	-	-
		H (-)	0.85±0.17	-	-	1.14±0.05	-	-
		DNA concentration (ng μL^{-1})	1.00±0.00	-	-	13.67±3.06	-	-
	Soil characteristics	**MWD (mm)**	0.82±0.03	-	-	1.55±0.02	-	-
		Soil N (mg N g^{-1} soil)	0.75±0.04	-	-	1.25±0.06	-	-
		FF (%)	51.00±1.00	-	-	43.00±1.00	-	-
Time 6 months	*Root traits*	**Root biomass (g)**	-	1.05±0.34	5.13±1.36	-	4.09±1.43	17.53±2.03
		SRL absorptive roots (m g^{-1} soil)	-	34.92±4.72	14.77±7.87	-	23.25±2.98	10.25±0.57
		Diameter absorptive roots (mm)	-	0.09±0.02	0.27±0.04	-	0.10±0.02	0.26±0.01
		Absorptive roots C:N	-	90.78±15.53	20.12±2.42	-	51.48±10.73	20.23±1.31
	Micorbial characteristics	**GMA (μgC-CO_2 g^{-1} soil h^{-1})**	6.77±0.72	7.41±2.50	5.98±1.06	7.41±1.72	11.69±1.02	19.06±4.21
		H (-+	0.78±0.07	0.93±0.08	0.90±0.04	1.16±0.01	1.17±0.00	1.17±0.00
		DNA concentration (ng μL^{-1})	4.00±2.22	3.00±1.41	5.20±1.64	18.40±5.90	18.40±5.59	19.00±4.32
	Soil characteristics	**MWD (mm)**	0.84±0.10	0.78±0.07	0.89±0.08	1.64±0.08	2.17±0.20	2.07±0.29
		Soil N (mg N g^{-1} soil)	0.63±0.05	0.65±0.08	0.65±0.05	1.19±0.14	1.12±0.10	1.23±0.10
		FF (%)	49.84±1.17	50.62±1.16	50.77±0.58	42.24±1.26	42.01±1.31	41.87±1.42
Δ t0-t6	*Micorbial characteristics*	**GMA (μgC-CO_2 g^{-1} soil h^{-1})**	3.00±2.22	-0.16±2.73	-1.59±1.53	-1.27±2.59	3.01±2.19	10.39±4.63
		H (-)	-0.07±0.18	0.08±0.18	0.06±0.17	0.02±0.05	0.03±0.05	0.03±0.05
		DNA concentration (ng μL^{-1})	3.00±2.22	2.00±1.41	4.20±1.64	4.73±6.64	4.73±6.37	5.33±5.29
	Soil characteristics	**MWD (mm)**	0.02±0.1	-0.03±0.08	0.08±0.08	0.1±0.08	0.62±0.20	0.52±0.29
		Soil N (mg N g^{-1} soil)	-0.11±0.07	-0.1±0.09	-0.1±0.07	-0.06±0.15	-0.13±0.12	-0.02±0.12
		FF (%)	-0.01±0.02	-0.01±0.01	0.00±0.01	0.00±0.02	-0.01±0.02	-0.01±0.02

For Δt0-t6 the red values indicate a loss in 6 months, black value a gain

Table 2: Statistical analysis of the effect of treatments (soil and species) on on root, microbial and soil characteristics analyzed after 183 days of experiment for *M. sativa, L. perenne* sowed on tospoil and subsoil, plus bare soil controls. Root traits: Root traits: Root biomass (g), specific root length (SRL, m g^{-1}), diameter of absorptive roots (mm), adsorptive roots C:N ratio; Microbial characteristics: global metabolic activity (GMA, µgC-CO_2 g^{-1} soil h^{-1}), Shannon metabolic diversity (H), concentration of DNA in solution as proxy for microbial biomass (DNA, ng µL^{-1}); Soil characteristics: mean weight diameter of aggregates (MWD, mm), soil nitrogen content (Soil N, mg N g^{-1} soil), percentage of fine fraction <20µm in soil (FF, %).. Data where normal according to the Shapiro-Wilk test and the ANOVA test was utilized to asses statistical differences.

	Soil	Variable	df	F	*p*
Effect of treatment	Topsoil	Root biomass	1,7	136.8	**<0.001*****
		SRL absorptive roots	1,6	73.33	**<0.001*****
		Diameter absorptive roots	1,6	224.6	**<0.001*****
		Absorptive roots C:N	1,7	32.61	**<0.001*****
		GMA	2,11	24.19	**<0.001*****
		H	2,11	5.925	**0.0179 ***
		DNA concentration	2,11	0.018	0.983
		MWD	2,11	9.953	**0.00341 ****
		Soil N	2,11	1.168	0.347
		FF	2,11	0.034	0.967
	Subsoil	Root biomass	1,8	42.13	**<0.001*****
		SRL absorptive roots	1,7	22.98	**0.00198 ****
		Diameter absorptive roots	1,7	64.44	**<0.001*****
		Absorptive roots C:N	1,7	104	**<0.001*****
		GMA	2,11	0.914	0.429
		H	2,11	6.827	**0.0118 ***
		DNA concentration	2,11	2.181	0.159
		MWD	2,11	2.263	0.15
		Soil N	2,11	0.079	0.925
		FF	2,11	0.662	0.535
Effect of Soil	*M.sativa*	Root biomass	1,7	120.9	**<0.001*****
		SRL absorptive roots	1,6	1.314	0.295
		Diameter absorptive roots	1,6	0.098	0.765
		Absorptive roots C:N	1,7	0.006	0.939
		GMA	1,7	46.22	**<0.001*****
		H	1,7	150.6	**<0.001*****
		DNA concentration	1,7	44.35	**<0.001*****
		MWD	1,7	80.22	**<0.001*****
		Soil N	1,7	129.6	**<0.001*****
		FF	1,7	159.8	**<0.001*****
	L.perenne	Root biomass	1,8	21.33	**<0.001*****
		SRL absorptive roots	1,7	18.3	**0.00366 ****
		Diameter absorptive roots	1,7	0.738	**<0.001*****
		Absorptive roots C:N	1,7	20.3	**0.00278 ****
		GMA	1,8	12.57	**0.00757 ****
		H	1,8	40.75	**<0.001*****
		DNA concentration	1,8	35.61	**<0.001*****
		MWD	1,8	220.4	**<0.001*****
		Soil N	1,8	72.42	**<0.001*****
		FF	1,8	132.1	**<0.001*****
	Bare soil	GMA	1,7	0.398	0.548
		H	1,7	149	**<0.001*****
		DNA concentration	1,7	18.83	**0.0034 ****
		MWD	1,7	178.9	**<0.001*****
		Soil N	1,7	56.59	**<0.001*****
		FF	1,7	45.36	**<0.001*****

Table 3: Pearson's correlation coefficients (r) showing relationships between microbial characteristics, root variables, soil structural characteristics and New, Old C, and ΔC in different soil C pools. Root traits: Root biomass (g), specific root length (SRL, m g^{-1}), diameter of absorptive roots (mm), absorptive roots C:N ratio; Microbial characteristics: global metabolic activity (GMA, μgC-CO2 g^{-1} soil h^{-1}), Shannon metabolic diversity (H), concentration of DNA in solution as proxy for microbial biomass (DNA, ng μL^{-1}); Soil characteristics: mean weight diameter of aggregates (MWD, mm), soil nitrogen content (Soil N, mg N g^{-1} soil), percentage of fine fraction <20μm in soil (FF, %). Data where normal according to the Shapiro-Wilk test and the ANOVA test was utilized to asses statistical differences.

	Root biomass (R_bio)	Specific root lenght of absorptive roots (SRL_AD)	Diameter of absorptive roots (Diam_AD)	C:N ratio of absorptive roots(C:N_AD)	Global metabolic activity (GMA)	Shannon metabolic diversity index (H)	Microbial DNA concentration (DNA)	Aggregates mean weight diameter (MWD)	Soil nitrogen (Soil_N)	Fine fraction percentage (FF)
$NewC_{POM}$	**0.77*** **	**-0.69** **	0.59**	**-0.66** **	**0.57* **	0.34	**0.53* **	0.36	0.43	-0.36
$NewC_{finePOM}$	**0.57* **	-0.4	0.06	-0.39	**0.71*** **	**0.90*** **	**0.87*** **	**0.97*** **	**0.94*** **	**-0.94*** **
$NewC_{silt}$	**0.60** **	-0.37	0.06	-0.35	**0.73*** **	**0.86*** **	**0.85*** **	**0.94*** **	**0.94*** **	**-0.90*** **
$NewC_{silt+clay}$	**0.83*** **	**-0.61** **	0.45	**-0.57* **	**0.84*** **	**0.69** **	**0.71*** **	**0.74*** **	**0.82*** **	**-0.76*** **
$NewC_{SUM}$	**0.88*** **	**-0.73*** **	**0.56* **	**-0.69** **	**0.76*** **	**0.57* **	**0.71*** **	**0.61** **	**0.69** **	**-0.62** **
$OldC_{POM}$	0.25	-0.08	-0.08	-0.04	0.38	**0.53* **	0.38	**0.57* **	**0.59** **	**-0.57* **
$OldC_{finePOM}$	**0.58** **	-0.3	0.14	-0.17	**0.51* **	0.36	0.33	0.38	0.43	-0.38
$OldC_{silt}$	0.06	-0.2	0.05	-0.17	-0.09	0.05	0.05	-0.02	-0.11	0.02
$OldC_{silt.clay}$	**-0.55* **	0.28	0	0.21	**-0.71*** **	**-0.83*** **	**-0.70*** **	**-0.87*** **	**-0.87*** **	**0.86*** **
$OldC_{SUM}$	-0.44	0.24	-0.07	0.23	**-0.59** **	**-0.57* **	**-0.60** **	**-0.58** **	**-0.59** **	**0.56* **
ΔC_{POM}	0.02	-0.13	0.24	-0.17	-0.16	-0.36	-0.16	-0.39	-0.38	0.39
$\Delta C_{finePOM}$	**-0.47* **	0.22	-0.13	0.09	-0.37	-0.17	-0.37	-0.17	-0.24	0.18
ΔC_{silt}	0.01	0.16	-0.04	0.13	0.17	0.05	0.17	0.12	0.21	-0.12
$\Delta C_{silt+clay}$	**0.60** **	-0.33	0.05	-0.26	**0.74*** **	**0.83*** **	**0.74*** **	**0.87*** **	**0.89*** **	**-0.87*** **
ΔC_{SUM}	**0.72*** **	**-0.52* **	0.33	**-0.50* **	**0.74*** **	**0.63** **	**0.74*** **	**0.66** **	**0.70*** **	**-0.65** **

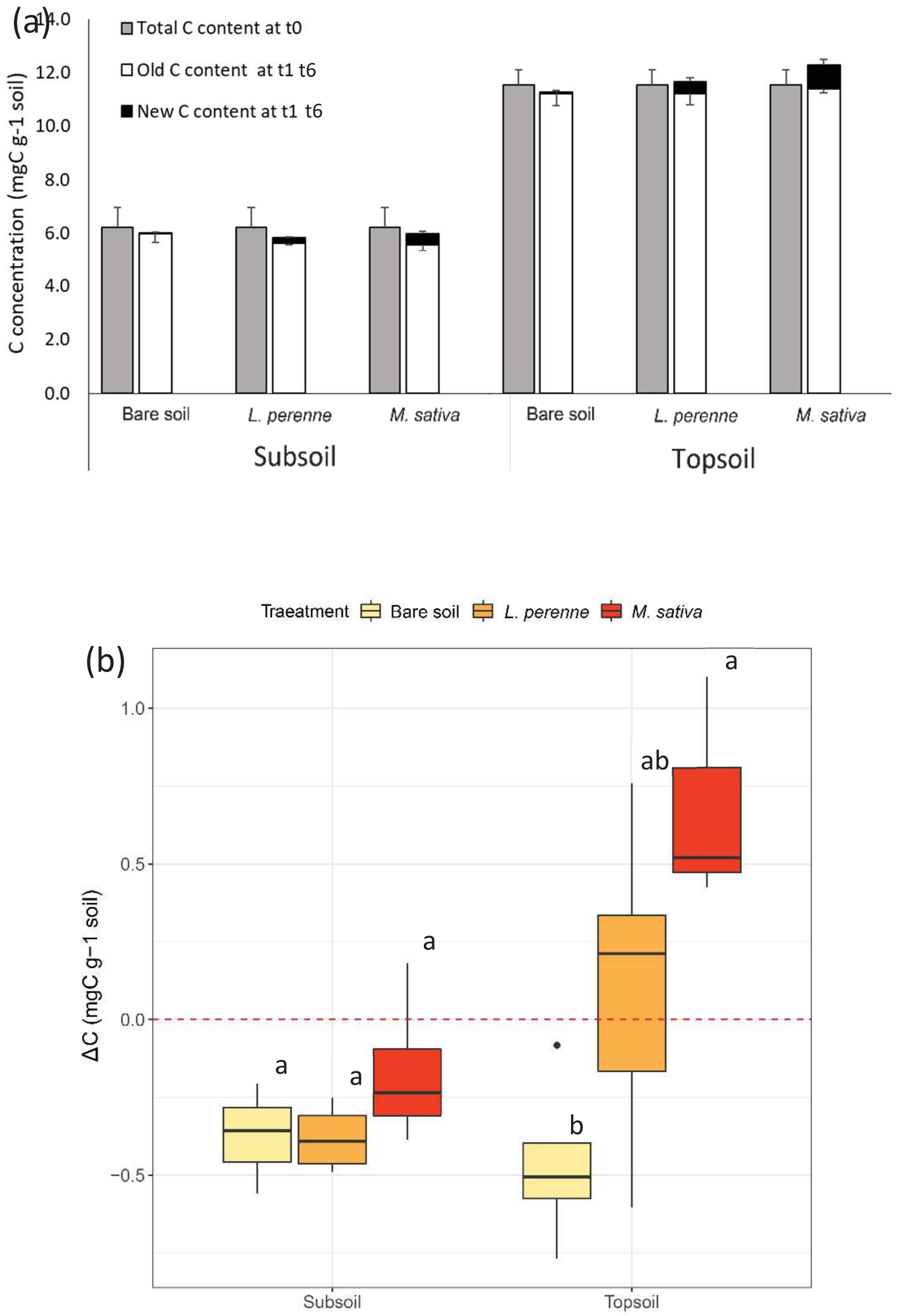


Figure 1. Total soil carbon (C) concentration (a) and concentration changes (b) among different soil types and vegetation treatments from *t0* (experiment set-up) to *t6* (harvest, i.e. 183 days after). In (a): total C concentration at t0, corresponding to old C concentration at t0, are all identical within each soil type. In (b), , for each boxplot, the lower edge of the box corresponds to the 25th percentile data point, while the top edge of the box corresponds to the 75th percentile data point; the upper and lower vertical lines corresponds to the 90th and 10th percentile data points, respectively; the horizontal line within the box represents the median and black dots indicate outliers. Letters above the boxplots indicate statistically significant differences ($p < 0.05$*) between species and controls according to Tukey HSD test.

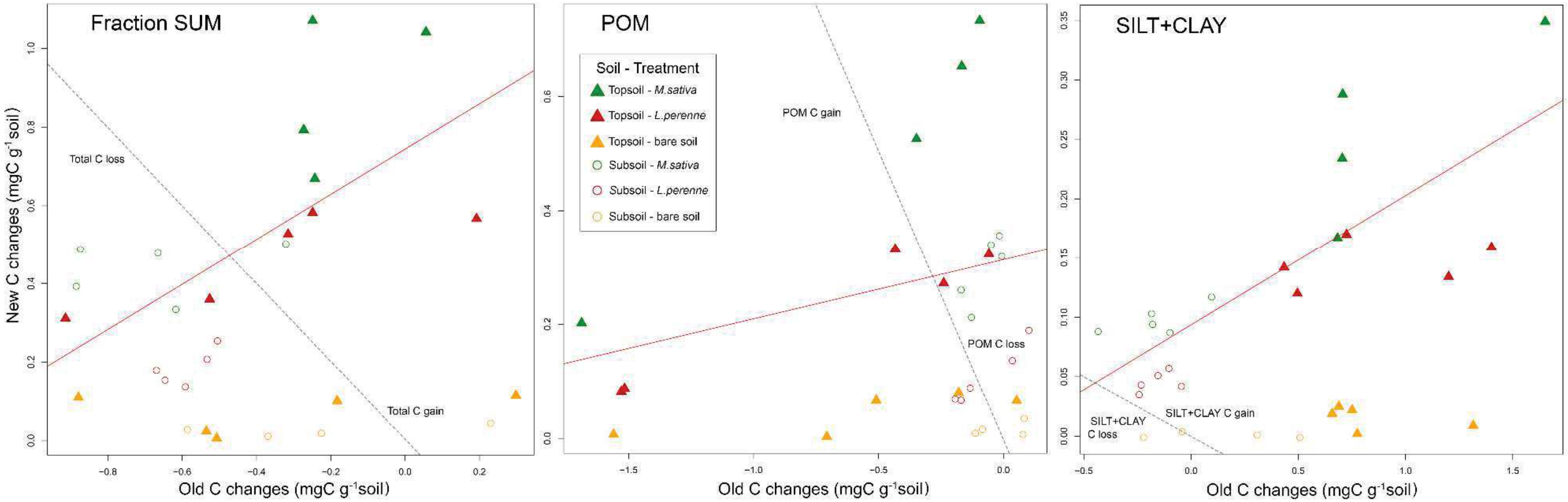


Figure 2. Relationships between the loss of OldC and gain in NewC in soil for (a) the sum of old and new C in every pool, (b) the POM pool and (c) the SILT+CLAY pool. The red solid line shows correlation between old c decrease and new c input for the vegetated treatment (without considering bare soil control). The grey dashed line shows the divide of the data between C gain (on the right) and loss (on the left) on sum of fraction, POM and SILT+CLAY pools.The red line show the liear correlations between data.

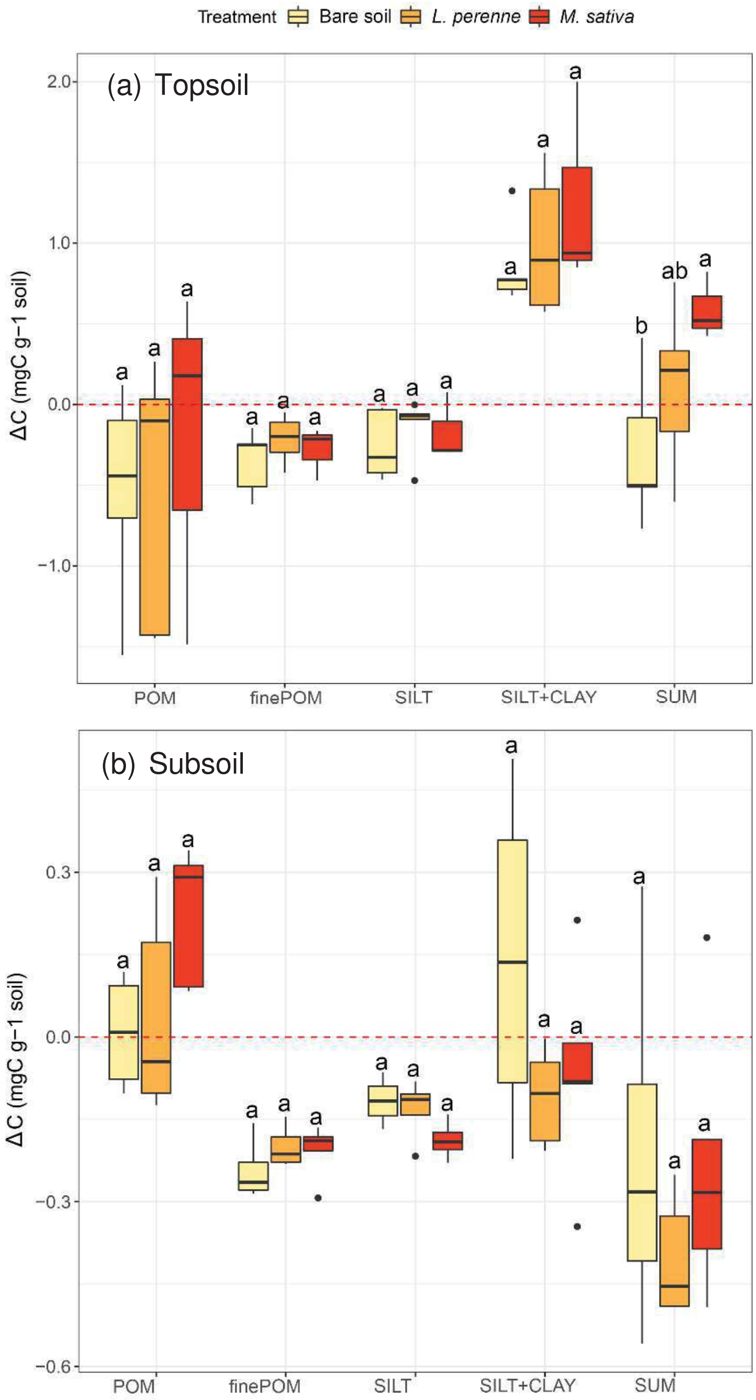


Figure 3: Comparison of the difference in carbon (ΔC) after 6 months in different soil C pools and for each treatment in a) subsoil and b) topsoil. In each boxplot, the lower edge of the box corresponds to the 25th percentile data point, while the top edge of the box corresponds to the 75th percentile data point. The line within the box represents the median and black dots indicate outliers. Different letters above the boxplots indicate statistically significant differences ($p < 0.05$) between families and controls according to Tukey HSD test.

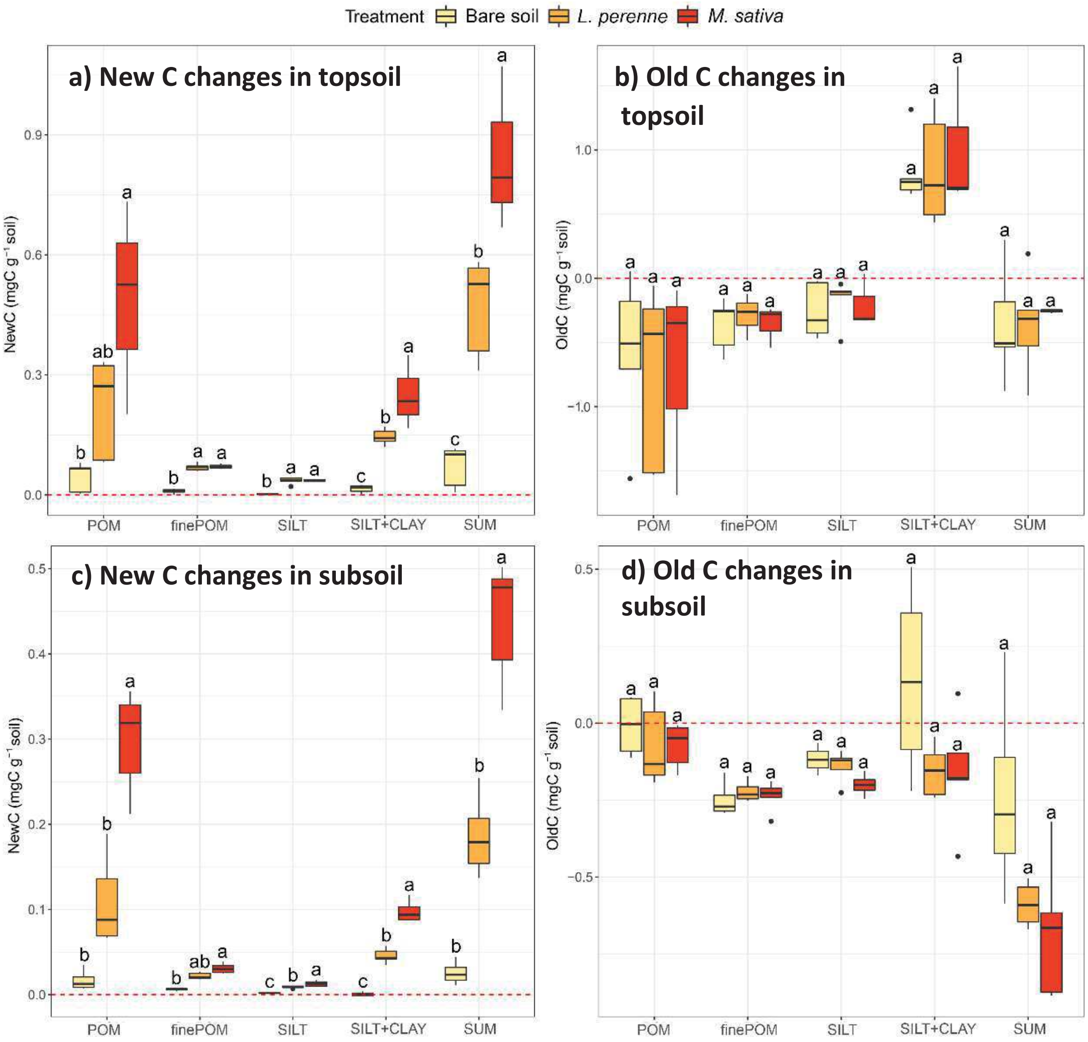


Figure 4: Gain of new C and changes in old C in 4 different C pools (POM, finePOM, SILT, SILT+CLAY) and in the total bulk soil (as sum of the different pools, Sum) for bare soil control (light yellow), *L. perenne* (orange), and *M. sativa* (red). a) shows the fluxes of new C in topsoil, b) the fluxes of old C in topsoil, c) the fluxes of new C in subsoil, and d) the fluxes of old C in subsoil. In each boxplot, the lower edge of the box corresponds to the 25th percentile data point, while the top edge of the box corresponds to the 75th percentile data point. The line within the box represents the median and black dots indicate outliers.Different letters above the boxplots indicate statistically significant differences ($p < 0.05$) between species treatments according to Tukey HSD test.

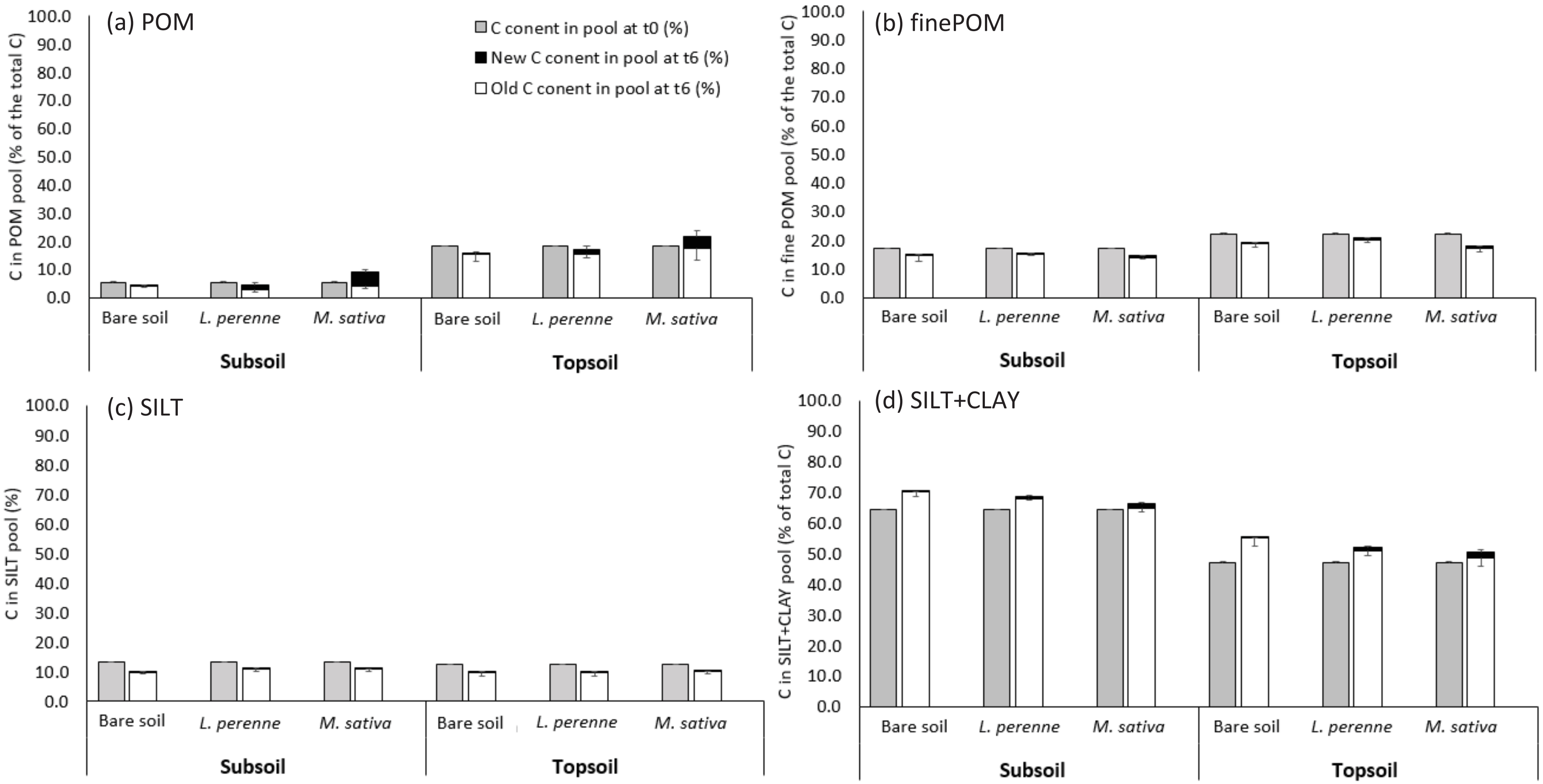


Figure 5: C concentration in % of total C in the different soil C pools at t0 and t6 (after 183 days of experiment). At t6 the C is divided in % of new C in soil (black) and old C in soil (white). (a) shows the C concentration in % in POM pool, (b) in fine POM pool, (c) in SILT pool, and (d) in SILT+CLAY pool.

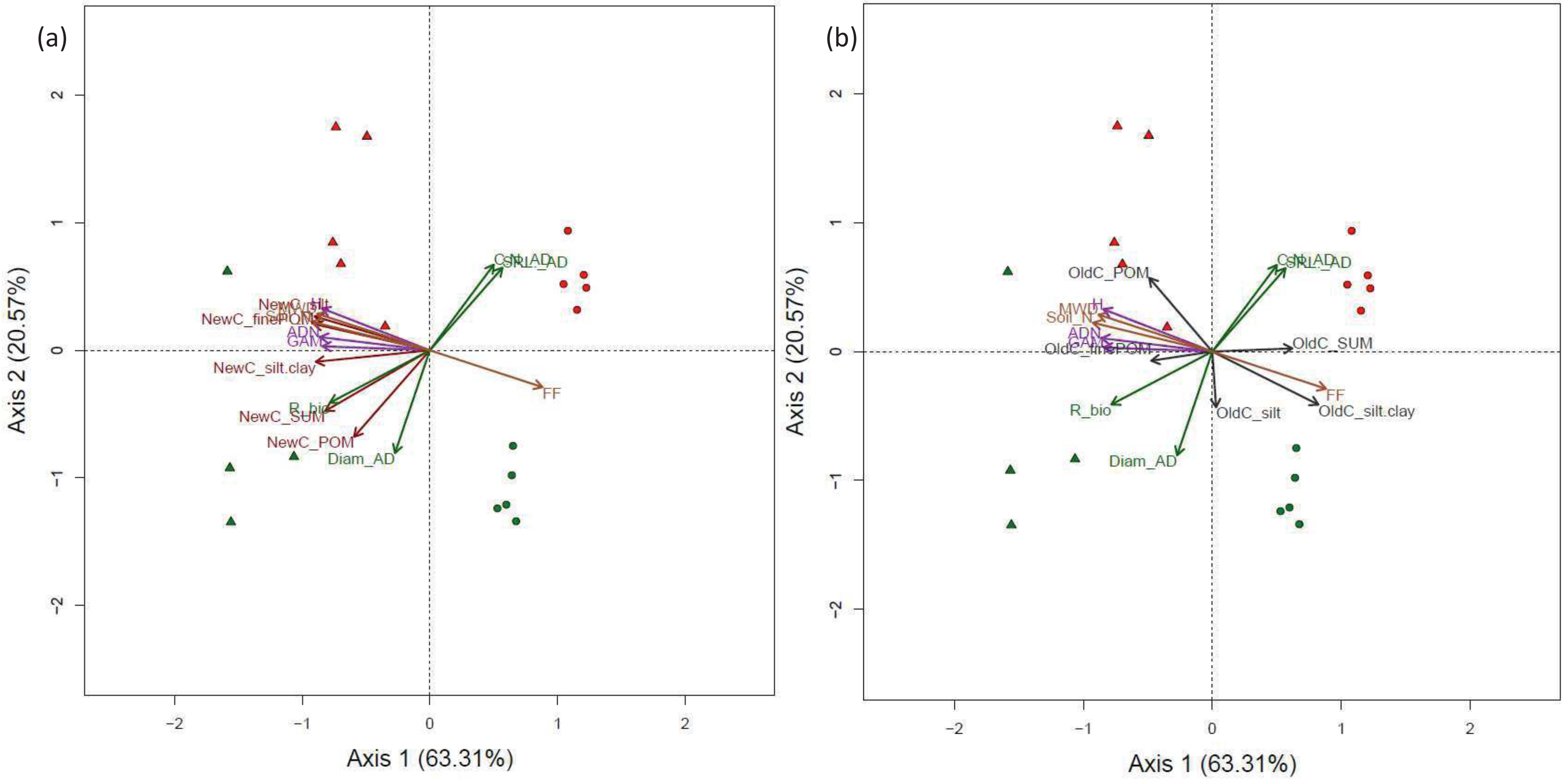


Figure 6: Principal Component Analysis of (a) new C input and (b) old C fluxes in different soil C pools and root traits (SRL_AD: specific root length of absorptive roots, C:N_AD: C:N ratio of absorptive roots, R_bio: root biomass and Diam_AD: diameter of absorptive roots), microbioal processes indicators (ADN: concentration of DNA as proxy for microbial biomass, GMA: global betabolic activity, H: Shannon metabolic diversity),and soil structure indicators (MWD: mean weight diameters of aggregates, Soil_N: nitrogen content in soil, and FF: percentage of fine fraction <20 mμ) in soil. Triangles are topsoil and dots subsoil. Green is *M.sativa* and red is *L.perenne.* (a) and (b) shows the same PCA analysys, but in (a) only the arrows of new C are shown, while in (b) only the arrows for old C, for a better comprehension of the graph.

SUPPLEMENTARY MATERIALS

Method S1: Soil fractionation according to Gavinelli et al. (1995) methodology

Gavinelli et al. (1995) methodology:

1. Soil is sieved at 2mm and 40g are collected for fractionation
2. Soil presoaked overnight at 4 °C in 300 mL of deionized water with 0.5g HMP (sodium metaphosphate)
3. Shaken with 5 agate balls (d 10 mm) in a rotary shaker, maximum frequency for 2h in case of sandy soils, 6h for other soils.
4. Soil suspension wet sieved through a 200 µm
5. Fraction remaining on sieves (2000-200 µm) washed with water in a bowl for POM separation via flotation, while the remaining >200 µm fraction is collected in a beaker for further farctioning
6. The POM is separated from the sand fractions by submerging the 2000-200 µm fraction in deionized water. The POM will float while the sand will drown. Carefully collect the POM using a sieve and separate it from the sand (**coarse POM fraction**). The sand fraction is collected in a glass beaker after separation from POM (sand fraction)
7. >200 µm suspension is sieved at 50 µm and the 200-50 µm is gently washed with deionized water from the sieve and collected in a glass beaker (**finePOM fraction**)
8. >50 µm suspension sonicated for 10 minutes
9. >50 µm suspension sieved with 20 µm screen and 50-20 µm is gently washed with deionized water from the sieve and collected in a glass beaker (**coarse SILT fraction**)
10. Transfer of >20 µm suspension in 1 L glass cylinder and add water to bring the volume to 1 L
11. >20 µm suspension shaken by hand (30 tumbling) and collection of 100 ml immediately after (aliquot for the **fine SILT+CLAY fraction**)
12. The resulting beakers containing the soil suspension of the different fractions are collected and oven dried at 60 °C until all the water evaporates

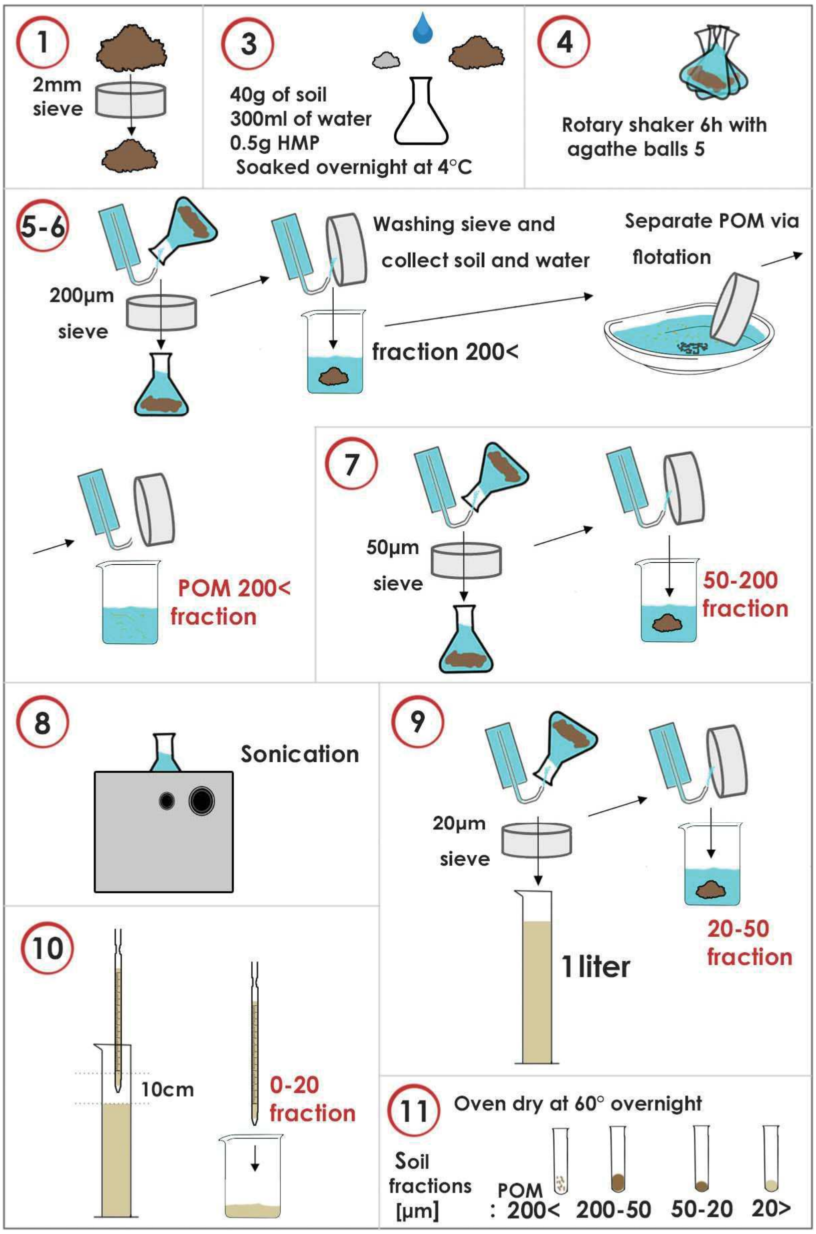


Figure S1: Soil fractionation according to the Gavinelli et al. (1995) methodology

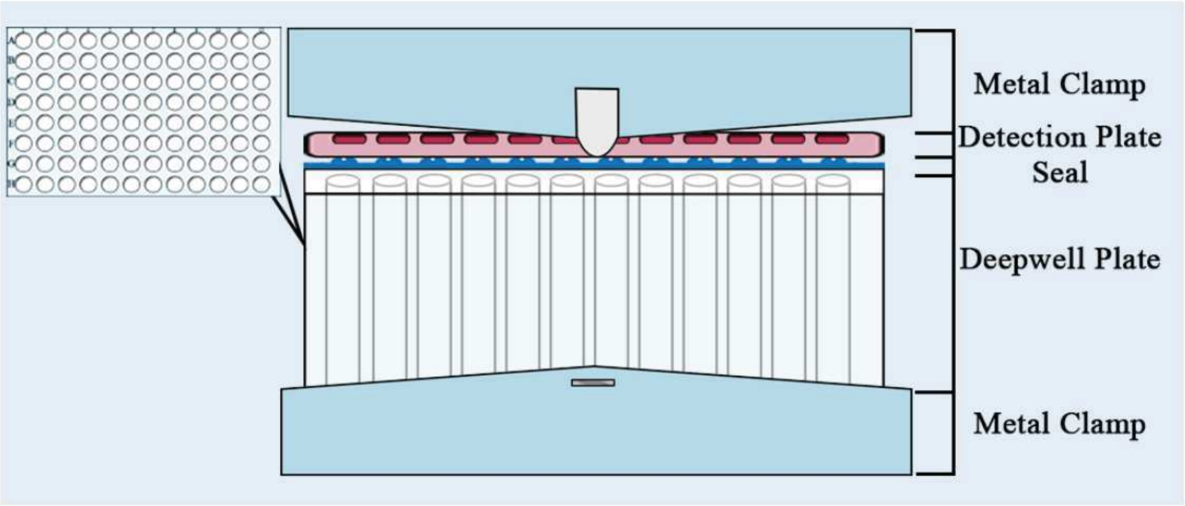


Figure S2 (ONICA et al.2018): scheme of a MicroResp system

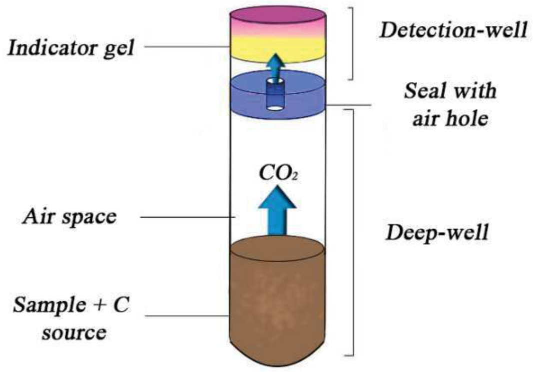


Fig. S3 (ONICA et al.2018): scheme of the functioning of a deep well detection system. Soil is placed in a deepwell and sealed, the CO2 respired and accumulating in the detection well change the colour of the detection gel according to the equation [4.5]

Method S2

The principle of the MicroResp is to utilize a colored detection gel (Cresol red) that change color when changing pH or increasing the CO2 concentration according to the equation:

$$CO_2\ (gaz) + H_2O + HCO_3^- \leftrightarrow 2CO_3^{2-} + 3\ H^+ \qquad [4.5]$$

When the pH diminishes, the Cresol red turns from pink to yellow. (Fig. S3)

The first step in the process is to prepare the detection gel in the MicroResp plaques. The preparation of detection gel is a fundamental step to assess the calibration curve of each detection plaque and calibrate the results based on the gel preparation, where $\%CO_2 = A + B / (1 + D * DO_{norm})$. First an Agar solution is prepared, mixing 3g of agar with 100ml of deionized water, mixed in an autoclave for 20mminutes at 120°C. After the indicator solution is prepared mixing 0.315 g $NaHCO_3$ (final: 2.5 mM), 16.77 g KCl (final: 150 mM) and 18.75 mg cresol red (final: 12.5 µg ml-1 or 20 ppm), in 900 ml of deionized water and brought at 65°C to dissolve. The 1000ml solution is transferred to an open bottle and stored at 4°C for 6 months maximum. To prepare the gel 100ml of the 3% agar solution are melted and kept at 60°C. Separately, other 200ml of the indicator solution are melted at 6°°C while stirring, and after mixed with the agar solution. The mixture is then distributed in the detection plaques, 150µl of solution for each one of the 96 detection well of each plaque. To avoid the formation of bubbles the gel is kept throughout the process in a baker of boiling water and the tip of the pipette used to distribute the solution is preheat in boiling water. When the gel is solidified (2-3h) the plaques are stored in a dessicator at room temperature in a dark room with a baker of soda and a cup of water for 2-3 days to allow each microplate to reach CO2 equilibrium. After they are covered in parafilm.

After the detection plaque are prepared a calibration process is needed for every new detection plaque. 12 strips (8 wells each) made for calibration containing the indicator gel are scanned used a Victor 1420 Multilabel Counter (PerkinElmer, Massachusetts, USA) to asses the initial DO590 (DOinitiales). Twenty-four 150ml serum vials are prepared, each containing ½ a strip of detection gel (4 wells), and injected with known CO2 concentration with a syringe in order to have a CO2 concentration range (from 0% CO2 concentration to 4% CO2 concentration, 6040 vol CO2 (µl), increasing the concentration in each vial of 0.1%). The strips are incubated for 2h at 25°C to achieve balance with the CO2 in each serum vial. After 2h the concentration of CO2 is assessed in the vials using the GC-microcatha measurement. The detection strips are then retrieve and immediately read at 590nm to assess the DOfinales. Finally the calibration is finalized as follow:

- Normalize DOdata: $DO_{norm} = DO_{final}/DO_{initial} * average(DO_{initial})$
- Draw the DO_{norm} vs $[CO_2]$ calibration curve in %.
- Fit the curve (rectangular hyperbola regression $\%CO_2 = A + B / (1 + D * DO_{norm})$

The next step in the MicroResp protocol is to prepare the different substrate solutions. The idea is to give 1.5 mg of C for each g of dry soil (substrate saturation) and reach a humidity level of 80% of field capacity. For each soil type is therefore necessary to determine 1) the field capacity in g of water per g of soil and 2) the soil mass distributed in each well by the MicroResp filling device. Field capacity for the soil was calculated at 28.3%. Three plaques for each soil where filled with the MicroResp filling device and weighted to estimate the average soil content in each MicroResp well, set at 0.5±0.04g well^{-1}. 1.2ml of solution have been added to each MicroResp well. The solution have been prepared using miliQ water and sterilized using 0.45 µm paper filters, stored in sterilized falcon tubes at 4°C.

The substrates used for the MicroResp analyses were chosen based on their biological properties and are reported in Table S1. In every detection plate an extra substrate with pure MillQ water were added as control. In each plaque (96 wells) we tested 2 samples, one in each half of the plaque, for a total of 48 wells per samples organized as follow: 3 sub replicates per substrate (15 substrate) plus 3 sub replicate per the millQ water control (Fig.S4).

Each sample was prepared as follow:

- Identify the deepwell plate and the sample used
- Tare the deepwell plate
- Hide half of the filling device, place it above a deepwell plate, covered with plastic sheeting, fill half of the filling device with the ground and remove the excess with a spatula.
- Pull the plastic sheet to drop the soil into the wells, then weigh and record the mass of soil used for the half plate (48 wells). Tare again before filling the second half of the plate with the other soil sample.

The samples are analyzed as follow:

- At time 0 the substrate are added using a multichannel pipette to each wells of the deepwell plate, cover with the parafilm and incubated at 25°C in the dark for 2h
- Before the end of the 2h incubation, the $DO_{590}t0$ of each detection placed is taken with a Victor 1420 Multilabel Counter (PerkinElmer, Massachusetts, USA). After two hours the detection plate is placed above the analysis plate with and sealed with a clamp. Resume incubation at 25°C for an additional 4 hours.
- At time 6h: the detection plate are detached from the deepweell and immediately read using a Victor 1420 Multilabel Counter (PerkinElmer, Massachusetts, USA) to determine the $DO_{590}t6$

For the data analysis the following steps were taken:

- Retrieve DO data at t0 and t6 for each plate. Check that the coefficient of variation of the DO_{590} of each detection half plate at t0 does not exceed 5% (otherwise, remove the outliers DO values).
- Normalize the data: DO_{norm} = DOt6/DOt2*average (DOt2)
- Convert DO_{norm} to % CO2 from calibration data: %CO2 = A + B / (1 + D * DO_{norm}). The data for the calibration curve were A = -0.29, B = -0.87, D = -7.72
- Convert these %CO2 to SIR (µg C-substrate g-1 soil h-1) according to the incubation time and soil mass in each well.

The aim of the MicroResp is to characterize the differences in functional activity of microbiological communities. The activity was assessed on soil sampled and t0 and t6.

Table S1: Substrates used for the MicroResp

Code	Substrate	Interest and biological properties	Reference	Formula
Carbohydrates :				
GLU	D-glucose	Potential comparison with SIR and cellulose compound	Reactive to different soils	C6H12H6
XYL	Xylan	Compost of hemicellulose, changes strongly according to the seasons	Reactive to different soils	(C5H8O5)n
CEL	Cellulose	plus dur à dégrader et comparaison potentielle avec résultats DCP	Bérard	$(C_6H_{10}O_5)_n$
Amino acid				
ASP	L-Asparagine	Reactive to different soils		C4H8N2O3
SER	L-Serine	Reactive to different soils		C3H7NO3
LYS	L-Lysine	Reactive to different soils		C6H14N2O2
GLY	Glycine	responds well when decomposed results Berard, precursor ac uric	Bérard, article	$C_2H_5NO_2$
GLUT	L-Glutamine	Linked to the metabolism of nitrogen and ammonia fixation on glutamic acid	données terrain	$C_5H_{10}N_2O_3$
Nac	N-acetyl glucosamine	Chitin monomer, found in insects exoskeleton and fungi	Dalmonech	$C_8H_{15}NO_6$
Carboxylic acid (more recalcitrant)				
OX	Oxalic acid	Root and exudates compontent linked with Malic acid	From field trial, Dalmonech, Bérard	C2H2O4
UR	Ureic acid	extruded by isopods (and diplopods)	From field trial, Dalmonech, Bérard	C5H4N4O3
MAL	Malique acid	Root and exudates compontent, useful in fermentation processes		C6H6O5
Phenolic acid (strongly recalcitrant)				
CAF	Cafeic acid	Close to rosemaric acid (extruded by Lamiaceae)	Bérard	$C_9H_8O_4$
SYR	Syringic acid	produit de la dégradation de pigments végétaux la malvidine	Dalmonech	C 9 H 10 O 5
VAN	Vanillic acid	produit de dégradation de la lignine par les champignons	Oren	$C_8H_8O_4$

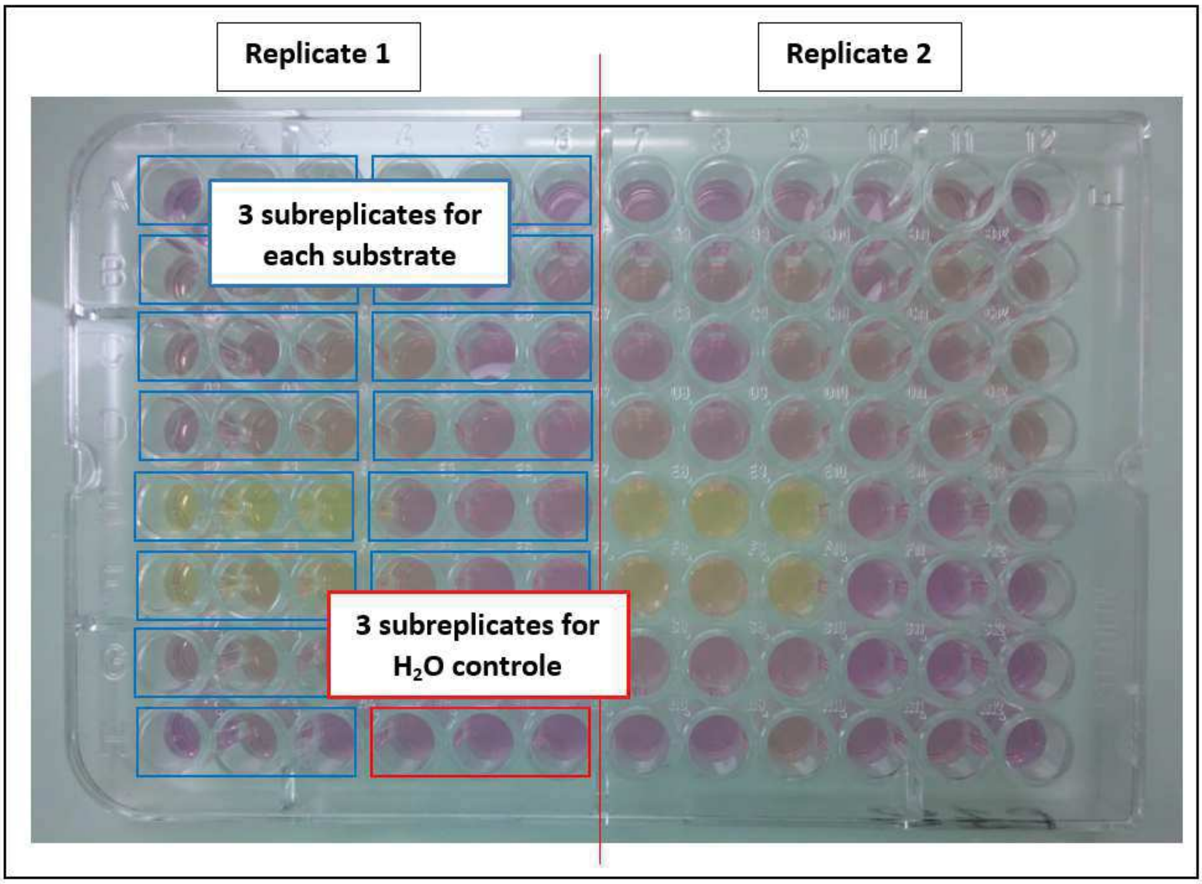


Figure S4: Scheme representing the organization of the MicroResp detection plate, showing the three replicates for each substrate used, the three for the H2O control and the separation of 2 replicates analyzed in the same MicroResp system.

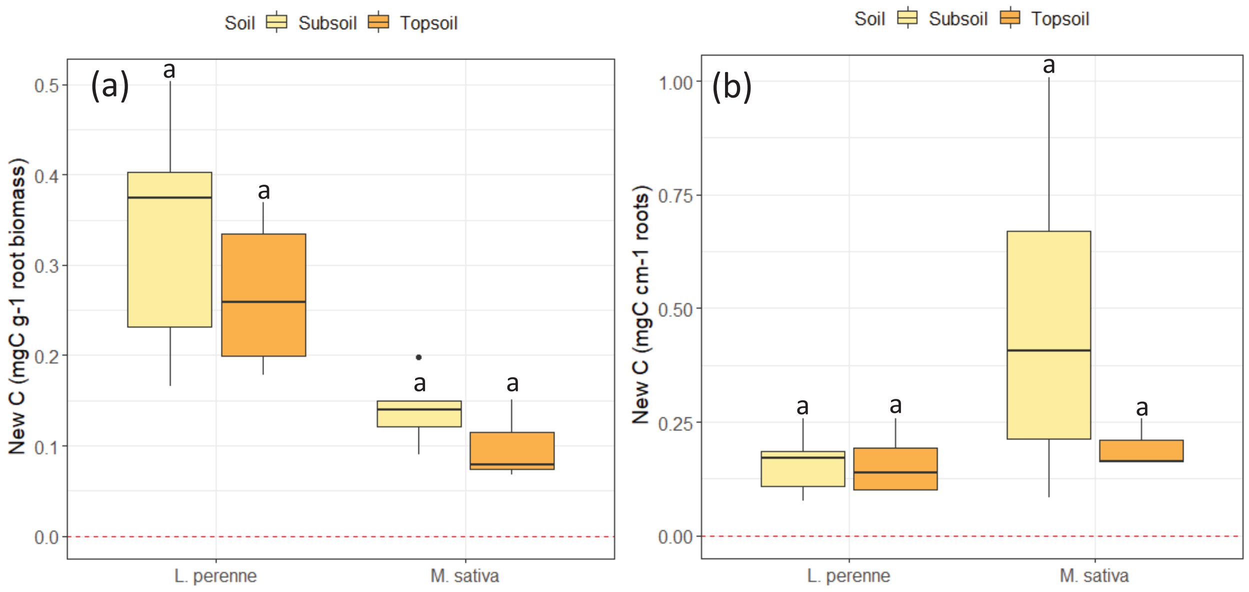
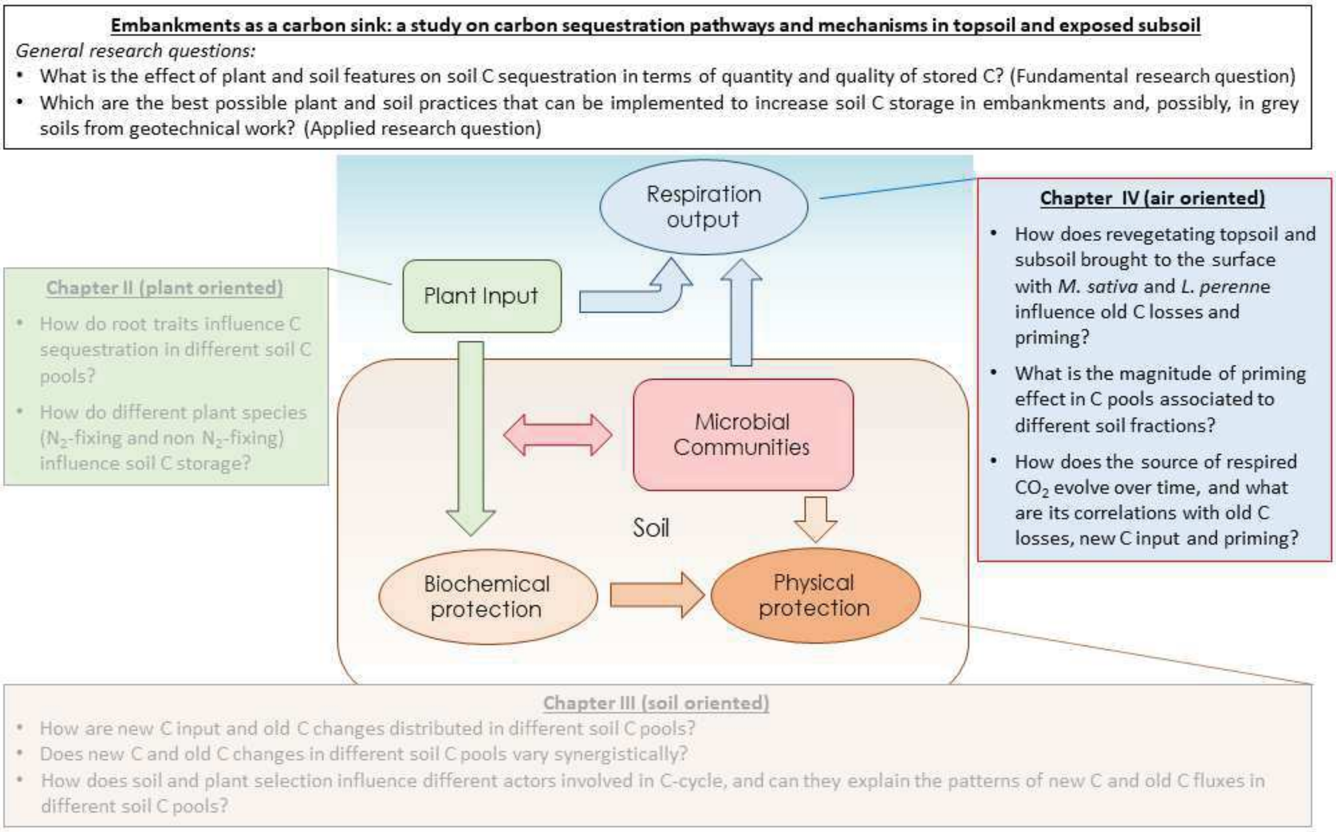


Figure S5: (a) New C moved in the SILT+CLAY fraction in subsoil (light ocra) and tospoil (orange) for g of root biomass for the two different treatments (*L. perenne, M. sativa*) in 183 days of experiment. (b) New C moved in the SILT+CLAY fraction in subsoil (light ocra) and tospoil (orange) for cm of root for the two different treatments *(L. perenne, M. sativa*) in 183 days of experiment. , for each boxplot, the lower edge of the box corresponds to the 25th percentile data point, while the top edge of the box corresponds to the 75th percentile data point; the upper and lower vertical lines corresponds to the 90th and 10th percentile data points, respectively; the horizontal line within the box represents the median and black dots indicate outliers. Letters above the boxplots indicate statistically significant differences ($p < 0.05$*) between species and controls according to Tukey HSD test.

Chapter IV: Soil quality drives the priming effect and plant species refine it



Embankments as a carbon sink: a study on carbon sequestration pathways and mechanisms in topsoil and exposed subsoil

General research questions:

- What is the effect of plant and soil features on soil C sequestration in terms of quantity and quality of stored C? (Fundamental research question)
- Which are the best possible plant and soil practices that can be implemented to increase soil C storage in embankments and, possibly, in grey soils from geotechnical work? (Applied research question)

Chapter IV (air oriented)

- How does revegetating topsoil and subsoil brought to the surface with *M. sativa* and *L. perenne* influence old C losses and priming?
- What is the magnitude of priming effect in C pools associated to different soil fractions?
- How does the source of respired CO_2 evolve over time, and what are its correlations with old C losses, new C input and priming?

Chapter II (plant oriented)

- How do root traits influence C sequestration in different soil C pools?
- How do different plant species (N_2-fixing and non N_2-fixing) influence soil C storage?

Chapter III (soil oriented)

- How are new C input and old C changes distributed in different soil C pools?
- Does new C and old C changes in different soil C pools vary synergistically?
- How does soil and plant selection influence different actors involved in C-cycle, and can they explain the patterns of new C and old C fluxes in different soil C pools?

In Chapters II and III we investigated the C storage in different C pools and its relationship with soil, root and microbiological characteristics. However, vegetation can also have a negative effect on soil C storage increasing the preexistent old C mineralization and loss compared to bare soil - the so called ‘priming effect’. In Chapter IV we aim to tackle this aspect, and study the how plant species and soil showing contrasting characteristics influence the priming effect, analyzing soil respiration origin and changes in soil old C in bulk soil and different soil C pools, and describing the main factors that influence our plant-soil system: microbiological substrate preference and N competition.

4.1. INTRODUCTION

Using vegetation to increase soil C sequestration in soil is recognized as an efficient method to mitigate atmospheric CO_2 content. Accordingly, national and supranational organizations designed international programs to promote C storage in soil e.g., the 4p1000 initiative (www.4p1000.org, Minasny et al. 2017). The net input of C in an ecosystem is jointly determined by fresh biomass input of new C via plant biomass turnover and labile rhizodeposition (new C) and losses of old preexistent C in soil (old C) (Kuzyakov and Domanski, 2000; De Deyn et al., 2008; Lal 1994; Smith et al., 2000; De Deyn et al., 2008). Both processes are not independent, but can have strong interactions. Especially, concerns about the fate of the preexisting old C loss due to such a fresh new C input from plants have been raised increasingly. Such a phenomenon, called the priming effect (PE), refers to input of labile C from plants that can accelerate (positive PE) or decelerate (negative PE) mineralization and losses of preexistent old C from soil (Kuzyakov et al., 2000, Fig. 1).

The PE depends on the nature of the substrate consumed by the soil microorganisms, which could be altered by plant and soil conditions (Hamer and Marschner, 2005; Shahzad et al., 2015; Huo et al., 2017). Therefore, understanding the impact of plant and soil characteristics on the amount of primed C has become a key research objective. Among the diverse factors affecting C sequestration, soil particle size and associated C pools is considered as a major factor affecting PE. The commonly used classification of soil C pools associated to soil particle size fractions in literature refers to four C pools. First C in particulate organic matter (POM) (C_{POM}, 2000-200µm), and C in fine POM ($C_{finePOM,}$ 200-50µm) originating from plant litter debris at different levels of degradation, and less protected from degradation (Kögel-Knabner, 2002). After the pools more stable due to their organomineral binding with fine soil particles: C in coarse SILT (C_{SILT}, 50-20 µm) and C in fine SILT + CLAY ($C_{SILT+CLAY}$, <20µm), deriving from exudation and processed C from microbiological communities in form of exudates, exopolysaccharides and microbial necromass(Sollins et al., 1996; von Lützow et al., 2006; Cotrufo et al. 2013, Liang et al., 2017). For example, Huo et al. (2017), found that rhizospheric PE was significantly greater in finely grained soil. Surprisingly, the effect of soil particle size fraction on PE has seldom been investigated (but see Ohm et al. 2007; Perveen et al., 2019), nor the interactions with soil type and plant species. Perveen et al. (2019) tested the effect of 35 different soils coming from all over the world and from different land uses. They found no effect of landuse on PE, but a significant effect of soil type, with positive priming effect increasing in any soil with increasing C and N content (Perveen et al., 2019). However, the incubation experiment was carried on in a heavily artificial environment, without the presence of living plants and with the addition of a nutrient solution to all the soils . The addition of an N rich solution excluded the possibility of competition between plants and microbes for N and the possible reduction of positive PE from this competition (‘competition hypothesis’ from Cheng and Kuzyakov, 2005; Perveen et al., 2019).

In a framework of soil C sequestration, both soils that differ in initial old C quantity in different soil C pools, and species of contrasted root growing strategies, have been shown to have significant effects on the sequestration of C in soil and relative size of C pools (De Deyn et al., 2008; Prieto et al. 2016; Poirier et al. 2018;. Henneron et al. 2019 ; Perveen et al., 2019). We argue that, to better understand the effects of revegetation on C-cycle and storage, even the influence of soil and species on priming effect needs to be examined, considering the changes of old C in different C pools related to soil particle size fractions.

To quantify the PE, knowing the fate of old C in soil is essential and usually performed using a stable isotopic labelling approach (Kuzyakov, 2006). Monitoring respired CO_2 reflecting the source of the consumed substrate and the remaining old C changes in soil are two major means to assess the PE. When plants are growing in an atmosphere constantly enriched with ^{13}C atmosphere, the input of plant derived C has a higher abundance of ^{13}C. Consequently, the abundance of ^{13}C in respired CO_2 ($A^{13}C$, %) depends on the mineralized C source: a higher $A^{13}C$ if the main respiration source is the consumed plant new C input, and a lower $A^{13}C$ if the source is the preexistent old C in soil (e.g. Fontaine et al. 2004, 2007; de Graaf et al. 2010). Another effective way to study priming effect is assessing the losses of preexistent old C in soil with and without vegetation. When analyzing the C in bulk soil, the ^{13}C signal also allows the differentiation between preexistent old C, and fresh new C derived from plant input. Comparing losses of old C in a vegetated soil allows for the quantification of the priming effect and whether it is positive or negative PE.

On newly constructed road embankments, subsoil is increasingly used to replace topsoil that is stripped off during the construction process. Compared to topsoil, subsoil contains less C, but the old C present is more stable than in topsoil for several reasons. C in subsoil is associated with the finest soil particles and stabilized via organomineral interactions (i.e., SILT and SILT+CLAY) (Eusterhues et al., 2005; Chabbi et al., 2009). Subsoil has less microbial biomass (Taylor et al. 2002; Andersen and Domsche 1989; Ekklund et al. 2001), and activity due to oxygen limitation (Rumple and Kögel-Knabner, 2010), and reduced plant inputs (Fontaine et al. 2007) increasing C residence time. Finally, physical separation of microbes and C decrease the possibility for C mineralization (Von Lützow et al. 2006; Holden and Fierer 2005). Subsoil excavation, mixing and revegetation alter all of these protection mechanisms. How the revegetation influences PE and the fate of old C in subsoil, especially in the SILT+CLAY pool remains unclear. To the best of our knowledge, no studies on the priming effect of subsoil brought to the surface have been performed.

We aim at comparing the priming processes in two soils with same origin but contrasting characteristics (topsoil with typical fertility, high microbial biomass and nitrogen (N) content versus subsoil with low fertility, microbial biomass and N content). The soils were vegetated with two herbaceous species: the di-nitrogen (N_2) fixing species *Medicago sativa* L., and the non N_2-fixing grass *Lolium perenne* L.. Soil respiration, changes in new C, old C and the priming effect for total C and that in each C pool were quantified. We hypothesize that (i) topsoil will have higher losses of old C due to greater root biomass and microbial biomass and activity; however, (ii) subsoil will have a greater positive priming effect because it is very highly disturbed compared to the initial conditions, and (iii) the C priming effect will differ among soil fractions due to different protection potential.

4.2. METHODOLOGY

4.2.1. *Experimental setup*

Soil used for growing plants was excavated from Pisciotta (Italy, 40°07’N 15°14’E/40.116667°N) at two depths of the same soil profile: topsoil (0-30cm depth) and subsoil (110-140cm depth). The soil is a clay loam soil (USDA) with a slightly different granulometry between topsoil and subsoil (topsoil: 27.3% clay, 31.1% silt, 41.6% sand; subsoil: 34.8% clay, 36.8% silt, 28.4% sand). The pH in topsoil was 7.0 and in subsoil was 8.4.

Air dried soil was crushed and sieved to 5mm to homogenize it. We mixed and divided the soil in four sections (quartiles) and 36 different pots were prepared collecting one scoop of soil from each section until the desired weight in each pot has been reached (Fig. S1). We added 6.9 kg of soil into each pot. During the preparation, three soil samples were removed and put aside for chemical analyses. These samples represent the initial soil, or time zero (t0). Inside each pot, a 60 mm deep plastic ring with a diameter of 80 mm was fitted that could be closed with an airtight dome for subsequent measurements of soil respiration (Fig. S2).

N_2-fixing *Medicago sativa* L. and non N_2-fixing *Lolium perenne* L. were sowed as monocultures with exactly the same pattern. In each pot, three seeds were put at six equidistant spots. After germination, one seedling was removed with scissors at ground level, at each spot. For each soil type (i.e. top- and subsoil) and species, six replicate containers were prepared and six bare containers per soil type were used as controls (n = 36 in total)

Containers were placed into three identical microcosms at the Ecotron growth facility at Montpellier, France (http://www.ecotron.cnrs.fr/) (Fig. S3). In each microcosm, two replicates of all treatments, i.e., 12 pots, were placed randomly to avoid any effect of microcosm on plant growth and soil processes. Plants were grown at a constant air temperature of 21°C and at 80% humidity (to reduce the soil water loss by evapotranspiration). Artificial light was provided by three lamps (Gavita PRO 300

LEP 02, Netherlands) in each microcosm with a 12h day/night cycle, shifted to allow air sampling at the same moment of the plant's circadian rhythm (data not shown in this study, Fig. S4). A shade was placed on the lamps and the distance of the lamps from the plants was adjusted to achieve the most possible homogenous light intensity on the foliage (300 µmol m^{-2} s^{-1}). Soil moisture was kept at 45 ± 10% of the soil water holding capacity for the entire duration of the experiment. A system of plastic pipes was installed in the chamber to allow irrigation without having to open the chamber and disturb the ^{13}C concentration (Fig.S5). Pots were irrigated every 2-3 days, according to their evaporation rate. However, with the growth of plants, the increase in biomass and in transpiration had to be considered to calculate the amount of water needed to keep the soil at the desired moisture content. For this reason, every 2 weeks, pots (in correspondence with the air sampling) were removed from the chamber, weighted and randomized inside the chambers. Knowing the amount of water added in the previous 2 weeks, the initial soil moisture content, and the final soil moisture content, we were able to calculate the increase in evapotranspiration every 2 weeks and adjust the amount of water needed (data not shown).

After the germination of seedlings, the atmosphere was enriched with ^{13}C, reaching a concentration of 2% (approximately two times higher than the natural ^{13}C abundance of 1.1%, in other words $\delta^{13}C$ of CO_2 in the chamber was roughly +760, as compared to the ambient -8). The air enrichment with ^{13}C was infused during the photoperiod and the ^{13}C infusion stopped during the night period. The experiment was carried out for 183 days, starting the 29 September 2017 and ending the 31 March 2018.

4.2.2. Air sampling

Air sampling rings were built with two openings in their belowground section to allow root growth in their perimeter, and a double ring structure (one inside the other) that could be filled with water. Inserting the plastic dome inside the double ring structure filled with water ensures an airtight sealing, allowing soil respiration to accumulate inside the chamber (the plastic ring cover had an area of 8.5cm and a height of 6cm, for a volume of 340 cm^3) (Fig. S6). Every two weeks, we assessed the percentage of ^{13}C in the respired CO_2.

To conduct the air sampling, ^{13}C enrichment was stopped 24h before the sampling procedure to allow the ^{13}C accumulated in soil to leak out of macropores and cracks that could pollute the results.

The day of the sampling, each chamber was open and the air sampling of the time 0 (t0) was performed as soon as the photoperiod stopped. The protocol for the air sampling consisted of:

1. Pour water in the ring (Fig. S7a)
2. Close the ring with the plastic dome (Fig. S7b)
3. Insert the syringe in the rubber cap of the plastic dome and collect 5ml of air to set the reference time 0 (t0) (Fig. S7c)
4. Immediately transfer the collected 5ml sample from the syringe to an Exitainer under vacuum to store gas (Fig. S7d)
5. After 2 hours of incubation, without moving the dome, insert the syringe in the rubber cap of the dome, collect 5ml of gas enriched with the soil respiration, and transfer it in the exitainer following steps 3 and 4. This sample will represent the Time 1 (t1) air sample, as the amount of CO_2 in the chamber atmosphere after a 2h incubation period.
6. Samples analyzed with an elemental analyzer Isoprime100 coupled with an Elementar Varo Isotope Cube

Results from the isotope analyzer provided the CO_2 concentration in ppmV at time 0 (CO_{2t0}) and time 2 hours (CO_{2t1}). The abundance of ^{13}C in respired CO_2 was gave in $\delta^{13}C$, according to the equation:

$$\delta^{13}C = \frac{R_{sample}}{R_{VPDB}} - 1 \qquad [1]$$

Where R_{sample} is the carbon isotope ratio of the sample ($^{12}C/^{13}C$) and R_{VPDB} the ratio of the international standard reference Vienna Pee Dee Belemnite ($R_{VPDB} = 0.0111802$, Werner and Brand, 2001). The $\delta^{13}C$ was adjusted according to the CO_2 concentration of the analyzed samples as $\delta^{13}C_{SR}$:

$$\delta^{13}C_{SR} = \frac{(\mathrm{CO}_{2t1} * \delta^{13}C_{t1}) - (\mathrm{CO}_{2t0} * \delta^{13}C_{t0})}{(\mathrm{CO}_{2t1} - \mathrm{CO}_{2t0})} \qquad [2]$$

Where $\delta^{13}C_{t1}$ is the isotopic composition of CO_2 at after 2 hours of soil respiration and $\delta^{13}C_{t0}$ the isotopic composition at time 0.

To calculate the fractional abundance of ^{13}C in the respired CO_2 ($A^{13}C$), first the carbon isotope ratio was derived from [1] as follows:

$$R_{sample} = \left(1 + {}^{\delta^{13}C_{SR}}/_{10^3}\right) * R_{VPDB} \qquad [3]$$

Finally, to calculate the isotope abundance $A^{13}C$ (%):

$$A^{13}\mathrm{C} = R_{sample}/(1 + R_{sample}) \qquad [4]$$

To calculate the percentage of CO_2 derived from fresh plant input mineralization (fPlant) first the soil derived CO_2 concentration (CO_{2C}) in µmol have been calculated as:

$$CO_{2C} = \Delta\mathrm{CO}_{2P} \times \frac{P \times Vc}{R \times T} \qquad [5]$$

Where ΔCO_{2P} is the CO_2 concentration in (ppmV) is the difference of CO_2 concentration in the sampling chamber (in ppmV) at time 0 and after 2h of incubation time; P the atmospheric pressure in Pa; Vc the volume of the chamber in m^3; R the ideal gas constant 8.314 J K^{-1}; and T the temperature in K.

After the amount we calculated the quantity C(C_Q in µg)in the respired CO_2 as:

$$C_Q = (CO_{2C} 12 \times (1 - A^{13}\mathrm{C})) \times (CO_{2C} \times 13 \times A^{13}\mathrm{C}) \qquad [6]$$

Where 12 and 13 are the atomic weight of ^{12}C and ^{13}C. The ^{13}C amount ($^{13}C_Q$ in µg) in the respired CO_2 have been determined as:

$$^{13}C_Q = C_Q \times E^{13}C \qquad [7]$$

Where the $E^{13}C$ is the excess of ^{13}C (in %) compared to the bare soil control at the beginning of the experiment t0 ($E^{13}C = A^{13}C$ at time x - $A^{13}C$ of bare soil control at time 0, equal to 1.076 in topsoil and 1.082 in subsoil). After the plant derived C (pC in µg) was calculated as:

$$pC = \frac{^{13}C\mu g}{E^{13}Catm} \qquad [8]$$

Where $E^{13}Catm$ is the excess of ^{13}C in the chamber atmosphere (average of +0.8%). Finally, to calculate the percentage of C in CO_2 deriving from plants C input (f_{plant}, in %):

$$f_{plant} = \frac{pC}{C\mu g} \qquad [9]$$

The design of the ring did not allow to calculate in a reliable way the amount of respired C due to several technical issues. The clay soil, being kept at 45% of soil water content, formed a superficial crust that did not allowed the soil respiration to freely flow in the plastic dome used for sampling, reducing the respiration rate. Moreover, the presence of cracks that act as preferential pathways for gas dispersion made the diffusion of respiration unreliable. However, the percentage of 13C in air was reliable independently from the amount of respired CO2, and allowed the calculation of the percentage of new and old C in the respired CO2.

4.2.3. Soil and biomass sampling

The volume of soil in each pot (20 x 20 x 10 cm^3) was divided in two halves vertically with a saw. One half was air dried and used for the soil analysis and microbial measurements, and the other half was used for the measurement of plant traits. Plants were cut at the root collar to divide aboveground and belowground biomass. The resulting mixed sample of soil and roots were placed on a 2 mm sieve and carefully washed to disperse the soil, and the plant individuals were divided (if possible). Above- and belowground biomass was collected, oven dried at 60°C, and weighed to determine dry weight.). Following McCormack *et al.* (2015), we visually separated and sampled transporting (long, thick, high-order roots (>3) and absorptive roots (short, thin, low-order roots 1 – 3),, finely ground and analyzed with an Elementar Varo Isotope Cube to determine their C and $\delta^{13}C$ signal.

The soil half used for soil sampling was subsequently divided into shallow soil (0-3.5 cm) and deep soil (3.5-10 cm). Deep soil was air dried, crushed, mixed, and divided into four sections. One 5 ml scoop from every section was collected to form a composite subsample, then sieved at 2 mm. Three subsamples were collected for each replicate pot, and analyzed with an elemental analyzer Isoprime100 coupled with an Elementar Varo Isotope Cube, to determine C content, nitrogen (N) content and $\delta^{13}C$ signal. Samples collected at time 0 and at the end of the experiment (after 183 days, *t6*) were analyzed. The difference between t0 and t6 gave the changes in C after 6 months (ΔC).

Simultaneously, 40g of soil from the same deep layer of the pot of the bulk soil samples were collected and fractioned after Gavinelli et al. (1995) (See Chapter III: Method S1, Figure S1). The resulting five fractions (POM: >200µm, finePOM: 200-50µm, SILT: 50-20 µm, SILT+CLAY: <20µm) were analyzed for C and $\delta^{13}C$ with an elemental analyzer Isoprime100 coupled with an Elementar Varo Isotope Cube. The sum of C in different fractions represents the total C in the fraction sample (SUM). A subsample of 0.1 g was taken from each 40 g sample and analyzed without fractioning to determine the total C in the bulk sample. The difference between total C in bulk soil and the sum of C in the different soil fractions was used to assess the correctness of the fractionation and was equal to 93.3%.

The increased $\delta^{13}C$ signature of the atmosphere in the microcosm chamber, allowed the calculation of the proportion of C stored in soil directly derived from the input of *M. sativa* and *L. perenne* (new C), to differentiate it from the carbon already present in the soil at the beginning of the experiment (old C). To calculate the proportion, an isotope mixing model (after Balesdent and Mariotti 1996) was used:

$$\%Cnew = \frac{\delta(t1)-\delta(t0)}{\delta B-\delta(t0)} \quad [10]$$

Where %Cnew is the percentage of new carbon in the measured SOC of a specific fraction, $\delta(t1)$ is the $\delta^{13}C$ signature of the SOC measured in a specific fraction at the end of the experiment (t1), $\delta(t0)$ is the

$\delta^{13}C$ signature of the SOC of a specific soil fraction before the experiment (t0), δB is the $\delta^{13}C$ signature of the new C input in the system, in our case, the signature of the root biomass (i.e., the mean signature of absorptive and transport roots). The choice of root biomass as the $\delta^{13}C$ reference for C input was made since root material was considered the main source of fresh C, given that litter was removed every two weeks from the pots. Multiplying the total soil C by %Cnew provides the amount of new C in mgC g^{-1} soil.

Since the changes in carbon in the system (ΔC in mgC g^{-1} soil) are composed of the two fluxes: input of new C (ΔC_{NEW}) and changes in oldC, the changes of preexistent carbon in soil (ΔC_{OLD}) were calculated as:

$$\Delta \mathrm{C}_{OLD} = \Delta \mathrm{C} - \Delta \mathrm{C}_{NEW} \qquad [11]$$

The effect of vegetation regarding the losses of old C (priming, PE in in mgC g^{-1} soil) was calculated as:

$$\mathrm{PE} = \Delta \mathrm{C}_{OLD}\mathrm{V} - \Delta \mathrm{C}_{OLD}\mathrm{BS} \qquad [12]$$

Where $\Delta C_{OLD}V$ is the change in old C in the vegetated soil fraction (in mgC g^{-1} soil), while $\Delta C_{OLD}BS$ is the mean of old C changes in the bare soil controls (in mgC g^{-1} soil). If priming has positive values (positive PE) – it means that vegetation increases old C mineralization, with the value corresponding to an increased loss of old C in vegetated soil compared to bare soil. Likewise, if priming has negative values (negative PE), it means that vegetation decreases old C mineralization and losses, with the value corresponding to a decrease in old C loss in vegetated soil compared to bare soil.

4.2.4. Statistical analysis

The normal distribution of residues was verified using a Shapiro-Wilk test (αp = 0.05). If data were normally distributed, a one way analysis of variance (ANOVA) was performed to test the effects of soil type (topsoil versus subsoil) and plant species (*M. sativa*, *L. perenne*, bare soil) on C priming and $A^{13}C$ in respired CO_2.

If data were not normal a Kruskal – Wallis test was used. Linear regressions ΔC, new C, old C and priming with $A^{13}C$ in respired CO_2 were analyzed and R^2 and *p* values calculated. All the statistical analyses were performed using the open-source statistical environment ‘R’, version 3.4.3 (R Development Core Team, 2017) using the packages *vegan* and *Hmisc*. (Oksanen et al. 2019, Harrel 2007).

4.3. RESULTS

4.3.1. *Soil characteristics and changes in carbon content*

A positive increase in C in bulk soil after six months was found in topsoil planted with *M. sativa* only (ΔC, Table 1). However, on subsoil, a net loss of total ΔC over 6 months was found. In bare soil and soil planted with *L. perenne*, losses of total ΔC were higher in topsoil than subsoil, with the greatest loss in bare topsoil (Tables 1, 2). In bulk soil, new C was significantly greater in topsoil than in subsoil (Tables 1, 2) and regardless of soil type, new C input was always greater in soils planted with *M. sativa* compared to *L. perenne* (Table 2).

Based on bulk soil data, the losses of old C in topsoil are the highest in bare soil compared to *L. perenne* and *M. sativa*, which are not statistically different (Table 1, Table 2). In subsoil, old C losses were significantly lower than in topsoil, with the most losses in soil planted with *M. sativa*, compared to bare soil and *L. perenne* (Table 1).

The losses of old C in SUM of fractions were lower in topsoil and not significantly different among fractions or between species and bare soil (Table 1). In subsoil, SUM was comparable in soils planted with *L. perenne* and *M. sativa*, however the losses in bare soil are lower in the SUM compared to bulk soil data.

After 6 months we observed that vegetation significantly influenced N content in both subsoil and topsoil when sowed with *M. sativa*, compared to bare soil. Soil N content at t6 for *L. perenne* did not show any significant change compared to that at t0 (Fig. S8).

4.3.2. *Priming effect*

In bulk soil that had vegetation present, PE was negative in topsoil and there was no significant effect of species (Fig. 2a). In bulk soil subsoil planted with *M. sativa*, PE was positive and old C loss was significantly greater than bare soil, but in subsoil planted with *L. perenne*, old C loss was not significantly different from bare soil and there was no PE (Fig. 2b). In topsoil SUM, PE was negative but was not significant different between plant species, although it had a lower intensity compared to bulk soil (Fig. 2a). In subsoil, the PE in SUM was positive, although there were no diferences among species (Fig. 2b). In topsoil fractions, PE was negative except in SILT+CLAY, and no sigificant differences occurred in fractions between *L. perenne* and *M. sativa*, except in fine POM (Fig. 2a). In subsoil, priming was positive in each fraction, with the highest effect in SILT and SILT+CLAY fractions, and the only difference between *L. perenne* and *M. sativa* occurred in the POM fraction (Fig. 2b).

4.3.3. Evolution of ^{13}C abundance in respired CO_2 ($A^{13}C$) over 6 months

There was a significant effect of both soil type (Kruskal-Wallis, $p < 0.001$) and vegetation treatment (Kruskal-Wallis, $p < 0.001$) on the abundance of ^{13}C in respired CO_2 ($A^{13}C$). In topsoil, the abundance of ^{13}C in the respired CO_2 increased over 6 months, with the highest $A^{13}C$ from *L. perenne* (2.02%) and *M. sativa* on 20/02/18 (1.99%), while in bare soil, $A^{13}C$ was greatest on 09/02/18 (1.96%). In tospoil there was a significant effect of treatment (Kruskal-Wallis, $p < 0.001$) (Fig. 3a,b,c). Over six months, in topsoil planted with *M. sativa*, the abundance of ^{13}C in CO_2 increased from 1.55±0.05 % to 1.73±0.03 % (with an increment of +11.2%). In the same period, the abundance of ^{13}C in CO_2 from *L. perenne* incraesed from 1.51±0.07 %to 1.7±0.02 % (with an increment of +13%), and bare soil increased from 1.43±0.13 % to 1.61±0.07 % (+13%).

In subsoil, the highest peak of $A^{13}C$ was found under *M. sativa* the 20/02/18 (1.49±0.07%), followed by bare soil on 06/03/18 (1.41±0.07%) and *L. perenne* on 23/01/18 (1.37±0.04) (Fig. 3d,e,f). The effect of treatment was significant (Kruskal-Wallis, $p < 0.001$), with the highest $A^{13}C$ under *M. sativa*. No sigificant differences between bare soil and that planted with *L. perenne* were found. In subsoil, $A^{13}C$ of soil respiration under *M. sativa* increased from 1.31±0.01% to 1.41±0.05% (with an increment of +9%) and bare soil from 1.26±0.01% to 1.32±0.03% (with an increment of +5%), while *L. perenne* decreased from 1.32±0.04% to 1.31±0.02% (with an decrement of -0.2%), however the decrease was not significant (Fig. 3e).

4.3.4. Evolution of ratio of CO2 derived from fresh plant new C input (f_{Plant})

There is a significant increase of respired CO_2 derived from fresh plant inputs (f_{Plant}) in the vegetated treatments over the 6 month period. Topsoil had a +152% increase of f_{Plant}, and subsoil had a +84% increase. When observing the trend over time, topsoil had a greater increase than subsoil in f_{Plant} (Fig. 4; $p < 0.001$)

4.3.5. Correlations between OldC loss, NewC input, priming and $A^{13}C$

In bulk soil, both new C and old changes in soil and the $A^{13}C$ were positively and significantly correlated with the $A^{13}C$ of respired CO_2 (all data grouped together). Data points from different species were clearly seggregated, with more changes in new C and old C and $A^{13}C$ in respired CO_2 for *M. sativa* (Fig. 5a,b). In the respired CO_2, $A^{13}C$ was negatively correlated with priming effect (Fig. 5c; Kruskal - Wallis, $p < 0.01$).

4.4. DISCUSSION

We observed a strong influence of soil type on old C stability and soil priming for both total C and that in the SILT+CLAY pool, which masked the effect of plant species. Revegetating topsoil results in a negative priming effect, with a lower mineralization of old C compared to the bare soil control. However, in subsoil, the priming effect depends on the species, with no priming effect under *L. perenne*, and a positive priming effect under *M. sativa,* that increased old C losses compared to the bare soil control. We will tackle these effects separately for the sake of clarity, but they are nonetheless closely linked and the C-cycle depends on a feedback mechanism between soil, plants and microbial communities.

4.4.1. Subsoil and topsoil revegetation: identifying the substrate preference of microbial communities

The priming effect was highly negative in topsoil, but was marginally positive in subsoil. On topsoil a high input of fresh new C allowed microbiological communities to change the substrate preference for energy and nutrients requirements from old C in soil to fresh new C inputted by plants. This phenomenon seems to support the Preferential Substrate Utilization hypothesis (Cheng, 1996; Cheng and Kuzyakov, 2005). However, negative PE is ususally for a short period of time; and after positive PE starts (Cheng, 1996; Kuzyakov and Cheng, 2001, 2004; Wang et al., 2016). It is therefore crucial to have long term experiment with living plants to assess the PE over a longer period of time. Subsoil had less N than topsoil, resulting in reduced plant development (Chapter III, this thesis), and a consequent lower input of labile C into soil from rhizodeposition. As subsoil is subjected to long-term limitation of nutrients, microbial functioning is decreased, thus promoting the development of oligotrophic communities with high metabolic diversity (Blagodatskaya and Kuzyakov, 2008). This set of conditions makes subsoil a perfect candidate for positive priming effect. Specific dormant microbial groups can be activated by the input of low available substrates, such as oxalic acid, that have a high impact on community shift, and synthetize a broad variety of enzymes that promote old C decomposition and a positive priming (Falchini et al., 2003; Landi et al., 2006; Blagodatskaya and Kuzyakov, 2008). Finally, fungi have been shown to play an important role in C degradation and priming (Panikov 1995; Bell et al. 2003; Blagodatskaya and Kuzyakov, 2008). The input of fresh C might activate spore or cysts dormant in subsoil, which can penetrate previously inaccessible micro zones with hyphae development (Blagodatskaya and Kuzyakov, 2008). The low amount of labile C input, together with the low N availability, will not be enough to shift the increased microbial metabolism towards labile C input in a significant way, and microbial communities will increase old C mineralization to acquire energy and nutrients (De Graaf et al., 2010). In topsoil, instead, the higher biomass development and the consequent high input of labile fresh C from plants will enhance fungal: bacterial gene copy ratios (Griffiths et al., 1998; Broeckling et al., 2008; Chiginevaa et al., 2009). Our data showed higher microbial abundance and diversity, as well as a higher level of activity (Chapter III, this thesis), corresponding well to such a phenomenon. The higher inputs of labile C in the system may, nevertheless, shift the microbial preferential consumption from preexistent old C to fresh C input (as shown in Fig. 5), and result in a negative priming effect. This phenomenon is well supported by the A^{13}C and percentage of plant derived C in respired CO_2, that was always higher in topsoil compared to subsoil.

Experiments investigating substrate preference or competition mechanisms effect on PE have usually been performed in controlled incubation conditions (e.g., Fontaine et al., 2007; De Graaf et al., 2010). Our study is novel in that plants were grown in different types of non-sterilised soil. Also, we demonstrate that the SILT and SILT+CLAY C pools played a pivotal role in determining the amount and trajectory of PE. Particularly in subsoil, where PE was positive in SILT and SILT+CLAY C pools, thus questioning the point of view that these C pools are highly stable (Chapters II and III, this thesis).However, SILT+CLAY C pools is very reactive to input of new C (Chapter III, this thesis) and Fontaine et al. (2007) showed how input of fresh C in subsoil ca increase the mineralization of stable C and lead to positive PE. Our results suggest that SILT+CLAY pool is stable when conditions are not abruptly changed: in topsoil positive PE is present only in POM. An abrupt change of conditions, however, can bring to instability of C associated with fine soil fractions and the resulting PE, as observed in subsoil SILT and SILT+CLAY.

4.4.2. The impact of plants on the two soil types: competition for nitrogen

The effect of vegetation on PE was largely influenced by soil type. We did not observe any significant difference in PE between topsoil planted with *M. sativa* or *L. perenne*. Plant species had more influence on PE in subsoil, despite the disparity between the results from bulk soil and from fractionation. *L. perenne* better mitigated the undesired positive PE than *M. sativa*, especially in bulk soil, where no PE occurred in subsoil sown with *L. perenne*. This result on of plant species effect on PE in subsoil, however limited, is in line with the competition hypothesis (Cheng and Kuzyakov, 2005). When plants are grown on an N poor soil, mineralization of old SOC from microbial communities can be reduced due to more efficient N removal by plant roots, that hinders microbial activity, resulting in a negative priming effect (Cheng & Kuzyakov, 2005). This phenomenon can explain the effect of *L. perenne* on positive PE mitigation in subsoil. Increased rhizodeposition will increase old C consumption and can result in a positive PE. This mechanism is exactly what we observe when planting soil with *M. sativa* which has a higher biomass development than *L. perenne* on subsoil (Chapter III, this thesis), and therefore lowers rhizodeposition (Fu and Cheng, 2002; Cheng et al., 2003; Dijkstra et al., 2006). Moreover, *M. sativa* is associated with *Rhizobium* bacteria that allow fixation of N_2 directly from the atmosphere, and rely less on N mining for growth. In this case, the 'competition effect' is avoided because *M. sativa* can acquire N from a different source. To test this hypothesis, we analyzed the difference in N content in soil between time 0 and time 6 months under the three different treatments and two soils (Fig. S8). We observed that bare soil and *L. perenne* do not significantly differ from t0 and between each other. However, *M. sativa* increased the amount of soil N, strengthening our hypothesis that competition for N was decreased. Also, substrates from fresh plants' new C input become available, and it increases microbial activity but do not permit a substrate preference switch, resulting in higher mineralization of old C and a positive priming effect (De Graaf et al., 2010). These results are supported in several studies, where N-rich rhizodeposition is believed to be linked with higher PE (Fu and Cheng, 2002; Cheng et al., 2003; Cheng and Kuzyakov, 2005). In topsoil, such a phenomenon was not observed. We speculate that higher fertility levels and rhizodeposition level (Chapter III, this thesis) mask the competitive effect between roots and microorganisms, providing enough nutrients via plants fresh C deposition to the microorganisms to allow them not to have to rely on mining soil C and compete with plants (Cheng and Kuzyakov, 2005; De Graaf et al., 2010).

Such a framework considering both soil and vegetation features incorporate the two hypotheses: the Substrate Preferential Utilization hypothesis and the Competition hypothesis (Fig.6), sopprting the reconciliation reconciliation between the two proposed by Cheng and Kuzyakov (2005): in fertile soils, the substrate preference will drive the PE, while in poor soil competition will shape the trajectory and magnitude of PE.

4.4.3. The priming effect and its implication in practice

From an applied point of view, when revegetating soil in geotechnical constructions, especially subsoil, it is necessary to consider soil fertility, as it will affect i) biomass development, ii) nutrient competition in soil and fresh substrate availability and, consequently, iii) the priming effect. In nutrient poor subsoils, the use of non N_2-fixing species (e.g. *L. perenne*) will result in a low priming effect. A possible solution to avoid the priming effect when revegetating subsoil (or nutrient poor soils in general) could be to couple inoculation of microbial strains that consume labile C with N fertilization, to increase fertility and nutrient availability, and try to switch the microbial consumption from preexistent oldC to new C.

4.5. CONCLUSIONS

We examined the priming effect in a crossed experimental design with two soil types and two plant species. We highlighted the complex interactions between the two sources of factors and demonstrated the importance of soil quality (in terms of N content and microbiological activity and biomass) in determining the trajectory and magnitude of PE over that of plant species. When soil quality is high, such as topsoil, positive PE can be mitigated and negative PE can occur thanks to high fresh new C input. However, in N-poor subsoils, old soil C, especially the stable old C in the SILT+CLAY pool, can be susceptible to the PE, depending on the competition between plants and soil microorganisms. Therefore, plant species could play a non-negligible role in influencing the tendency and magnitude of PE.

Our results suggest that topsoil, with higher rhizodepositions, allows microbial communities to switch from consuming old C to new C mineralization, resulting in a negative priming effect. In subsoil, microbes will mine old C for nutrients due to low new C input. Competition for N is fundamental to shape the priming effect, and in poor subsoil, *L. perenne* had no priming effect due to N competition between plants and microbes. Therefore, when a soil is severely limited in nutrients the competition effect will be predominant; while when conditions are not so limiting the substrate preference will dominate. These findings are in line with the reconciliation of hypothesis from Cheng and Kuzyakov (2005). The $A^{13}C$ and its correlations with old C losses and priming helps to understand the processes in different soils, but alone this is not enough to investigate the effects of priming. Old C losses of vegetated and bare soil control need to be taken into account.

REFERENCES

Andersen, T.H., Domsche, K.H. 1989. Ratios of microbial biomass carbon to total organic carbon in arable soils. Soil Biology and Biochemistry21:471–479

Balesdent, J. & Mariotti, A. 1996. Measurement of soil organic matter turnover using I3C natural abundances. In: Mass Spectrometry of Soils (eds T.W. Boutton & S. Yamasaki), pp. 83-1 11. Marcel Dekker Inc., New York.

Bell, J.M., Smith, J.L., Bailey, V.L., Bolton, H 2003. Priming effect and C storage in semi-arid no-till spring crop rotations. Biology and Fertility of Soils 37:237–244

Blagodatskaya, E., Kuzyakov, Y., 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: Critical review. Biology and Fertility of Soils 45, 115–131.

Broeckling, C.D., Broz A.K., Bergelson, J., Manter, D.K., Vivanco, J.M., 2008 Root exudates regulate soil fungal community composition and diversity. Appliedand Environmental Microbiology 74: 738–744

Chabbi, A., Kögel-Knabner, I., Rumpel, C., 2009 Stabilised carbon in subsoil horizons is located in spatially distinct parts of the soil profile. Soil Biology and Biochemistry41:256–271

Cheng W., 1996 Measurement of rhizosphere respiration and organic matter decomposition using natural 13C 263–268.

Cheng, W., Johnson, D.W., Fu, S., 2003 Rhizosphere effects on decomposition: controls of plant species, phenology, and fertilisation. Soil Science Society of America Journal 67, 1418e1427

Cheng, W., Kuzyakov, Y., 2005 Root effects on soil organic matter decomposition. In: S. Wright, S., Zobel, R. (Eds.), Roots and Soil Management: Interactions Between Roots and the Soil. Agronomy Monograph No. 48, American Society of Agronomy, Madison, Wisconsin, USA 119–143.

Chiginevaa, N.I., Aleksandrovab, A.V., Tiunovc, A.V., 2009. The addition of labile carbon alters residue fungal communities and decreases residue decomposition rates. Applied Soil Ecology 42: 264–270.

De Deyn, G.B., Cornelissen, J.H.C., Bardgett, R.D., 2008. Plant functional traits and soil carbon sequestration in contrasting biomes. Ecology Letters 11, 516–531.

De Graaff, M.A., Classen, A.T., Castro, H.F., Schadt, .CW., 2010. Labile soil carbon inputs mediate the soil microbial community composition and plant residue decomposition rates. New Phytologist 188, 1055–1064.

Dijkstra, F.A., Cheng, W.X., Johnson, D.W., 2006. Plant biomass influences rhizo- sphere priming effects on soil organic matter decomposition in two differently managed soils. Soil Biology and Biochemistry 38, 2519e2526.

Ekklund, F., Ronn, R., Christensen, S. 2001. Distribution with depth of protozoa, bacteria and fungi in soil profiles from three Danish forest sites. Soil Biology and Biochemistry33:475–481

Eusterhues, K., Rumpel, C., Ko¨ gel-Knabner, I., 2005b. Organo–mineral associations in sandy acid forest soils: importance of specific surface area, iron oxides and micropores. European Journal of Soil Science 56, 753–763

Falchini, L., Naumova, N., Kuikman, P.J., Bloem, J., Nannipieri P. 2003. CO2 evolution and denaturing gradient gel electrophoresis profiles of bacterial communities in soil following addition of low molecular weight substrates to simulate root exudation. Soil Biology and Biochemistry35:775–782

Fang, C., Moncrieff, J.B. 2005. The variation of soil microbial respiration with depth in relation to soil carbon composition. Plant and Soil 268:243–253

Fontaine, S., Bardoux, G., Abbadie, L., Mariotti, A., 2004. Carbon input to soil may decrease soil carbon content. Ecology Letters 7, 314–320.

Fontaine, S., Barot, S., Barré, P., Bdioui, N., Mary, B., Rumpe, C. 2007. Stability of organic carbon in deep soil layers controlled by fresh carbon supply. Nature 450, 277–280.

Fu, S. and Cheng, W. 2002. Rhizosphere priming effects on the decom- position of soil organic matter in C4 and C3 grassland soils. Plant Soil 238, 289–294.

Gavinelli, E., Feller, C., Larré‐ Larrouy, M.C., Bacye, B., Djegu, Z., Nzila, J.D., 1995. Routine method to study soil organic matter by particle‐ size fractionation: Examples for tropical soils. Soil Science Plant Analysis 26(11&12), 1749-1760

Griffiths, B.S., Ritz, K., Ebblewhite, N., Paterson, E., Killham, K., 1998.: Ryegrass rhizosphere microbial community structure under elevated carbon dioxide concentrations, with observations on wheat rhizosphere. Soil biology and Biochemistry. 30, 315±321

Hamer, U., Marschner, B., 2005. Priming effects in soils after combined and repeated substrate additions, Geoderma 128, 38–51

Harrell, F.E., 2007. Package ‘Hmisc’. Harrell Miscellaneous

Holden, P.A., Fierer, N. 2005. Microbial processes in the vadose zone. Vadose Yone Journal 4, 1–21

Huo, C., Luo, Y., Cheng, W., 2017. Soil Biology & Biochemistry Rhizosphere priming effect : A meta-analysis. Soil Biology and Biochemistry 111, 78–84.

Kuzyakov, Y., Domanski, G. 2000.. Carbon input by plants into the soil. Review. Journal of Plant Nutrition and Soil Science 163, 421–431.

Kuzyakov, Y., Friedel, J.K., Stahr, K., 2000. Review of mechanisms and quantifcation of priming effects . Soil Biology and Biochemistry 32, 1485-1498

Kuzyakov, Y., Cheng, W.X., 2001. Photosynthesis controls of rhizosphere respiration and organic matter decomposition. Soil Biology & Biochemistry 33, 1915–1925.

Kuzyakov, Y., Cheng, W.X., 2004. Photosynthesis controls of CO2 efflux from maize rhizosphere. Plant and Soil 263, 85–99.

Kuzyakov, Y., 2006. Sources of CO2efflux from soil and review of partitioning methods. Soil Biology and Biochemistry 38, 425–448.

Lal, R, 2004. Soil carbon sequestration impacts on global change and food security. Science 304: 1623-1627. 304.

Landi, L., Valori, F., Ascher, J., Renella, G., Falchini, L., Nannipieri, P. 2006. Root exudate effects on the bacterial communities, CO2 evolution, nitrogen transformations and ATP content of rhizosphere and bulk soils. Soil Biology and Biochemistry38:509–516

Le Bissonnais, Y., 2016. Aggregate stability and assessment of soil crustability and erodibility: I. Theory and methodology. European Journal of Soil Science 67, 11–21.

Liang, C., Schimel, J.P., Jastrow, J.D., 2017. The importance of anabolism in microbial control over soil carbon storage. Nature Microbiology 2, 1–6.

Minasny, B., Malone, B.P., McBratney, A.B., Angers, D.A., Arrouays, D., Chambers, A., Chaplot, V., Chen, Z.S., Cheng, K., Das, B.S., Field, D.J., Gimona, A., Hedley, C.B., Hong, S.Y., Mandal, B., Marchant, B.P., Martin, M., McConkey, B.G., Mulder, V.L., O'Rourke, S., Richer-de-Forges, A.C., Odeh, I., Padarian, J., Paustian, K., Pan, G., Poggio, L., Savin, I., Stolbovoy, V., Stockmann, U., Sulaeman, Y., Tsui, C.C., Vågen, T.G., van Wesemael, B., Winowiecki, L., 2017.. Soil carbon 4 per mille. Geoderma 292, 59–86.

. Ohm, H., Hamer, U., Marschner, B. 2007. Priming effects in soil size fractions of a podzol Bs horizon after addition of fructose and alanine. Journal of Plant Nutrition and Soil Science. 170: 551–559.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., Szoecs, E., Wagner, H., 2019. Package 'vegan'. Community Ecology Package

Panikov, N.S. 1995. Microbial growth kinetics. Chapman & Hall, London

Perveen, N., Barot S., Maire V., Cotrufo M.F., Shahza T., Blagodatskaya T., Stewart C.E, Ding W., Siddiq, Dimassi B, Mary B, Fontaine S., 'Universality of priming effect: An analysis using thirty five soils with contrasted properties sampled from five continents', Soil Biology and Biochemistry journal, 134, 162-171

Rumpel, C., Kögel-Knabner, l., 2011. Deep soil organic matter-a key but poorly understood component of terrestrial C cycle. Plant and Soil 338, 143–158.

Shahzad, T., Chenu, C., Genet, P., Barot, S., Perveen, N., Mougin, C., and Fontaine, S., 2015. Contribution of exudates, arbuscular mycorrhizal fungi and litter depositions to the rhizosphere prim- ing effect induced by grassland species. Soil Biology and Biochemistry 80, 146–155, 2015

Smith, P., Powlson, D.S., Smith, J.U., Falloon, P., Coleman, K., 2000. Meeting Europe's climate change commitments: Quantitative estimates of the potential for carbon mitigation by agriculture. Global Change Biology 6, 525–539.

Taylor, J.P., Wilson, B., Mills, M.S., Burns, R.G., 2002. Comparison of microbial numbers and enzymatic activities in surface soils and subsoils using various techniques. Soil Biology and Biochemistry 34, 387-401

von Lutzow, M., Kogel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B., Flessa, H. 2006. Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions—a review. European Journal of Soil Science, 57, 426–445

Wang, X., Tang, C., Severi, J., Butterly, C.R., Baldock, J.A., 2016. Rhizosphere priming effect on soil organic carbon decomposition under plant species differing in soil acidification and root exudation. New Phytologist 211, 864–873

FIGURES AND TABLES

Table 1: ΔC is the difference of C content in bulk soil between time 0 and time 6 months in mgC g^{-1} soil. New C is the input of C in bulk soil deriving from the vegetation growth during the 6 months of the experiment in mgC g^{-1} soil. Old C is the losses of old C in bulk soil. The last column shows the losses of old C in the sum of fractions data. ΔC new C, and old C have been calculated for each treatment (*M.sativa*, *L. perenne* and bare soil) and each soil (topsoil: 0-30cm depth, subsoil: 110-140cm depth). Different letters next to the average value indicate statistically significant differences ($p < 0.05$) between species or families according to Tukey HSD tests.

	Data set:	Bulk soil data			SUM of fractions
Soil	Treatment	**ΔC** (mgC g^{-1} soil)	**New C** (mgC g-1 soil)	**Old C** (mgC g-1 soil)	**Old C** (mgC g-1 soil)
Topsoil	bare soil	(c) -1.60±0.20	(c) 0.10±0.05	(b) -1.70±0.15	(a) -0.36±0.44
	L. perenne	(b) -0.48±0.33	(b) 0.68±0.09	(a) -1.16±0.27	(a) -0.36±0.40
	M. sativa	(a) 0.01±0.41	(a) 1.22±0.16	(a) -1.21±0.29	(a) -0.26±0.02
Subsoil	bare soil	(b) -0.50±0.07	(c) 0.04±0.03	(ab) -0.54±0.08	(a) -0.24±0.35
	L. perenne	(a) -0.26±0.08	(b) 0.22±0.03	(b) -0.49±0.06	(ab) -0.59±0.07
	M. sativa	(a) -0.17±0.18	(a) 0.45±0.11	(a) -0.61±0.1	(b) -0.67±0.23

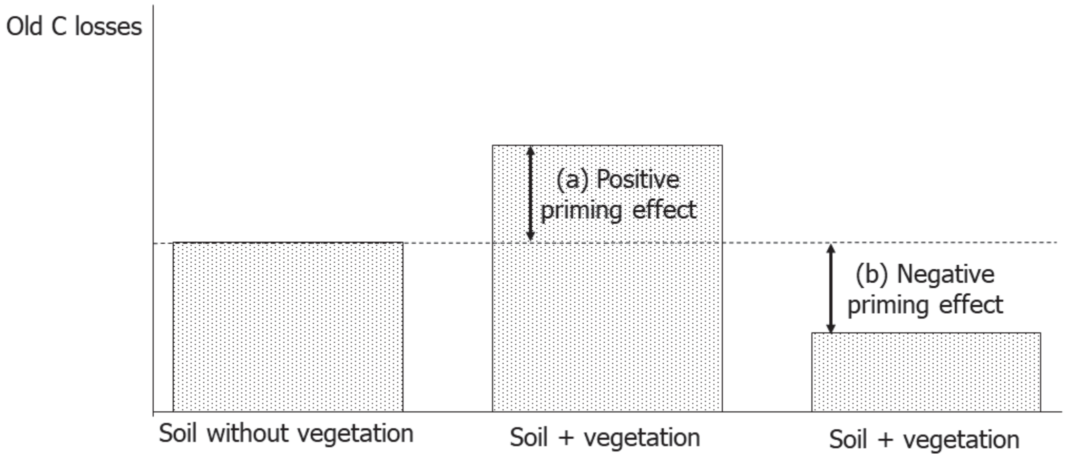


Figure 1: Graphic explanation of positive priming effect (a) and negative priming effect (b).

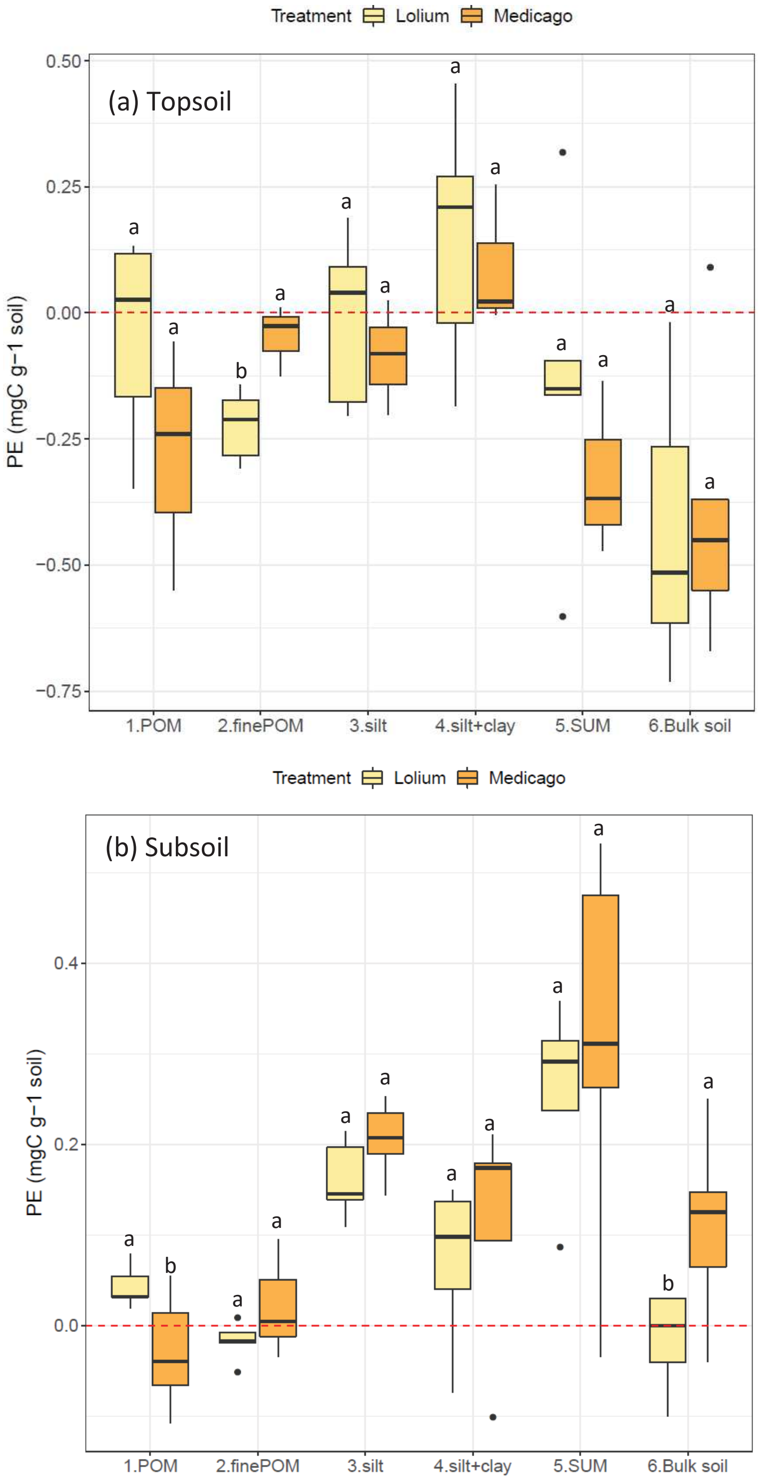


Figure 2: Comparison of priming effect after 6 months of revegetation between different soils ((a) topsoil and (b) subsoil) for each vegetated treatment (*L. perenne* light yellow*, M. sativa* orange). The data presented shows the priming in the different soil fractions (1.POM, 2.finePOM, 3.silt, 4.silt+clay) the sum of the soil fractions (5.SUM) and the bulk soil data (6.Bulk soil). Negative values means a reduced loss of old C, positive values an increased loss of old C. In each boxplot, the lower edge of the box corresponds to the 25th percentile data point, while the top edge of the box corresponds to the 75th percentile data point. The line within the box represents the median. Different letters above the boxplots indicate statistically significant differences ($p < 0.05$) among species according to a ANOVA test

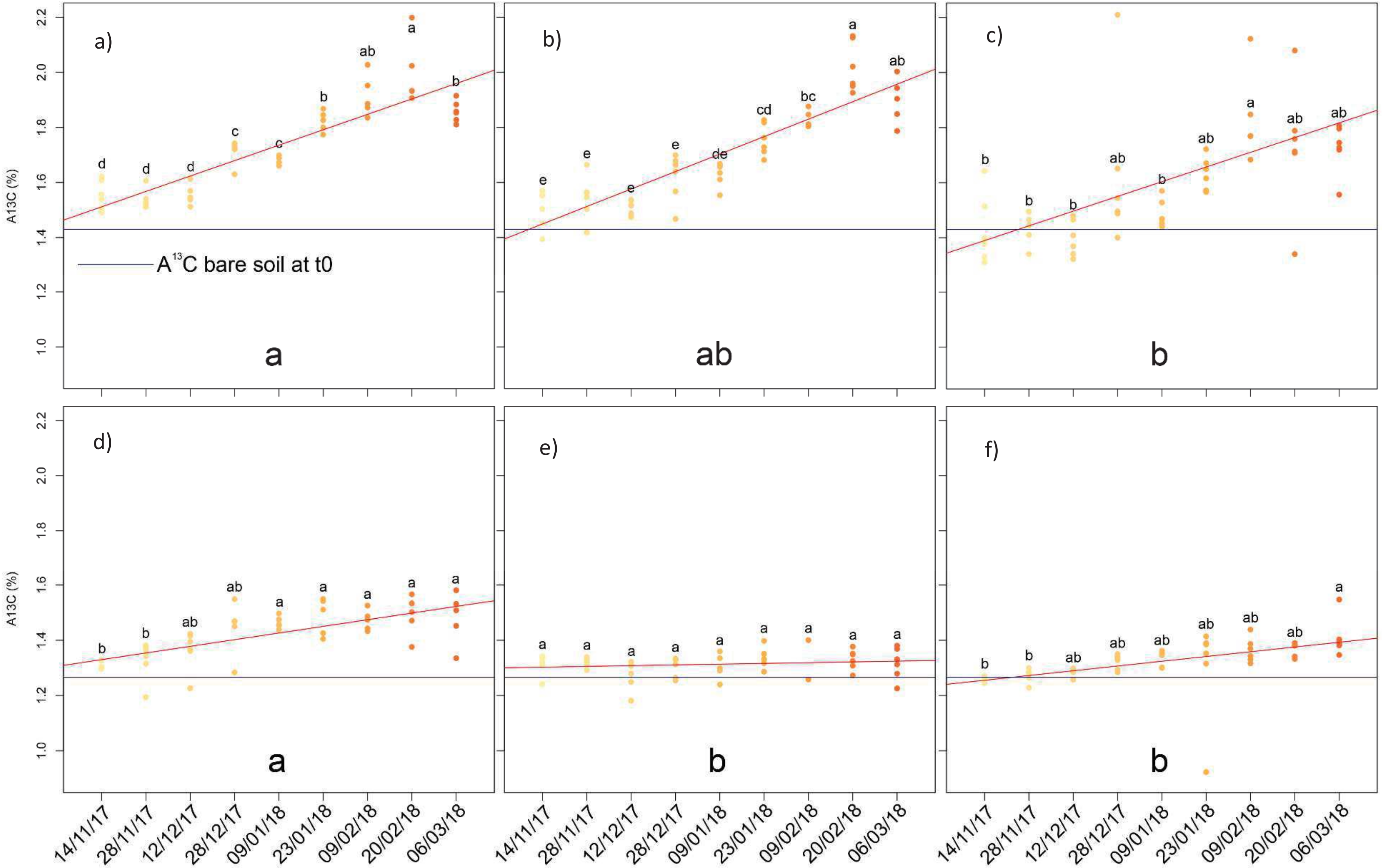


Figure 3: Evolution over time of $A^{13}C$, as the percentage of ^{13}C in the respired CO_2. The results shows the trend for a) topsoil vegetated with *M.sativa*, b) topsoil with *L.perenne* and c) topsoil bare soil control. The second row shows the results for d) subsoil sowed with *M.sativa*, e) subsoil with *L.perenne* and f) subsoil bare soil control. The different dots shows the A13C result for the single subsample of the treatment in shade of yellow/red according to the sampling time. Different letters above the dots indicate statistically significant differences ($p < 0.05$) according to a Tukey HSD test among different sampling dates. Different letters at the bottom of the graph indicate statistically significant differences ($p < 0.05$) according to a Tukey HSD test among different treatments.

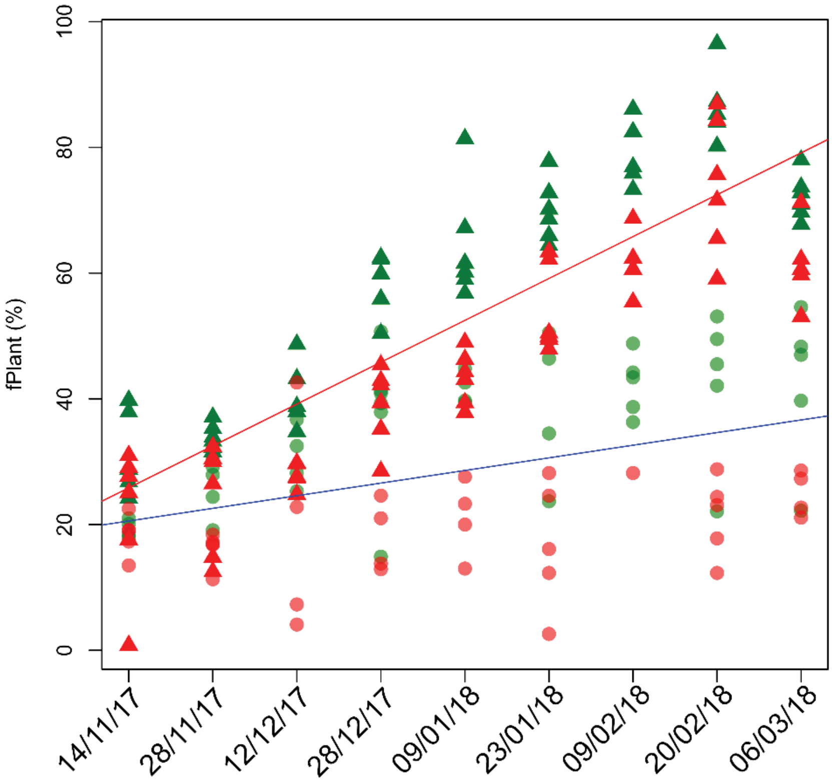


Figure 4: Percentage of C derived from mineralization of plant inputs in soil respired CO_2 (f_{Plant}) over 4 months in the two vegetated soils. Triangles represent topsoil and dots subsoil. Green represent *M. sativa* and red *L. perenne*, different saturations have been given to better differentiate the points. The red solid line represent the increase according to a linear model of the f_{Plant} in topsoil over the 4 months, the blue solid line in subsoil. The slopes of the linear models are significantly different, $p < 0.001$.

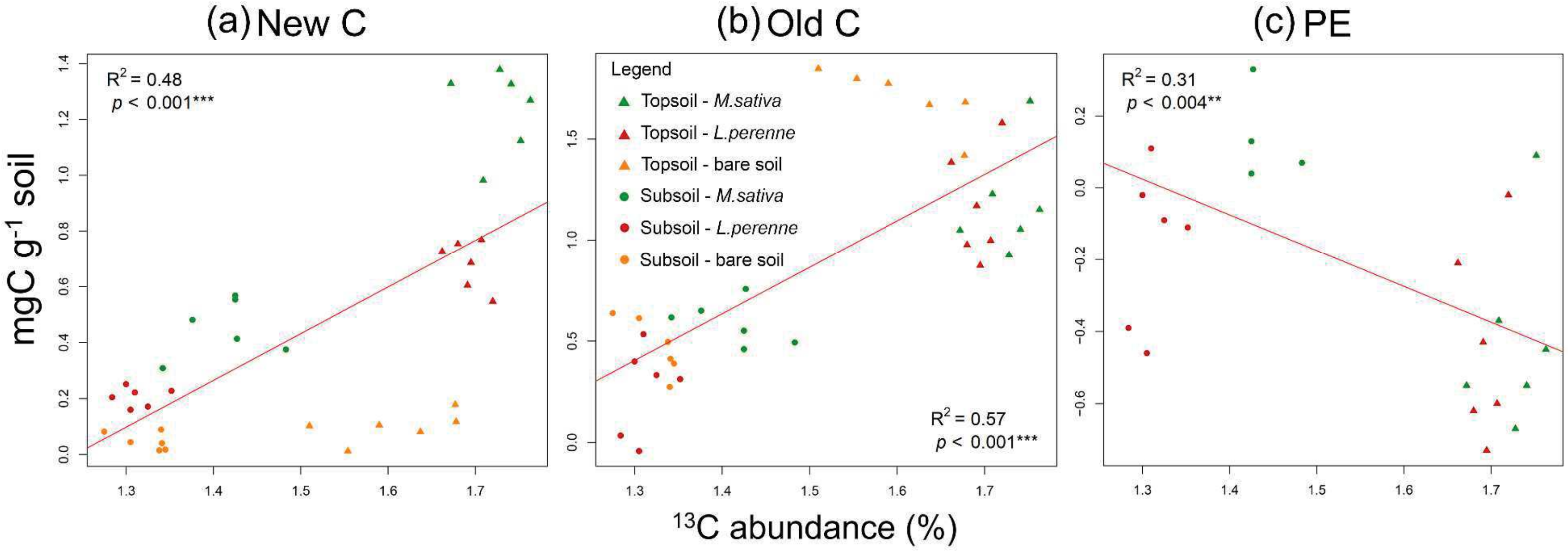


Figure 5: Correlations between a) New carbon inputs in soil, b) old SOC C losses (OldC) and c) priming effect (Priming), with abundance of ^{13}C in respired CO_2 (A^{13}C). Topsoil is represented by triangles, subsoil by dots, *M.sativa* is green, *L.perenne* red and bare soil control orange. The red line shows the correlation according to a linear model, and the significance (R^2, p) is shown in a corner box in every graph.

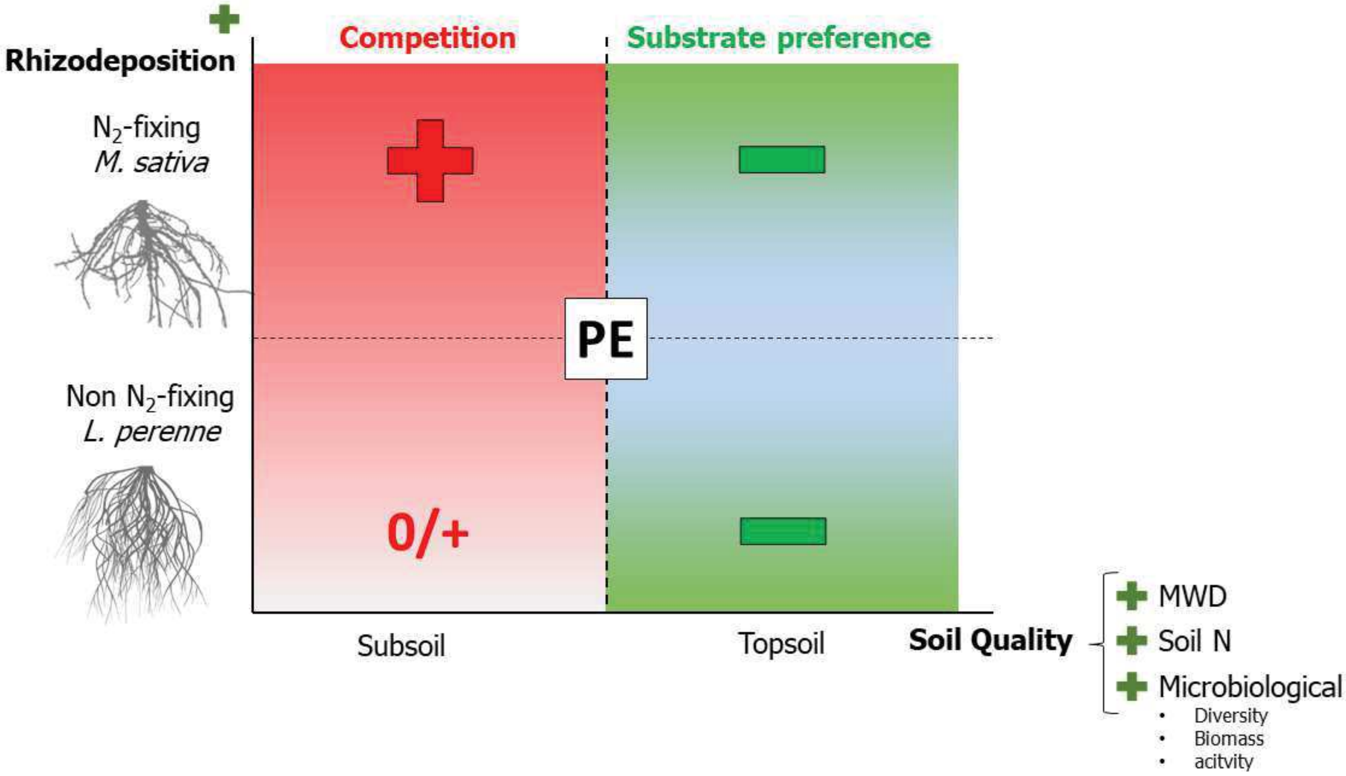


Figure 6: Reconciliation of Preferential Substrate Utilization hypothesis and competition hypothesis. a) shows the effect of soil, where the higher input in the fertile soil allow microbial communities to switch preference of substrate and decrease old C mineralization, while in subsoil, with low fertility and low input of fresh new C, competition drive the priming effect that is generally higher than topsoil. b) shows the species effect in the two soil conditions. In subsoil low rhizodeposition from *L. perenne* stimulate and competition for N hinder old C mineralization and result in a slightly negative priming effect. In topsoil, the N rich rhizodeposition from *M. sativa* increase the soil N content and decrease competition, allowing microbial communities to mine more efficiently old C and resulting in positive priming effect. In topsoil, contrary to what expected, we did not find any difference between *L. perenne* and *M. Sativa* in priming effect, suggesting a lower influence of rhizodeposition when the system is rich and efficiently colonized by roots.

SUPPLEMENTARY MATERIALS

Figure S1 : Pot preparation. Starting from left to right : Quartile of soil prior to filling the pots, weighting of the pots while filling and examples of two pots filled with the two different types of soil The soil has been added collecting one scoop of soil from each quartile and keep moving to the next quartile, in the same order, until the desired weight was reached.

Figure S2 : From left to right: example of the ring used for soil respiration analysis, ring with the plastic dome in place and ring inside the soil, the red crosses mark the spots were the seeds were planted

Figure S3 : Pots positioned in the growing chamber at a fix distance from lights in the Ecotron facilities

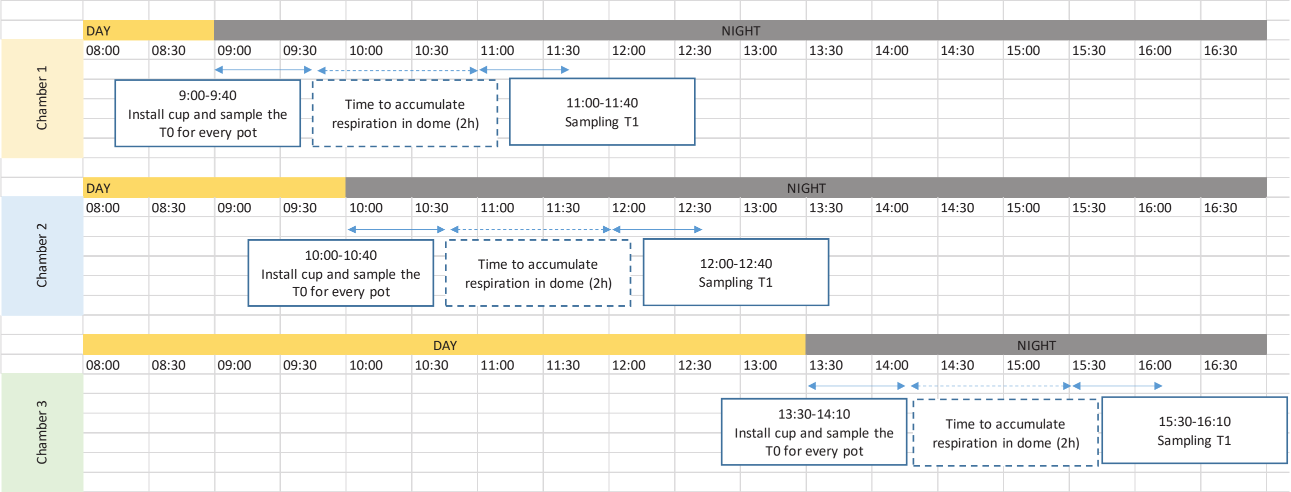


Figure S4 : Day and night cycles in the three different grow chambers with timing for air samples collection

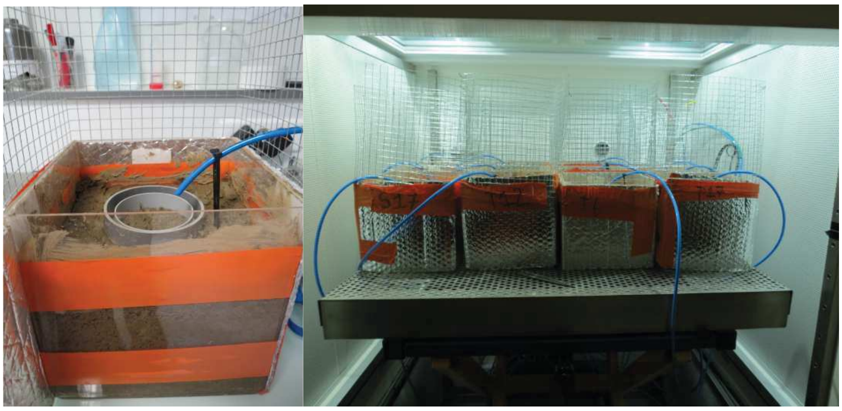


Figure S5 : plastic tubes used for irrigation, fix on the single pot on the left and positioned in the chamber on the right

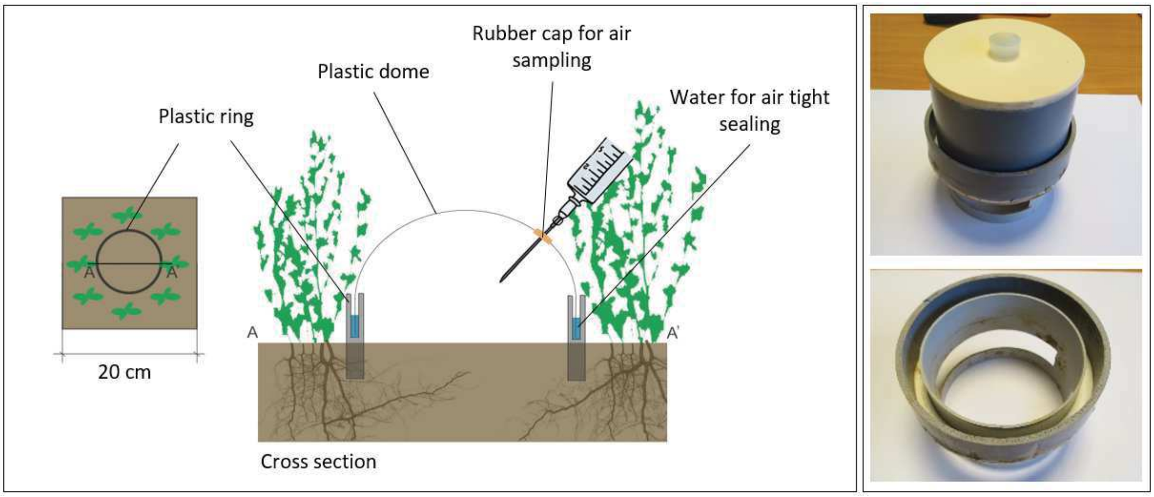


Figure S6 : Scheme of the plastic dome used for soil respiration analysis

Figure S7 : Air sampling protocol : a) fill ring with water, b) place the plastic cap on top, c) take a 5ml air samples using a syringe on the rubber cap and d) transfer the air taken in the airtight exitainers

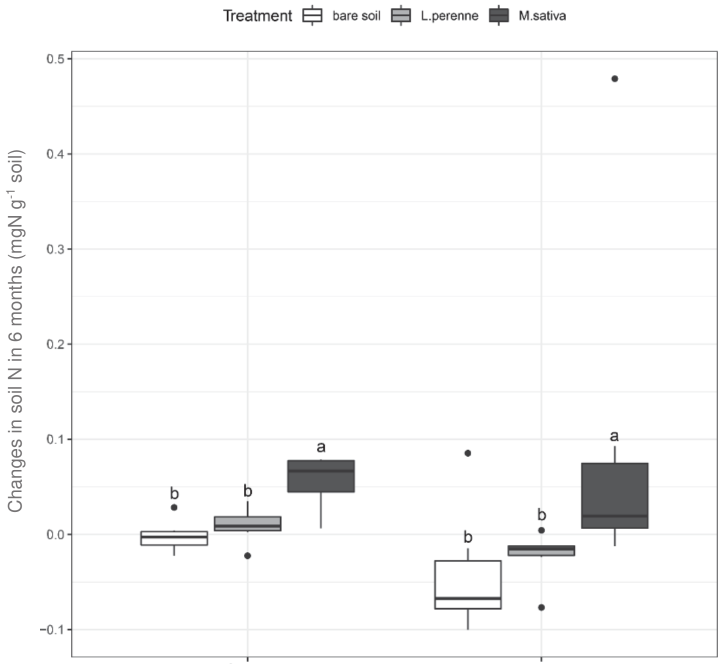


Figure S8: Comparison of N content in soil between the beginning of the experiment (t0) and the end (t6) in the two soils (subsoil and topsoil) and the three treatments (*M.satva, L.perenne*, and bare soil). No significant differences have been found between bare soil and *L.perenne*, which show no changes from the initial C content. *M.sativa*, instead, is significantly different compared to the other treatments, showing an increase of soil N. In each boxplot, the lower edge of the box corresponds to the 25th percentile data point, while the top edge of the box corresponds to the 75th percentile data point. The line within the box represents the median. Different letters above the boxplots indicate statistically significant differences ($p < 0.05$) among species and control according to a Tukey HSD test.

Chapter V: General discussion

5.1. Carbon quality matters: coarse particle pool versus fine particle pool

Assessment of C stock of an ecosystem usually considers total soil carbon, not the C in individual fractions. As a result, soil C is presented as a simple number at the plot, catchment, regional or national scale, e.g., the 4P1000 goal, that considers only total C (Minasny et al., 2017). Yet, the soil C pool is a chemically and physically complex system in which C compounds associated with different soil particle fraction sizes may greatly differ in stability and mean residence time. As a result, increasing attention has been paid to the understanding and characterization of soil organic C quality, here defined as the relative amount of fast-turnover particulate organic matter C fractions ($C_{POM,}$ $C_{finePOM}$) and stable clay and silt associated C fractions (C_{SILT} , $C_{SILT+CLAY}$) (Balesdent et al., 1998; Cotrufo *et al*., 2013; Cardinael et al., 2015). A soil with good C quality should be targeted in C sequestration practices, aiming to have a high proportion of stable C in the SILT+CLAY pool.

In both experiments of my thesis, soil particle size fraction related C pools were characterized to assess C quality besides C quantity, and proved to be fundamental in the understanding of the plant-soil system. In Chapter II and III, there was no species effect on the total amount of C stored in soil. However, the quality of C, i.e. its accumulation in different soil fractions, was influenced by the root traits of the different plant species analyzed (Ch. II/III) as well as the chosen soil type (Ch. III), and their effects on microbiological communities. In Chapter IV, appling the concept of 'priming effect' on fraction associated C pools enabled us to highlight that the positive priming in subsoil was mainly due to an increased mineralization of C in the SILT+CLAY pool, while topsoil showed a homogeneous negative priming among pools, allowing us to better disentagle the priming mechanisms in different soil types. All of these findings not only highlight the great importance of looking at C sequestration at the fraction scale, but also challenge the supposed high stability of the SILT+CLAY pool.

5.2. Carbon origin matters: new carbon versus old carbon

The labelling approach to distinguish new C input in soil has been widely applied and is a relatively new frontier in plant-soil studies examining soil C storage (i.e. Dijkstra and Cheng, 2007; Paush et al. 2013;

Haddix et al., 2016). In my thesis, the stable labelling approach during 183 days of plants growth allowed to disentangle plant-soil processes connected with soil C storage, and was an efficient way to study soil C storage. We observed how changes in soil C were mainly attributable to the input of new C. Also, in Chapter III we observed a positive synergy with new C input and old C losses, with higher new C input connected with lower old C losses. This result was in accordance with results from De Graaf et al. (2010), and supported the preference substrate utilization theory (Cheng and Kuzyakov, 2005).

Considering new C gains and old C changes in different soil fractions allowed us to further unveil mechanisms of soil C storage that would have been hidden without this double approach of isotope labelling and soil fractionation. In Chapter III there was a high response of POM and SILT+CLAY pools to the input of fresh new C from plants, highlighting the double pathway of new C accumulation in soil, from turnover in POM and exudation or microbial mineralization and deposition in the SILT+CLAY pool (Cotrufo et al., 2013). Moreover, the fractionation allowed us to underline how old C is active in tospoil, being mineralized and accumulating from the coarser fraction (especially POM in tospoil) to the fine SILT+CLAY pool. These changes in old C among pools would have been hidden by analysis of total C in bulk soil, and old C would have been wrongfully considered inactive.

New C and old C in fractions were only studied in Chapters III and IV, but the lesson learnt from these chapters could make us rethink results in Chapter II, in which new C and old C were not distinguished. If not considering old/new C, in both Chapter II and III, we observed that there was no significant effect of species in C storage in different fractions (ΔC; Chapter III, Fig. 2, 3). However, considering the new C and old C changes in different fractions, we were able to identify the effect of species on the input of new C (Chapter III, Fig. 4), that in ΔC was masked from the changes of old C and its accumulation in the SILT+CLAY pool. Not only distinguishing between old and new C dynamics helped us to understand the effect of species and the different behaviour of soil pools, but even to shed light on the relationships between factors involved in C-cycling and C storage in different pools. Comparing the correlations in Chapter III

Table 3 between microbial/soil and root characteristics and new C, old C and ΔC, underlined how new C accumulation was better predicted compared to ΔC (see also Henneron et al. 2019).

We, therefore underline the power of coupling the study of C sources with soil fractionation and related C pools, which helped us greatly to analyse the mechanisms behind soil C storage, as explained in the next sections.

5.3. Microbial community matters: priming and entombing

The microbial community is the factor shaping all the processes involved in C cycling. Our main goal was to investigate the influence of species and soil selection on soil C storage in topsoils and exposed subsoils. However, our findings highlight that the role of these two factors always indirectly pass through microbial community biomass and activity via two mechanisms:

i) the priming effect (Chapter IV) was the response of microbial communities to revegetation, shaping the losses of C in the system. The productivity of the system (based on soil fertility and plant species performance) will determine the substrate preference of microbial communities and the direction and magnitude of the priming effect (Cheng and Kuzyakov, 2005: De Graaf et al. 2010), as shown in Chapter IV. Moreover, when soil N was low, we found an effect of competition for nitrogen influencing the priming direction and intensity (Cheng and Kuzyakov, 2005). These results support the reconciliation of the Preferential Substrate Utilization Hypothesis with the Competition Hypothesis (Cheng and Kuzyakov 2005), and underline the pivotal role of microbial communities in the priming effect.

ii) the quality of C stored due to microbial transformations. The common view that microbial communities reduce C sequestration due to increased soil respiration is becoming increasingly redundant. In chapter II and III we found strong links of microbial activity and biomass with C sequestration in the fine SILT and SILT+CLAY fractions. In this regard, two main theoretical framework have been developed in the last years:

- Microbial Efficiency-Matrix Stabilization (MEMS) from Cotrufo et al. (2013). The main hypothesis behind this framework states that labile C from plant inputs is the main source for microbial exudates and exopolysaccharides, which are the precursors of stable SOM in aggregates and organomineral compounds.
- Soil microbial carbon pump (MCP) from Liang et al. (2017). Microbial communities are the main factors shaping the sequestration of C by 1) degrading via *ex-vivo* modifications the soil C, consuming labile POM and leaving high recalcitrant and persistent SOM in soil, 2) increasing via *in-vivo* turnover through their metabolism the stability of stored C, as microbial necromass and metabolites, i.e., the 'entombing effect'.

Microbial necromass was not measured in this thesis. However, in Chapter IV, I provide evidence of the entombing effect. The increase of soil C is mainly due to accumulation of new C in POM and in SILT+CLAY and mineralization of old C in the SILT+CLAY pool. These C increases were correlated with microbial activity and biomass. As predicted by the MEMS model, the quality of input influences the destination of C: with microbial activity enhanced by labile inputs, while recalcitrant input was stabilized via ex-vivo transformations. The transformed labile C by microorganisms as exopolysaccharides was then stabilized in the SILT and SILT +CLAY pools – via in vivo turnover that promoted 'matrix stabilization' via organomineral interactions, as part of the 'entombing effect'. We, therefore, argue that the MEMS and MPC theories are complementary (Fig. 1), and the use of fractionation enables us to further expand their understanding by quantifying the effects of the different pathways of stabilization (Fig. 2).

This thesis highlights the necessity to jointly consider priming and entombing effects as the two faces of the same 'microbial coin'. The balance between these two processes will affect the final C sequestration efficiency, as stated by Liang et al. (2017). We observed, however, how a higher microbial activity have a positive effect on both i) C entombing via necromass and exopolysaccharides deposition and ii) reducing the priming in fertile soil with high microbial biomass, suggesting an overall positive effect of microbial communities on C sequestration.

5.4. Root traits matter: N_2 fixing species (Fabaceae) vsnon N_2 fixing species (Poaceae)

Plants are widely recognized as the main factor influencing C input in soil via litter fall, root mortality and exudation (Six *et al.*, 2002; Derrien *et al.*, 2016; Sokol *et al.*, 2019). This thesis focused partly on the comparison between N_2-fixing species (*Fabaceae*) with non N_2-fixing species (*Poaceae*) commonly used for revegetating embankments in the South of France. Coupling isotopes labelling with fractionation techniques, we demonstrate the beneficial effect of N_2-fixing species and the specific root traits that species possessed in this study (low C:N, high hemicellulose and low lignin content, high root elongation rate, low absorptive root diameter, low SRL,a nd high biomass) on total C sequestration and its accumulation in stable C pools. The once common view that stable C storage is driven by selective preservation of recalcitrant compounds is once again challenged by these results. We found root biomass to be a better predictor of new C gain in every soil pool, more than any other root trait. The reason might be that morpho-physio-phenological (as SRL and C:N, and diameter) can be compensated by the effect of biomass, as performance traits (Violle et al., 2007). In soil planted with Poaceae species, C storage in the POM C pool was greater possibly due tissue recalcitrance inhibiting microbiological activity, microbiological biomass and overall mineralization. However, this effect was masked in N_2-fixing species with their higher root biomass and related C input. It is necessary to underline that N_2-fixing species are associated with *Rhizobium* bacteria. This association increases the N content in the root biomass, decreasing the C:N ratio and their recalcitrance. In addition, the symbiosis with *Rhizobium* bacteria increases microbial activity and the deposition of microbiological exopolysaccharides (Garcia et al., 2001). These effects overall increase the new C input in the system, especially in the SILT and SILT+CLAY (Cotrufo et al., 2013). For this reason, is important to consider that the root traits related to high labile C input in this thesis are characterized by an intrinsic high microbiological activity due to symbiosis of N_2 fixing species with *Rhizobium*. When studying Leguminous species and C sequestration, he effect of the symbiosis and the root raits connected with high labile input are therefore synergic in incresing new labile

C input, and difficult to disentagle. In general, root biomass and chemical traits (C:N ratio, and lignin/cellulose/hemicellulose ratio) were a better predictor for C storage compared to architectural traits. Recent studies have highlighted correlations between root economic spectrum (RES) and C storage (De Deyn et al. 2008; Roumet et al. 216; Prieto et al. 2016; Poirier et al. 2018; Henneron et al 2019). The main characterization of RES - as coordinate variation of root respiration rate, decomposability, and morphological and chemical traits related to C economy (Roumet et al., 2016) - is given by the distinction between fast growing, acquisitive species (e.g. Fabaceae) and slow growing, conservative species (e.g. Poaceae) (Chapter II, this thesis). Therefore, our findings are consistent with the bulk of literature that find N_2-fixing Fabaceae in the spectrum of acquisitive species characterized by high input of labile C in the soil, and N_2-fixing species Poaceae representing conservative species with low input of recalcitrant old C (Prieto et al., 2016; Henneron et al., 2019). These results support the correlations between RES and soil C sequestration potential, mediated by root growth strategies and different C economies (Roumet et al. 2016; Poirier et al. 2018; Henneron et al 2019). However, when studying RES correlations with C storage is important to differentiate between N_2 fixing and non N_2 fixing species.

5.5. Soil matters: a major factor in carbon-cycle regulation, but due to indirect effects

Soil fractions and the related C pools have shown to be fundamental and understand the C-cycle in soil. The results of this thesis show that soil type has the highest impact on both C storage and priming effect. Reduced root biomass in subsoil due to low fertility decreases the input of new C in every soil C pool and the transfer of C in the SIL+CLAY pool via microbial metabolic transformations (Cotrufo et al., 2013; Vidal et al., 2018). It also has a negative effect on priming, since labile C input are not high enough to allow substrate preferential switch of microbial communities (Cheng and Kuzyakov , 2005; De Graaf et al. 2010).

However, when observing the direct effect of soil characteristics on the soil C storage, it is surprising to observe that C saturation has no effect on the increase of protected C in the SILT+CLAY fraction. In our experiment, subsoil had a higher clay percentage and lower initial C content compared to topsoil, decreasing soil C saturation that should positively influence C storage in the SILT+CLAY C pool (Six et al., 2002; Eyles et al., 2015; Shahbaz et al., 2017). Under these premises, we expected a higher rate and amount, or at least relative amount, of C stored in fine SILT+CALY pool. However, topsoil had a higher increase in the SILT+CLAY C pool compared to subsoil in absolute terms, while in relative terms they were comparable. The reasons behind this behavior is attributable to plant biomass and microbial communities in different soils. A lower fertility of subsoil decreases the input of C via plant biomass. The decreased microbial activity and abundance decreased the input of processed C in the SILT+CLAY C pool. Without C input in the SILT+CLAY C pool, increased potential for organomineral interactions did not influence the amount of stored C. With this diagnosis, we could not claim that clay content and C saturation had no effect on potential C storage. However, we can affirm that they were less important to C input by plants and C metabolic transformation by microbial community in respect to soil C storage.

Soil N content had a high impact on soil C-cycle, increasing fertility and biomass production and, consequently, new C input. Regarding the priming effect in soil, we confirm that in a poor soil the competition for N reduced the consumption of C from microorganisms and had a positive effect on soil C storage (Cheng and Kuzyakov, 2005). We confirmed that N rich soils positively influenced soil C storage (Dou et al. 2016, Guo et al., 2019).

Aggregate stability had a significant effect on C storage due to a double feedback mechanism: new C input in the system participated in creating more stable aggregates that, in turn, protected the encompassed C (Tisdall and Oades 1982; Caesar-Tonthat 2002; Nichols and Wright 2005). The decrease of old C mineralization in SILT+CLAY C pools were the direct result of the higher physical protection of C in stable aggregates (Chevallier et al., 2004). Soil structure, and especially aggregates, seems to be the main direct soil effect that influences C stability, and more studies need to be developed on the subject.

5.6. Ecological engineering toward a carbon sequestration goal

I suggest that we do not only consider soil C storage potential from a point of view of mineralogy or clay content (Hassink 1992, Hassink al. 1997) or C saturation (Six et al. 2002), but we should pay more attention to soil health. More specifically, we need to assess its fertility levels, such as N content, aggregate stability and microbial community development (biomass and/or activity). Microbial diversity could also be an important indicator. These indicators are connected with higher input of C in soil via increased biomass production, transfer to the protected SILT+CLAY pool and negative priming due to the switch of substrate utilization. An overview of the effect of soil and plants on C sequestration can be found in Figure 2.

The use of fertile topsoil increases carbon accumulation when compared with poor subsoil and it is therefore desirable for revegetation of geotechnical soils. When revegetating fertile topsoil, fast growing N_2 fixing species with high input of labile C are more efficient to store C in the protected SILT+CLAY pool via higher root input and microbial turnover. Revegetating topsoil also induces a negative priming effect, increasing preexistent C stability.

However, the use of topsoil is not always possible. Some particular conditions, eg in quarries and minor road embankments, might require revegetation of subsoil. In this case, I advise to:

1) Fertilize soil to increase biomass production and C storage in soil. Fertilization is recognized to increase soil C storage in both unprotected and protected C pools (Dou et al. 2016, Guo et al., 2019). However, to my knowledge, no studies exist on the effect of fertilization on subsoil brought to the surface. Moreover, the C impact of different fertilizer production and transport needs to be compared with the benefits for C storage, or the final result might be detrimental for global C storage.
2) Inoculate with microbial communities: we argue that inoculation of bacteria and mycorrhiza (especially *Rhizobium* associated with Leguminous species to increase nodulation, and arbuscular mycorrhiza fungi) would increase the C input in the SILT+CLAY C pool. Li et al. (2016) found a decrease in C loss due to an increase of soil microbial biomass. However they did not consider the

different fluxes of C (New C and Old C), so it is not possible to assess if the increase of respiration was detrimental for C balance. Kuimei et al. (2012) observed an increased soil C sequestration with arbuscular mycorrhiza fungi inoculation in a reclaimed mine soil treated with coal gangue, fly ash and sludge.

The lower C saturation did not increase protected C storage in subsoil in our experiment, but is still promising for potential C storage if fertility and microbial requirements are met.

If neither fertilization nor microbial inoculation are possible, we suggest avoiding the use of N_2 fixing species, since the increase in microbial biomass connected with those species will result in a higher mineralization of old SOC.

Figure 3 shows a simple flowchart that provides suggestions for revegetating geotechnical soils and optimize C sequestration. However, it is to be noted that this flowchart is based on results that present a major shortcoming: the short timeframe of experiments. Long-term experiments are now much needed, to explore how the results from this thesis are influenced over time.

5.7. What research remains to be performed?

Countless opportunities for research are possible in the C storage domain. However, this work on C storage in revegetated geotechnical soil sparked some specific questions that I feel should to be tackled to have a more comprehensive view of the system, from both a mechanistic and an applied point of view.

- It would be vital to extend these studies on fractions and C sources on long term experiments. How subsoil evolves and ‘become topsoil’ is a fundamental aspect to be considered in studies of plant development, microbiological characteristics and soil aggregation etc. However, the cost and experimental setup makes it difficult to implement long-term constant isotope labeling experiment. A solution would be to use C_3 plants grown on soil planted only with C_4 plants (or vice-versa) as they have different isotopic signatures (Hobbie and Werner, 2004; Kuzyakov 2006).

- While in topsoil, research on aggregate protection and formation are numerous, they have not reached a complete consensus on the processes involved. In subsoil, instead, the role of aggregates in C protection and their formation processes remains still obscure. In Annex I, I present a preliminary work to investigate in depth the influence of soil structure on C protection in topsoil and exposed subsoil.
- In this thesis, I investigated the influence of microbiological communities on C-cycling and sequestration based on microbiological activity and biomass. However, refined identification of microbiological community structure and diversity would help unveiling key processes and factors in C-cycling. Studying the evolution of microbiological communities on subsoil brought to the surface would be fundamental to better understand its C sequestration potential, the processes behind it, and to have an insight into soil evolution.
- More studies have to be carried out on inoculation with different strains of fungi and bacteria to understand the mechanisms and influence of inoculation on C-cycling and its role as a ‘pump’ for complexed C in the protected silt and silt+clay C pools.
- Microbial communities influence most of the major processes involved in soil C storage, directly or indirectly. More precisely: i) total C input, as microbes area commonly used indicator for soil fertility and health (Waksman, 1922; Waksman and Starkey, 1924; Mader et al., 2002; Suzuki et al., 2005; Schloter et al., 2018), ii) quality of the stored C, determining if the C will accumulate in the unprotected C pool (when microbial abundance and activity is low) or if it will be metabolized and transferred in the protected C pool (when microbial abundance and activity is high) (De Deyn et al, 2008; Cotrufo et al., 2013; Liang et al. 2017), iii) total respired C (increased with increasing microbial activity) and priming effect (decreased when microbial activity is inhibited due to competition or a switch of substrate preference from old C to new C) (Cheng and Kuzyakov., 2005; De Graaf et al., 2010), and iv) increased aggregate formation and stability (Tisdall and Oades 1982; Caesar-Tonthat 2002; Nichols and Wright 2005). Relying on clay abundance or C saturation levels

to determine the quantity of protected C might be inaccurate, since they are not an indicator for soil health, and since microbial communities will determine the C input in the silt+clay C pool. For these reasons we state that, given the correlations between microbial activity and many of the key processes involved in C storage, further research should be carried out regarding the use of microbial characteristics as an indicator for potential soil C storage.

REFERENCES

Balesdent, J., Besnard, E., Arrouays, D., Chenu, C., 1998. The dynamics of carbon in particle-size fractions of soil in a forest-cultivation sequence. Plant and Soil 201, 49-57.

Caesar-Tonthat, T.C. 2002. Soil binding properties of mucilage produced by a basidiomycete fungus in a model system. Mycological Research 106, 930–937.

Cardinael, R., Chevallier, T., Barthès, B.G., Saby, N.P.A., Parent, T., Dupraz, C., Bernoux, M., Chenu, C., 2015. Geoderma Impact of alley cropping agroforestry on stocks , forms and spatial distribution of soil organic carbon — A case study in a Mediterranean context. Geoderma 259–260, 288–299.

Cheng, W., Kuzyakov, Y., 2005. Root effects on soil organic matter decomposition. In: S. Wright, S., Zobel, R. (Eds.), Roots and Soil Management: Interactions Between Roots and the Soil. Agronomy Monograph No. 48, American Society of Agronomy, Madison, Wisconsin, USA 119–143.

Chevallier, T., Blanchart, E., Albrecht, A., Feller, C., 2004. The physical protection of soil organic carbon in aggregates: A mechanism of carbon storage in a Vertisol under pasture and market gardening (Martinique, West Indies). Agriculture, Ecosystems and Environment 103, 375–387.

Cotrufo, M.F., Soong, J.L., Horton, A.J., Campbell, E.E., Haddix, M.L., Wall, D.H., Parton, W.J., 2015. Formation of soil organic matter via biochemical and physical pathways of litter mass loss. Nature Geoscience 8, 776–779.

Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Denef, K., Paul, E., 2013. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: Do labile plant inputs form stable soil organic matter? Global Change Biology 19, 988–995.

De Deyn, G.B., Cornelissen, J.H.C., Bardgett, R.D., 2008. Plant functional traits and soil carbon sequestration in contrasting biomes. Ecology Letters 11, 516–531.

De Graaff, M.A., Classen, A.T., Castro, H.F., Schadt, C.W., 2010. Labile soil carbon inputs mediate the soil microbial community composition and plant residue decomposition rates. New Phytologist 188, 1055–1064.

Derrien, D., Barot, S., Chenu, C., Chevallier, T., Freschet, G.T., Garnier, P., Guenet, B., Hedde, M., Klumpp, K., Lashermes, G., Nunan, N., Roumet, C., 2016. Stocker du C dans les sols : Quels mécanismes, quelles pratiques agricoles, quels indicateurs ? Étude et Gestion des Sols 23, 193–224.

Dijkstra, F.A., Cheng, W.X., 2007. Moisture modulates rhizosphere effects on C decomposition in two different soil types. Soil Biology and Biochemistry 39, 2264–2274.

Dou, X., He, P., Cheng, X., Zhou, W., 2016. Long-term fertilization alters chemically-separated soil organic carbon pools: Based on stable C isotope analyses. Scientific Reports 6, 1–9. doi:10.1038/srep19061

Eyles, A., Coghlan, G., Hardie, M., Hovenden, M., Bridle, K., 2015. Soil carbon sequestration in cool-temperate dryland pastures: Mechanisms and management options. Soil Research 53, 349–365.

Garcia, J.A.L., Barbas, C., Probanza, A., Barrientos, M.L., Manero, F.J.G. 2001. Low molecular weight organic acids and fatty acids in root exudates of two Lupinus cultivars at flowering and fruiting stages. Phytochem Analysis 12: 305–311.

Guo, Z., Zhang, Z., Zhou, H., Wang, D., Peng, X., 2019. The effect of 34-year continuous fertilization on the SOC physical fractions and its chemical composition in a Vertisol. Scientific Reports 9, 1–10.

Haddix, M.L., Paul, E.A., Cotrufo, M.F., 2016. Dual, differential isotope labeling shows the preferential movement of labile plant constituents into mineral-bonded soil organic matter. Global Change Biology 22, 2301–2312.

Hassink, J., 1992. Effects of soil texture and structure on carbon and nitrogen mineralization in grassland soils 126–134.

Hassink, J., Whitmore, A.P., Kubat, J., 1997. Size and density fractionation of soil organic matter and the physical capacity of soils to protect organic matter. European Journal of Agronomy 7 (1–3), 189–199.

Henneron, L., Cros, C., Picon-cochard, C., Rahimian, V., Fontaine, F., 2019. Plant economic strategies of grassland species control soil carbon dynamics through rhizodeposition. Journal of Ecology

Hobbie, E.A., Werner, R.A., 2004. Review and Synthesis bulk carbon isotope patterns in C3 and C4 plants: a review and synthesis. New Phytologist 161, 371–385.

Kuimei, Q., Liping, W., Ningning, Y., 2012. Effects of AMF on soil enzyme activity and carbon sequestration capacity in reclaimed mine soil. International Journal of Mining Science and Technology, 22, 553-557

Kuzyakov, Y., 2006. Sources of CO2efflux from soil and review of partitioning methods. Soil Biology and Biochemistry 38, 425–448.

Li, J.H., Zhang, J.i., Li, W.J., Xu, D.H., Knops, M.H.J., Du, G.Z., 2016. Plant functional groups, grasses versus forbs, differ in their impact on soil carbon dynamics with nitrogen fertilization. European Journal of Soil Biology 75, 79-87

Liang, C., Schimel, J.P., Jastrow, J.D., 2017. The importance of anabolism in microbial control over soil carbon storage. Nature Microbiology 2, 1–6. doi:10.1038/nmicrobiol.2017.105

Mader P, Fliessbach A, Dubois D, Gunts L, Fried P, Niggli U, 2002. Soil fertility and biodiversity in organic farming. Science 296, 1694-1697

Minasny, B., Malone, B.P., McBratney, A.B., Angers, D.A., Arrouays, D., Chambers, A., Chaplot, V., Chen, Z.S., Cheng, K., Das, B.S., Field, D.J., Gimona, A., Hedley, C.B., Hong, S.Y., Mandal, B., Marchant, B.P., Martin, M., McConkey, B.G., Mulder, V.L., O'Rourke, S., Richer-de-Forges, A.C., Odeh, I., Padarian, J., Paustian, K., Pan, G., Poggio, L., Savin, I., Stolbovoy, V., Stockmann, U., Sulaeman, Y., Tsui, C.C., Vågen, T.G., van Wesemael, B., Winowiecki, L., 2017.. Soil carbon 4 per mille. Geoderma 292, 59–86.

Nichols, K.A., Wright, S.F., 2005. Comparison of glomalin and humic acid in eight native U.S. soils. Soil Science 170, 985–997.

Pausch, J., Tian, J., Riederer, M., Kuzyakov, Y., 2013. Estimation of rhizodeposition at field scale: Upscaling of a 14C labeling study. Plant and Soil 364, 273–285.

Poirier, V., Roumet, C., Munson, A.D., 2018. The root of the matter: Linking root traits and soil organic matter stabilization processes. Soil Biology and Biochemistry 120, 246–259.

Prieto, I., Stokes, A., Roumet, C., 2016. Root functional parameters predict fine root decomposability at the community level. Journal of Ecology 104, 725–733.

Roumet, C., Birouste, M., Picon-Cochard, C., Ghestem, M., Osman, N., Vrignon-Brenas, S., Cao, K. fang, Stokes, A., 2016. Root structure-function relationships in 74 species: Evidence of a root economics spectrum related to carbon economy. New Phytologist 210, 815–826.

Schloter, M., Nannipieri, P., Sørensen, S.J., van Elsas, J.D., 2018. Microbial indicators for soil quality. Biology and Fertility of Soils 54, 1–10.

Shahbaz, M., Kuzyakov, Y., Sanaullah, M., Heitkamp, F., Zelenev, V., Kumar, A., Blagodatskaya, E., 2017. Microbial decomposition of soil organic matter is mediated by quality and quantity of crop residues: mechanisms and thresholds. Biology and Fertility of Soils 53, 287–301.

Six, J., Conant, R.T., Paul, E.A., Paustian, K., 2002. Stabilization mechanisms of soil organic matter : Implications for C-saturation of soils 155–176.

Sokol, N.W., Kuebbing, S.E., Karlsen-ayala, E., Bradford, M.A., 2019. Evidence for the primacy of living root inputs , not root or shoot litter , in forming soil organic carbon 233–246.

Suzuki, C., Kunito, T., Aono, T., Liu, C.T., Oyaizu, H., 2005. Microbial indices of soil fertility. Journal of Applied Microbiology 98, 1062–1074.

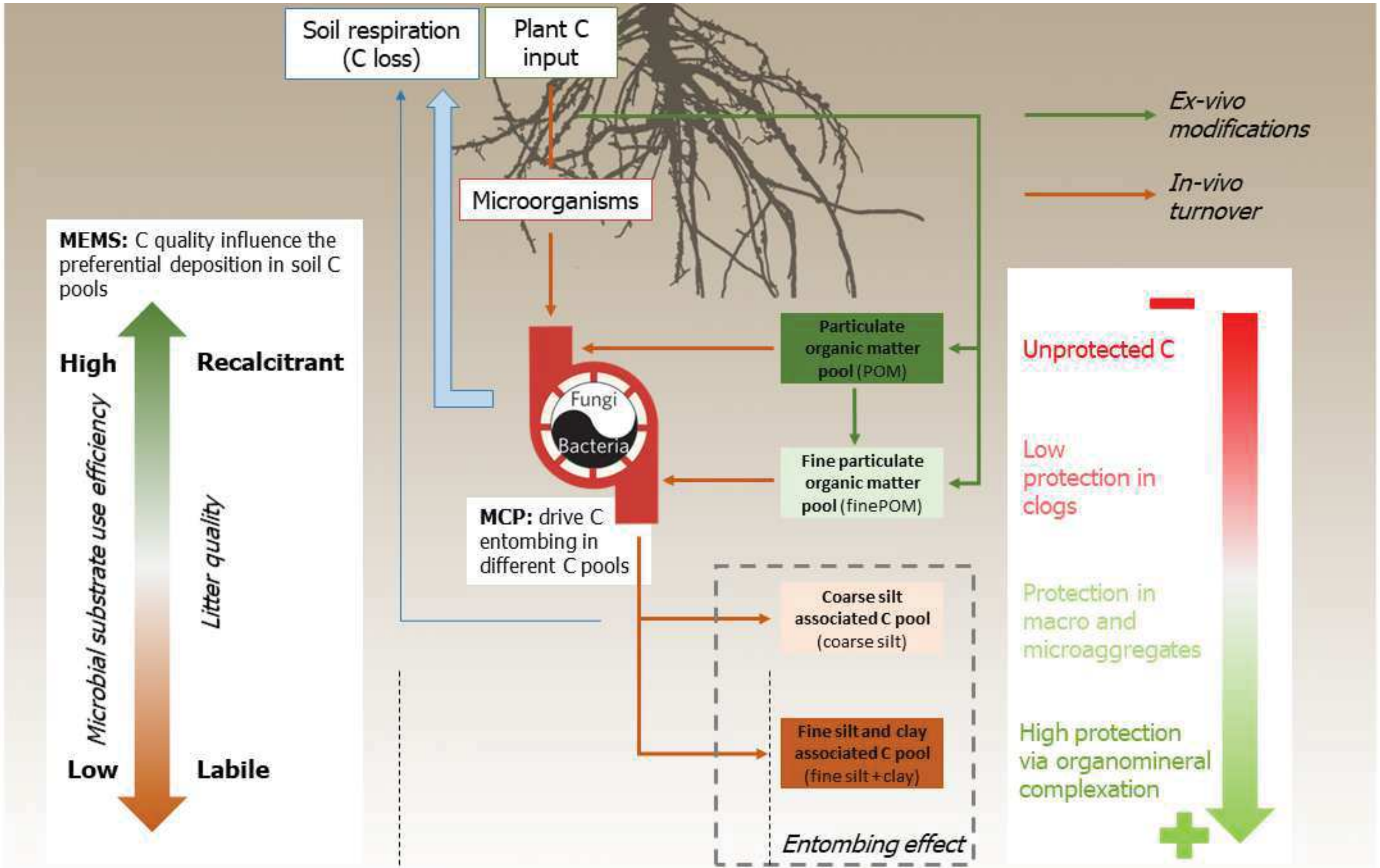
Tisdall, J.M., Oades, J.M., 1982. Organic matter and waterstable aggregates in soils. Journal of Soil Science 33, 141±163.

Vidal, A., Hirte, J., Bender, S.F., Mayer, J., Gattinger, A., Höschen, C., Schädler, S., Iqbal, T.M., Mueller, C.W., 2018. Linking 3D Soil Structure and Plant-Microbe-Soil Carbon Transfer in the Rhizosphere. Frontiers in Environmental Science 6, 1–14.

Violle, C., Navas, M.L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I. et al. (2007). Let the concept of trait be functional!. Oikos, 116, 882–892.

Waksman, S. A. and Starkey, R. L., 1924. Microbiological analysis of soil as an index of soil fertility: VII. Carbon dioxide evolution. Soil Science 17, 141-161.

Waksman, S. A., 1922. Microbiological analysis of soil as an index of soil fertility: III. Influence of fertilization upon numbers of microorganisms in the soil. Soil Science 14, 321-346.



MEMS framework (Cotrufo et al., 2013) allow to link the quality of the C input with the potential output in terms of SOC storage quality, via microbial substrate use efficiency.

MCP framework (Liang et al., 2013) shows the microbial processes shaping the C flows in soil via ex vivo modifications and in vivo turnover

The use of soil fractioning allows to quantify SOC in different soil C pools. Different pools are associated with different degrees of protection. SOC accumulation in different pools follow the pathways of MCP and is shaped by the quality of input as proposed by MEMS.

Figure 2: Conceptual framework illustrating complementarity between MEMS and MCP models, enriched by including the soil C pools. Primary plant's carbon inputs quality influence the final stabilization of stored C (MEMS, Cotrufo et al. 2013). The microbial carbon pump determine the entombing of C in the soil system, via ex-vivo modifications (green arrows) and in-vivo transformation (brown arrows) (MCP, Liang et al. 2017). Finally, the quality of C input will determine the C distribution in different soil C pools associated to soil fractions, through the microbial carbon pump. Labile C input will favor in-vivo turnover, increasing C in the silt and silt+clay fractions, protected in microaggregates and via organomineral interactions with fine silt and clay minerals. Recalcitrant C decrease in vivo turnover, and C accumulate mainly in unprotected POM and finePOM fractions via ex-vivo modifications.

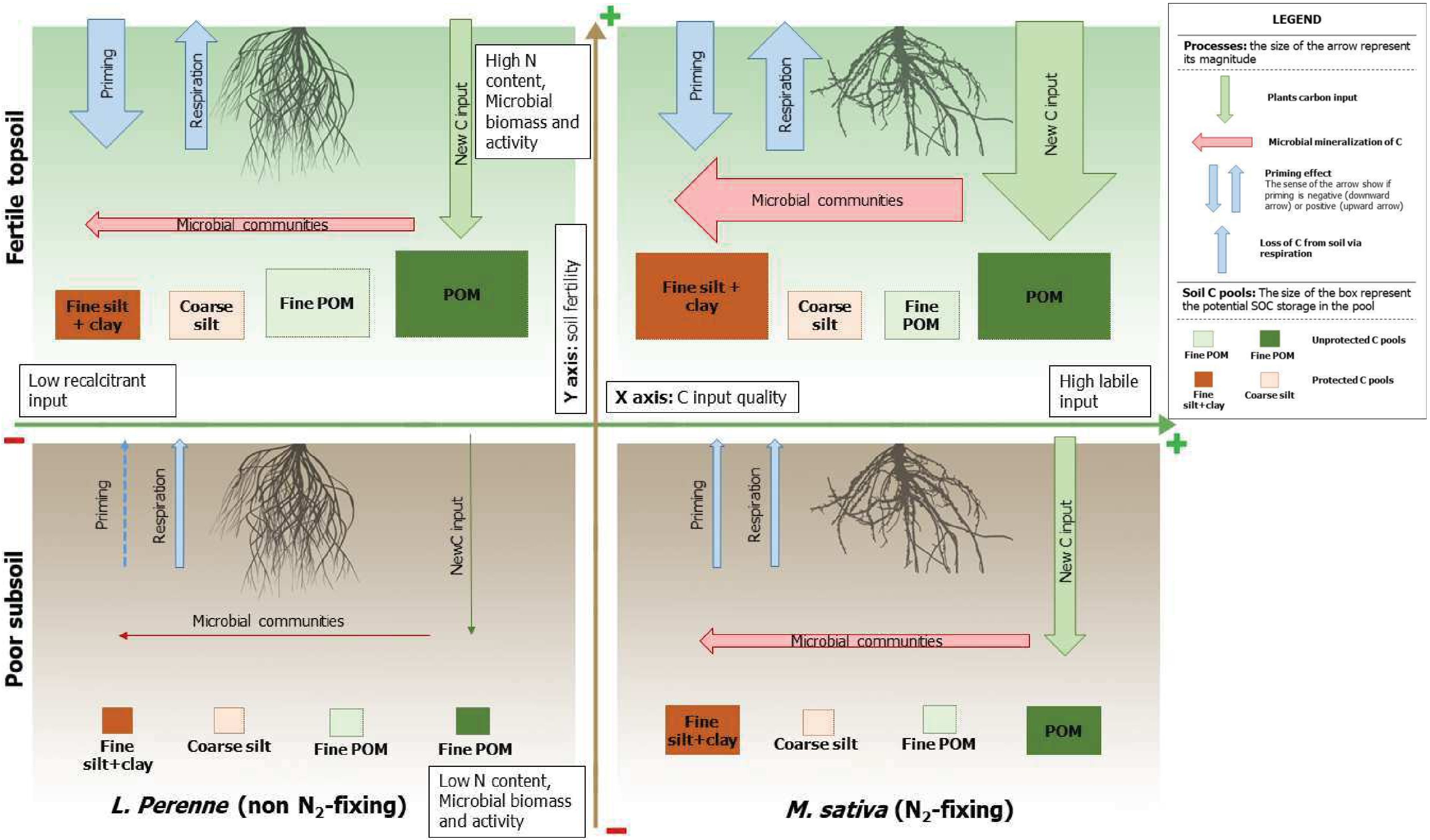


Figure 3: Scheme illustrating the changes in the C storage mechanisms along two man axes: x – Soil fertility and y – C input quality. Top right has the higher C storage potential, with high fertility soil revegetated with N2-fixing species, that have high labile C input, positively influencing POM and, most importantly, fine silt+clay fraction accumulation through high microbial activity. Top left corner shows the potential soil C storage of fertile soil revegetated with non N_2-fixing species. The lower input of recalcitrant C decrease microbial biomass and activity, and increase POM and finePOM accumulation via decreased mineralization. In fertile topsoil priming effect is high and negative. Bottom right corner shows the effect of revegetating poor subsoil with N2-fixing species: decreased C input due to fertility decreases the accumulation in the POM fraction, and decreased microbial biomass/activity its complexation in the protected silt+clay fraction. Priming effect is lower in intensity but positive; increasing the loss of preexistent SOC. Bottom left corner shows the effects of revegetation of poor subsoil with non N2-fixing species. Decreased input and microbial activity decrease the C accumulation in every soil fraction, however priming effect almost absent due to pant-microbes competition for nitrogen.

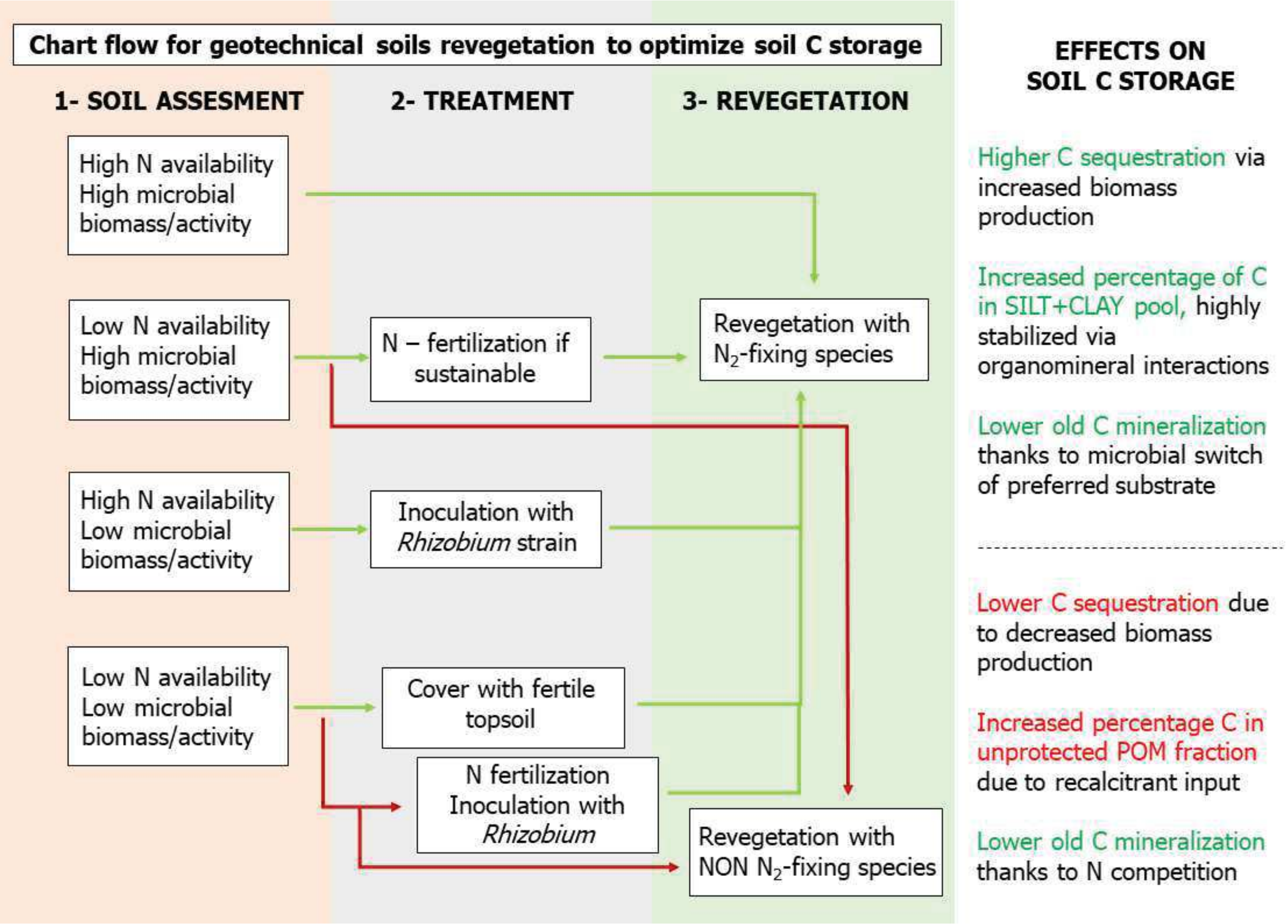


Figure 4: flowchart for soil revegetation to increase C sequestration in geotechnical embankments. First step is soil assessment in regard to N content and microbial biomass and activity. The second step shows the treatments to implement if N content is low (fertilization) or microbial communities are underdeveloped (inoculation). The fourth step shows the suitable species for revegetation given the soil conditions. The last ox the effects on C sequestration given the soil, the treatments and the plant species selected. Green arrow shows the suggested pathway to take, red arrow the alternative unadvised pathway if the first is not possible. This flowchart, however, is based on a short term experiment. Long term experiment should be implemented to improve it.

Annex I: Perspectives: the influence of vegetation on soil microstructure and its implications on soil carbon sequestration: a geotechnical approach

INTRODUCTION

A particular interest in sustainability has been voiced in both environmental and geotechnical disciplines, given the global climate change challenge that requires immediate action in multiple sectors. However, the research on sustainability remains largely confined in the peculiarity of each discipline, which has its specific assumptions and methodology characterizing the sustainability. We argue that a higher multidisciplinary approach is needed to hybridize research and find transdisciplinary methodologies and points of view on sustainability in every discipline. In this part of the research, we aim to bridge C storage research from a plant/soil science point with geotechnical engineering research. Soil microstructure have proven to be a good common ground between the two fields, since it is largely studied both with regards to C sequestration and in the geotechnical engineering field for characterization of the soil structural properties.

Soil structure has been proven to be central with regards to C sequestration, especially regarding the role of aggregates. Aggregates forms through binding of soil particles by fine roots and fungal hyphae (Tisdall and Oades 1982). Glycoproteins, polysaccharides, and mucilage, from plants cement their structure and influence their stability (Tisdall and Oades 1982; Caesar-Tonthat 2002; Nichols and Wright 2005). Aggregates occlude C in their structure, physically impairing the accessibility of microbes (O'Brien & Jastrow, 2013). The efficiency of aggregates C protection will depend on their stability and the amount of C stored inside their structure. Aggregates is a dynamics process, and the higher is their stability the higher hey will resist to disaggregation (Eyles et al., 2015). In soil sciences most of studies refers to three main classes: microaggreagets (0.02-0.2mm), macroaggregates (0.2-3mm) and clots (3-5mm). Aggregation in soil will deeply influence the void ratio, a common indicator used in geotechnical research to define soil structural characteristics. Aggregates structure gives a double porosity behavior to soil, with micropores characteristic of intra-aggregates structure, and macropores formed by the interaction between different aggregates (Koliji et al., 2008). In geotechnical studies, a common method used to investigate soil porosity and deriving void ratio is mercury intrusion porosimetry (MIP). MIP allow characterizing the cumulative and relative abundance of voids of different pore classes (Russo et al., 2016). The aggregation process and characteristics are expected to be correlated with void ratio in different pore classes. Soil porosity and connectivity also influence the possibility of microbes to be in contact with substrates and their consumption (McCarthy et al., 2008; Lugato et al., 2009). Vegetation can influence soil porosity in different classes due to i) root channels, ii) hyphae development, iii) wet and dry cycles, iv) cementation and clogging of micropores due to rhizodeposition and v) aggregation and disaggregation processes favored by plants influence (McCarthy et al., 2008; Lugato et al., 2009). Extensive studies have been implemented on aggregate formation and C protection. However, most of the studies see aggregates as 'building blocks of soil' (Malamoud et al., 2009) and overlook the more complicated structure deriving by their interactions. Moreover, studies on aggregate formation and C protection have seldom been implemented on subsoil, where the aggregate formation processes are still debated. Soil microstructure has great potential to shape C sequestration in soil, and we aim to assess evolution of aggregate characteristics due to revegetation in topsoil and subsoil brought to the surface. Moreover, we aim to assess pore ratio in soil and the influence of vegetation using MIP, and correlate it to aggregate characteristics, to better understand how aggregates shape soil structure. The use of MIP together with aggregate stability and C analysis will allow comparing methods characteristics of different disciplines and exploring possible exchanges and overlapping between these fields.

OBJECTIVES AND HYPOTHESIS

Our first objective is to investigate the influence of vegetation on microstructure using the MIP methodology. For this, we assessed bulk void ratio at time 0 and after 6 months of two soils showing contrasting characteristics (topsoil 0-30 cm depth and subsoil 110-140 cm depth) vegetated with *M. sativa* and *L. perenne*, plus bare soil controls. Comparing void ratio in bare soil control after 6 months of experiment (time 6) with the initial soil (time 0) will allow us to assess the effect of wet and dry cycles on microstructure (since soil was kept at 45% of water holding capacity with irrigation to compensate evaporation) and compare it with the effect of revegetation. Void ratio will be cumulated in different classes relative to different aggregates and processes as a proxy for: microaggregates porosity, macroaggregates porosity deriving from microaggregates interaction and clots porosity deriving from macroaggregates interactions.

Our second objective is to characterize aggregates and their characteristics for bare soil control and vegetated treatment with *M. sativa* and *L. perenne*. For this, we measured aggregate stability, quantity of C protected inside of aggregates, quantity of fresh new C inputted in different aggregates classes in 6 months, thanks to the constant CO_2 enrichment with ^{13}C.

Our final objective is to investigate the relationship between aggregate stability and void ratio in different pore classes, ii) between C protected in different aggregate classes and void ratio in different pore classes and iii) if new C input in different aggregate size influence macro- and microporosity.

We hypothesize that plants will increase macroaggregates and clots porosity due to channeling of roots and aggregates formation. However, vegetation might clots micropores due to rhizodeposition (McCarthy et al., 2008). In this case, aggregate stability will increase with increased void in macroaggregates and clots (due to aggregate formation and inter-aggregate porosity) while it will be negatively correlated with porosity in microaggregates, due to bioclogging from microbial activity that cement and increase aggregate stability (Ivanov and Chu, 2008). However, wet and dry cycle will probably drive the formation of soil structure.

Another hypothesis is that the protection of C will increase when decreasing the porosity (and void ratio) in macro and microaggregates, since the microbes will not have access to the occluded C. Finally, we expect that new C input is positively correlated with porosity in macroaggregates and clots (due to the role that fresh C input plays in aggregate formation, and root channeling connected with new C deposition) but negatively correlated with porosity in microaggregates, due to the clogging from rhizodeposition and microbiological exudation and exopolysaccharides. The analysis will be conducted using pearson's correlations between C protection and new C input in microaggregates, macroaggregates and clots, and the void ratio (as proxy for porosity) in three different pore classes.

STATE OF THE WORK

Analyses on aggregate stability, C protection in aggregates and new C input in different aggregates classes have been performed. A first MIP analysis campaign has been carried out to design the work. A second campaign to acquire MIP replicates is in progress and expected to finish by the end of October 2019. After, correlations with aggregate properties will be investigated to study the relations between vegetation, soil structure in terms of aggregation and porosity, and C protection. Methodology and preliminary results are presented in the following sections.

METHODOLOGY

Mercury intrusion porosimetry (MIP) curves and cumulative porosity for different pore classes

MIP test allow to characterize the porosity of the sample in an entrance pore diameter that ranges between 0.001 to 300μm. Abundance of pores of different diameters define the microstructure of the soil. Once grouped in different pore size classes, we believe to find correlations between pores and aggregate characteristics in soil.

Methodology:

1. 1-2 g of sample are dehydrated according to the freeze-drying method (Delage et al. 1984).
2. MIP test is performed in a double chamber Micromeritics Autopore III apparatus.
3. Place the sample in the filling low-pressure apparatus (dilatometer).
4. The samples is outgassed and under vacuum, and after filled by mercury. The chamber is at ambient absolute pressure.
5. Pressure is then rise up to 200 kPa using of compressed air
6. Chamber is depressurized and the samples were transferred to the high-pressure unit,
7. The pressure is then raised to 205 MPa following a previously set intrusion program. At any intrusion step a time sufficient to observe a quasi-static penetration of mercury was allowed.
8. A blank test is performed to corrections the results and prevent errors deriving from the compressibility of the intrusion system.
9. Finally, SEM analyses were performed on dehydrated samples in order to highlight their fabric.

Output: Intruded void ratio and pore size density function for different pore classes rangion from 0.001 to 300 μm

Aggregate stability

Aggregates provide physical protection to carbon. However, the degree of protection of carbon depends on their stability. The more stable are the aggregates, the more resilient will be to changes in environmental condition, decreasing their disaggregation and ensuring stable physical protection to the carbon occluded. Mean weight dimeter is a standard indicator for aggregate stability, as the mean diameter of aggregates that, starting from a 5-3mm diameter, have undergone a disaggregation process via wet and drying. The higher the MWD, the lower the disaggregation.

Methodology according to le Bissonais et al. (2006):

1. 20 g of soil collected and air dried
2. The sample is sieved first at 5mm and after at 3mm, to isolate the 3-5mm fraction
3. Aggregates are put in the oven for 24h so they are at the same matrix potential
4. 5g of 3-5mm fraction are weighted and gently immerse in a 250 cm3 beaker filled with 50 cm3 of ethanol for 10 minutes
5. Ethanol is sucked off with a pipette
6. Sample transferred in a 250cm3 Erlenmayer flask containing 50cm^3 of deionized water and brought to 200cm^3
7. Flask is agitated 20 times and left 30 minutes for sedimentation of coarse particles
8. Water sucked off with a pipette
9. Mixture of soil and water transferred to a 50µm sieve previously immersed in ethanol
10. Sieve gently moved 5 times to separate <50 µm from those >50 µm, use of ethanol for the wet sieving to reduce additional breakdown
11. >50 µm fraction is collected from the 50-µmsieve, oven-dried and gently dry-sieved by hand on a column of six sieves: 2000, 1000, 500, 200, 100 and 50 µm
12. Mass percentage of the different fraction is calculated, and for subtraction even the <50µm fraction
13. MWD is calculated as the sum of the mass fraction of soil remaining on each sieve after sieving multiplied by the mean aperture of the adjacent mesh

Output: MWD for different soils and treatments

Protected carbon: aggregate mineralization

Aggregates provide physical protection to carbon. However, not all the carbon is automatically protected inside the aggregates. The protection will depend on the porosity of the aggregates and the amount of microbial biomass enclosed in the aggregates itself. This will ultimately influence the capacity of microbes to get in contact with the C substrate and mineralize it. We aim to assess the degree of C protection in different aggregates sizes of the two different soils (topsoil vs subsoil) and three different treatments (*M. sativa*, *L. perenne*, bare soil). The soil nature will influence pore size, microorganisms' abundance and aggregate stability. First, to assess the unprotected C in aggregates we measure the amount of CO_2 released when incubating undisturbed aggregates of different classes. After, to assess the amount of total C (unprotected and protected) we finely grind aggregates the aggregates (to remove their physical protection on carbon) and assess the CO2 respired during incubation. The different between these two values (CO_2 deriving from protected C and CO_2 deriving from total C) will allow us to assess the amount of soil derived CO_2 that is protected in different aggregates classes (3-5 mm, 0.2-3 mm, 0.02-0.2 mm) for different soils (topsoil and subsoil) and species (*M.sativa*, *L.perenne* and bare soil) (Figure A1).

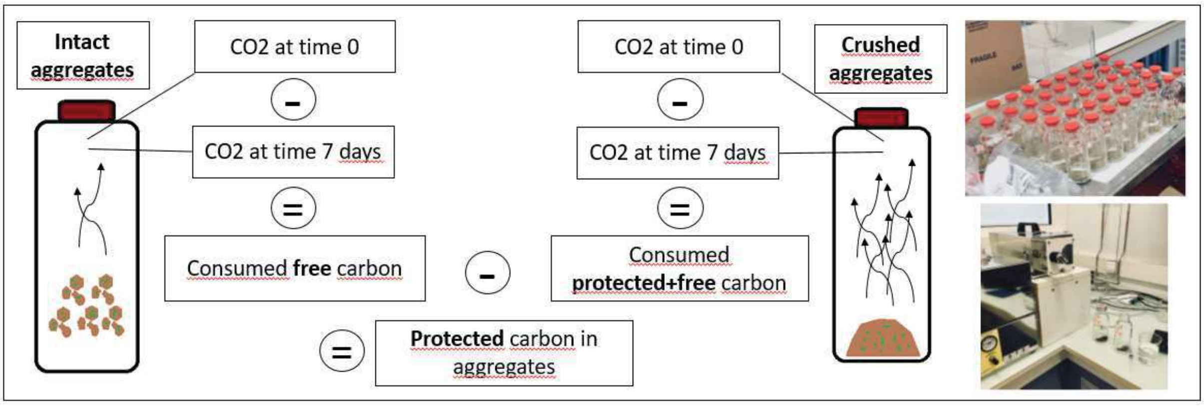


Figure A1: : scheme of the michrocatarometer methodology

Methodology:

1. Manually crush the soil and push it through a 5000 µm sieve (aggregates 3000-5000 µm fraction)
2. Sieve at 3mm and 0.2mm (aggregates < 200 µm fraction and 3 mm to 200 µm).
3. Collect the different fractions and separate them in half
4. Crush half one half of each fraction to obtain two subsamples: uncrushed aggregates and crushed aggregates (20g of aggregates for each sample)
5. Bring them to 75% of water holding capacity
6. Samples placed in 125 ml jars with parafilm allowing the interchange of gases (but not water) and incubated at 28 °C for 7 days.
7. Each sample was adjusted for soil moisture and, just after, the bottles were air tightly closed and measurements of respiration made. After 6 hours of incubation (without any gas interchange) measurements were made.
8. The differences of CO_2 between these two measurements gave the amount of respired CO_2 in 6h per treatment, soil and aggregate class.

Output: amount of respired CO_2 for crushed and uncrushed aggregates. The difference between these measurements represent the aggregates protected carbon in potential respiration. These results were available for 3-5mm, 0.2-3mm and 0.02-0.2mm aggregate classes.

Plant derived fresh carbon (new C) stored inside aggregate structures

It is well known how aggregates provides protection for C, however the aggregate formation processes are still debated. Especially in subsoil were little is known about aggregate structures and formation. C deriving from plants, often processed by microbes, is recognized as one of the main actors in aggregate formation. The input of C as plants' exudates and microbiological exudates and exopolysaccharides cement the mineral structure of the aggregate that will provide protection from microbial mineralization. We aim to investigate the pathways of C input in different aggregate classes to acquire information on aggregate formation and C protection in the two different soils.

Methodology:

1. A subsample from the aggregate abundance samples was taken, representative of the following aggregate classes:
    1) aggregates 2000-5000 µm (2-5 mm)
    2) aggregates 200-3000 µm (0.2-3 mm)
    3) micro-aggregates 20-200 µm (<0.2mm)
2. The subsample is finely ground with an agate mortar and stored
3. The sample were analyzed to asses SOC and ^{13}C with an elemental analyzer Isoprime100 coupled with an Elementar Varo Isotope Cube at INRA Nancy.
4. To calculate the proportion of NewC in aggregates, an isotope mixing model according to the work of Balesdent and Mariotti (1996) was used:

    $$\%Cnew = \frac{\delta(t1)-\delta(t0)}{\delta B-\delta(t0)} \qquad [1]$$

    Where %Cnew is the percentage of new carbon in the measured SOC of a specific aggregate fraction, $\delta(t1)$ is the δ^{13}C signature of the SOC measured in a specific aggregate fraction at the end of the experiment (t1), δ(t0) is the δ^{13}C signature of the SOC before the experiment (t0), δB is the δ^{13}C signature of the new C input in the system, in our case the signature of the root biomass (as the average of adsorptive and transport roots signature).
5. Multiply the total SOC for the %Cnew provides the amount of NewC in mgC g^{-1} soil.

Output: Amount of total SOC and NewC in the different classes (3-5 mm, 0.2-3 mm, 0.02-0.2 mm) for different soils (topsoil and subsoil) and species (*M.sativa*, *L.perenne* and bare soil).

PRELIMINARY RESULTS AND MAIN DISCUSSION POINTS

Mercury intrusion porosimetry (MIP) curves and cumulative porosity for different pore classes

Bare soil condition: Effect of wet and dry cycles

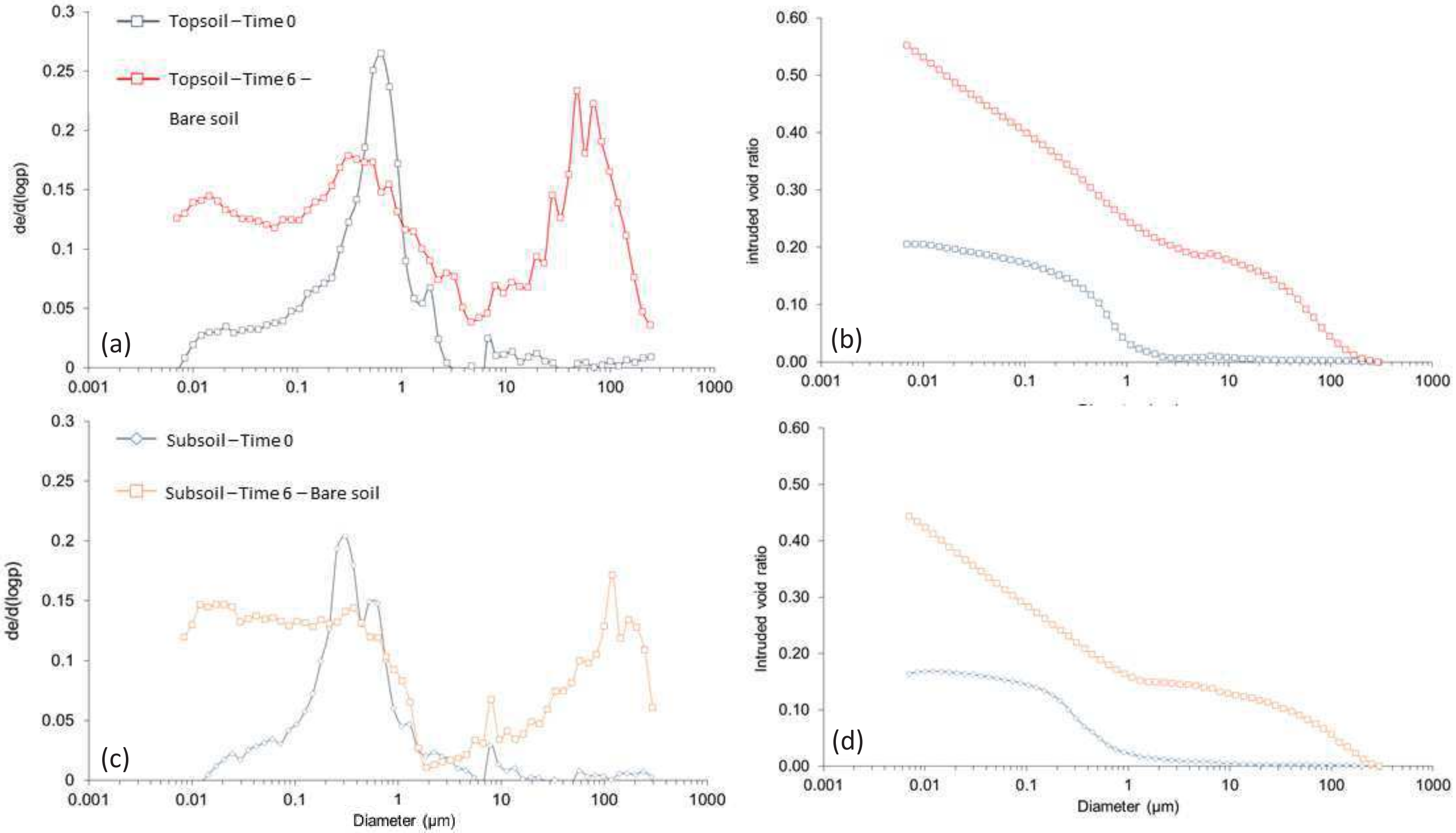


Figure A2: Evolution of microstructural voids in 6 months of wet and dry cycles, with no vegetation sowed. Figure (a) shows the pore size density function of topsoil at time 0 (blue boxes) and time 6 (red boxes) without vegetation. (b) shows the total intruded void ratio for different diameter classes in topsoil. (c) shows the pore size density function of subsoil at time 0 (blue boxes) and time 6 (orange boxes) without vegetation (d) the total intruded void ratio for different diameter classes in subsoil.

- Wet and dry cycles have a high effect on soil structures: soil structure pass from a mono-modal curve structure to a bi-modal curve structure in both soils.
- Void ratio highly increase in the micropores (<0.1 µm) and macropores (>10 µm) classes due to wet and dry cycles. Mesopores (0.1-10 µm) decrease during the 6 months experiment.
- The wet and dry cycles proved to influence both aggregates formation and stabilization processes (Shiel et al. 1988; Denef et al. 2001). We hypothesize that, after the soil preparation (that included crushing and sieving) the soil lost its primary microstructure. Wet and dry cycles increase aggregate formation and, consequently, the microporosity deriving from intra-aggregates structure and the macroporosity deriving from inter-aggregates interactions.

Vegetated treatment compared to bare soil after 6 months of growth

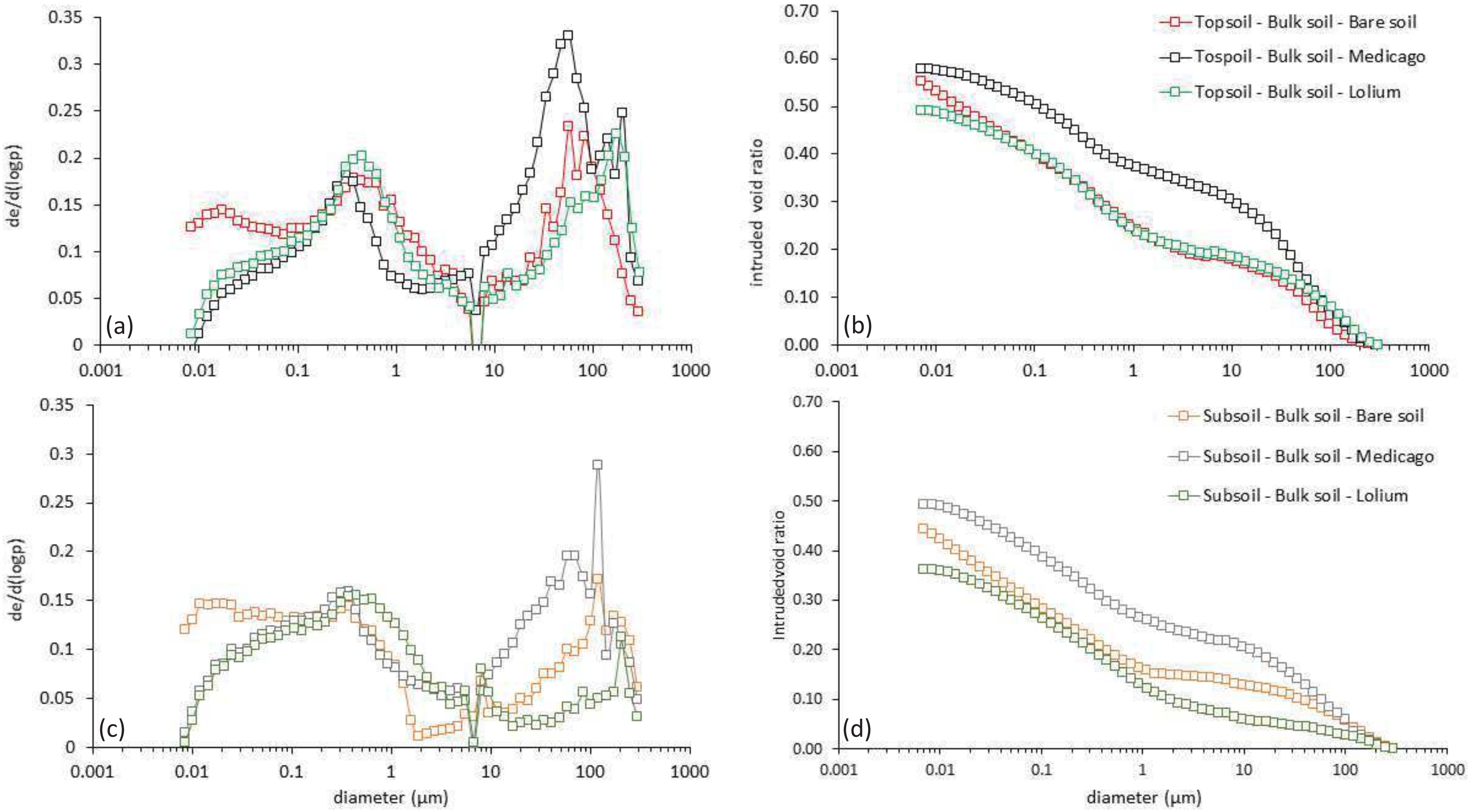


Figure A3: Evolution of microstructural voids in 6 months of soil vegetated with *M.sativa and L.perenne* compared to bare soil control. Figure (a) shows the pore size density function of topsoil in bare soil control (red boxes*)*, *M.sativa* (black boxes), and *L.perenne* (green box) after 6 months of growth. (b) shows the total intruded void ratio for different diameter classes in topsoil. (c) shows the pore size density function of subsoil in bare soil control (orange boxes), *M.sativa* (grey boxes), and *L.perenne* (dark green boxes) after 6 months of growth. (d)shows the total intruded void ratio for different diameter classes in subsoil.

- *M. sativa* show an increase in total porosity in both subsoil and topsoil, while *L.perenne* decrease the total porosity (Figure A3b,d).

- *M.sativa* increase the macroporosity in both soils (> 10 µm), while *L.perenne* show a decrease in macroporosity in bare soil (Figure A3a,c).
- Both *M. sativa* and *L. perenne* decrease the microporosity of the system (<0.1 µm) (Figure A3a,c).
- The increase in macroporosity due to *M.sativa* might be correlated with increased aggregate formation and interactions, and root channeling effect. The decrease of microporosity is imputable to exudates from microbial activity and plants that clog the micropores in aggregates. However, replicates are needed to verify these hypothesis.

Aggregate stability: Mean weight diameter (MWD)

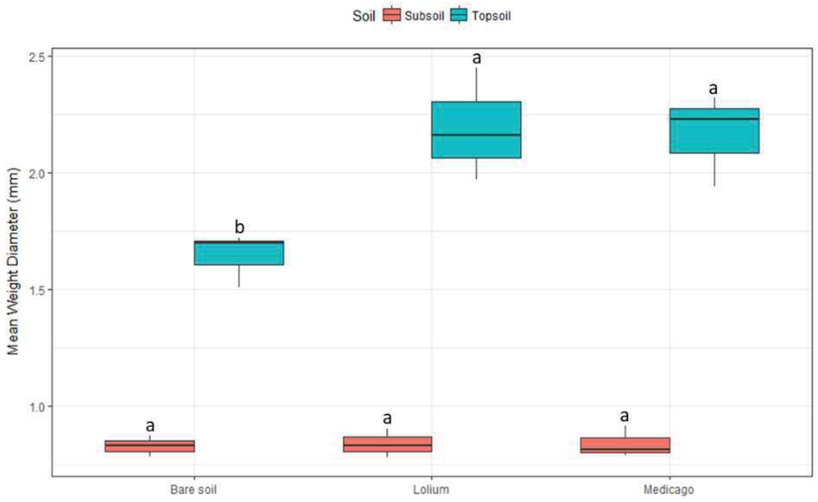


Figure A4: Mean weight diameter (MWD) in topsoil (blue boxplot) and subsoil (red boxplot) for the three different treatments (*M.sativa, L.perenne* and bare soil control) after 6 months of revegetation. In each boxplot, the lower edge of the box corresponds to the 25th percentile data point, while the top edge of the box corresponds to the 75th percentile data point. The line within the box represents the median and black dots indicate outliers. Different letters above the boxplots indicate statistically significant differences ($p < 0.05$) between families and controls according to Tukey HSD test.

- Subsoil and topsoil have a significant different MWD after 6 months of revegetation, with a higher aggregate stability in topsoil.
- No significant effect of vegetation in subsoil.
- In topsoil vegetated treatment have a significantly higher stability compared to bare soil control. However, we didn’t observe any effect of different vegetation.

Protected C in aggregates

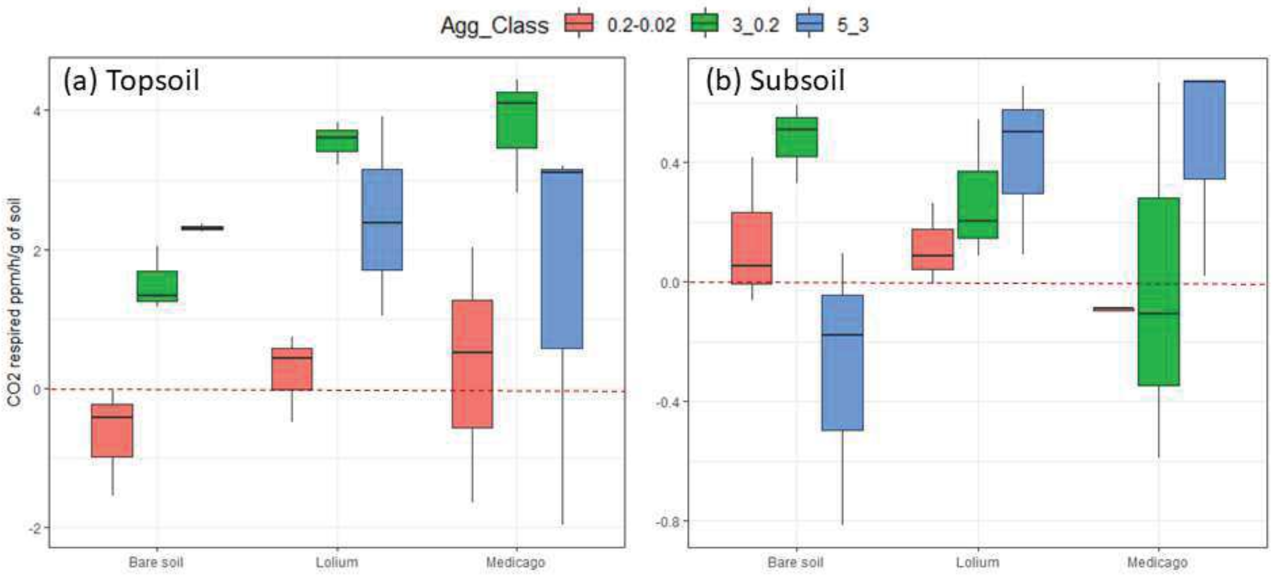


Figure A5: amount of CO_2 (respired ppm h^{-1} g^{-1} of soil) protected inside different aggregate classes (5-3 mm clogs in blue, 3-0.2 macroaggregates in green, 0.2-0.02 mm microaggregates in red) in (a) topsoil and (b) subsoil. The protected C is calculated as the difference between the respired $C0_2$ deriving from incubation of undisturbed aggregates (CO_2 deriving from unprotected C) and $C0_2$ deriving from incubation of crushed aggregates (CO_2 deriving from consumption of protected and unprotected C). In each boxplot, the lower edge of the box corresponds to the 25th percentile data point, while the top edge of the box corresponds to the 75th percentile data point. The line within the box represents the median and black dots indicate outliers. The red dotted line is the 0 line, meaning no protection of C in aggregates.

- In topsoil the higher C protection is found in the macroaggregates, with vegetation that increase the amount of protected C underlining the reactivity of this aggregate class to revegetation. In topsoil clots no effect of vegetation can be found, with increased standard variation in vegetated treatment but no differences with bare soil.
- In subsoil we observe a very different trend, with vegetation decreasing the C protection in the 3-0.2mm macroaggregates, while increasing the amount of protected C in the 5-3 clots, that did not presented any protection in bare soil control.

- In both topsoil and subsoil, microaggregates do not have any role in C protection, since no changes are observed when crushing them. However, since the crushing was done by hand in an agate mortar it is not sure that the structures were efficiently disaggregated, leaving a possible bias in the methodology.

Plant derived NewC input in aggregates

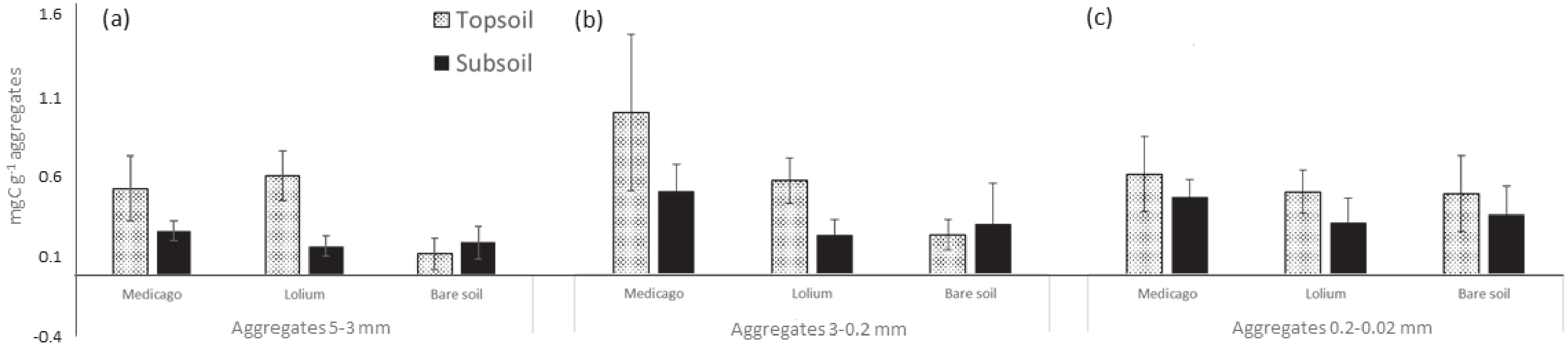


Figure A6: newC (mg new C g^{-1} aggregates) deriving from plant input in aggregates for gram of aggregates of different classes for the three analyzed treatment (*M.sativa, L.perenne*, and bare soil control) after 6 months in topsoil (light dotted bars) and subsoil (solid black bars). (a) Show the C concentration in the clogs (5-3mm diameter), (b) in the macroaggregates (3-0.2 mm), and (c) in the microaggregates (0.2-0.02mm). Bars represent the standard deviation.

- In clots (Figure 5A,a), vegetated treatment store more new C compared to bare soil, while in subsoil no significant differences seems to occur.
- The higher increase in C seem to be in macroaggregates (Figure 5A,b), higher in *M.sativa* compared to *L.perenne* and bare soil.
- In microaggregates, no effect of vegetation seem to influence the quantity of new C moved (Figure 5A,c).
- Bare soil control shows increase in NewC, probably due to mosses colonization that mineralized enriched CO2. The analysis of deeper layer of soil is needed to avoid the contamination.

FUTURE WORK

1. Replication of MIP results to confirm the trends.
2. MIP results will be separated in different pore classes to have cumulative data for void ratio in different pore classes relative to different aggregate sizes.
3. Implement principal component analysis and Pearson’s correlations between i) aggregate stability MWD, ii) amount of C protected in different aggregates classes in CO_2 equivalent, iii) new C input in different aggregate classes, and iv) void ratio in different pore classes representing microaggregates, macroaggregates and clots, in the two soils and for the different species.
4. Discussion on the effect of vegetation on soil structure and relationship between aggregate characteristics and soil structure in terms of void ratio. Use of the data to link the C input in aggregates with the soil structure formation, and the feedback between the structure and the C protection.
5. Results will help to understand the differences in C fluxes in aggregates in topsoil and subsoil brought to the surface and test the aggregate hierarchy theory of Tisdall and Oades (1982), widely accepted for topsoil, on subsoil brought to the surface. Preliminary results suggest that subsoil brought to the surface might not share the same aggregation processes than topsoil.

REFERENCES

Balesdent, J. & Mariotti, A. 1996. Measurement of soil organic matter turnover using I3C natural abundances. In: Mass Spectrometry of Soils (eds T.W. Boutton & S. Yamasaki), pp. 83-1 11. Marcel Dekker Inc., New York.

Caesar-Tonthat, T.C. 2002. Soil binding properties of mucilage produced by a basidiomycete fungus in a model system. Mycological Research 106, 930–937.

Delage, P., Pellerin, F.M., (1984). Influence de la lyophilisation sur la structure d'une argile sensible du Québec. Clay minerals 19 : 151-160

Denef, K., Six, J., Bossuyt, H., Frey, S.D., Elliott, E.T., Merckx, R., Paustian, K., 2001. 1-s2.0-S0038071701000761-main.pdf 33, 1599–1611.

Eyles, A., Coghlan, G., Hardie, M., Hovenden, M., Bridle, K., 2015. Soil carbon sequestration in cool-temperate dryland pastures: Mechanisms and management options. Soil Research 53, 349–365.

Ivanov, V., Chu, J., 2008. Applications of microorganisms to geotechnical engineering for bioclogging and biocementation of soil in situ. Reviews in Environmental Science and Biotechnology 7, 139–153.

Koliji, A., Vulliet, L., Laloui, L., 2008. New basis for the constitutive modelling of aggregated soils. Acta Geotechnica 3, 61–69.

Le Bissonnais, Y., 2016. Aggregate stability and assessment of soil crustability and erodibility: I. Theory and methodology. European Journal of Soil Science 67, 11–21.

Lugato, E., Morari, F., Nardi, S., Berti, A., Giardini, L., 2009. Relationship between aggregate pore size distribution and organic-humic carbon in contrasting soils. Soil and Tillage Research 103, 153–157.

Malamoud, K., McBratney, A.B., Minasny, B., Field, D.J., 2009. Modelling how carbon affects soil structure. Geoderma 149, 19–26.

McCarthy, J.F., Ilavsky, J., Jastrow, J.D., Mayer, L.M., Perfect, E., Zhuang, J., 2008. Protection of organic carbon in soil microaggregates via restructuring of aggregate porosity and filling of pores with accumulating organic matter. Geochimica et Cosmochimica Acta 72, 4725–4744.

Nichols, K.A., Wright S.F., 2005. Comparison of glomalin and humic acid in eight native U.S. soils. Soil Science 170, 985–997.

O'Brien, S.L., Jastrow, J.D., 2013. Physical and chemical protection in hierarchical soil aggregates regulates soil carbon and nitrogen recovery in restored perennial grasslands. Soil Biology and Biochemistry 61, 1–13.

Russo ,G., Rezza, A., Mancuso, C., Oliviero, V., D'Onza, F., Gallipoli, D., Wheeler, S., 2016. Microstructure analysis of laboratory and in-situ compacted silts. E3S Web of Conferences 9, 06003

Shiel, R.S., Adey, M.A., Lodder, M., 1988. The effect of successive wet/dry cycles on aggregate size distribution in a clay texture soil. Journal of Soil Science 39, 71–80.

Tisdall, J. M., & Oades, J. M. (1982). Organic matter and water-stable aggregates in soils. Journal of Soil Science, 33, 141–163.

Résumé exhaustif: Objectifs, résultats, conclusions générales

OBJECTIFS GENERAUX ET HYPOTHESES

Les objectifs généraux appliqués de la thèse sont :

i. Comprendre l'effet des caractéristiques des plantes et du sol sur la séquestration du carbone dans le sol en termes de quantité et de qualité (objectif fondamental)

ii. Identifier les pratiques possibles en matière de plantes et de sols qui peuvent être mises en œuvre pour augmenter le stockage du carbone dans les remblais des routes et ferroviaires et, éventuellement, dans les sols gris des travaux géotechniques (objectif appliqué)

Différentes questions spécifiques concernant les mécanismes fondamentaux du cycle C ont été abordés dans chaque chapitre de la thèse.

Chapitre II : Voie de la persistance : les caractéristiques des racines des plantes modifient l'accumulation de C dans différents réservoirs de carbone du sol par médiation microbienne

i. Objectif 1 : Comprendre les relations entre les caractéristiques des racines et l'accumulation de C dans différents bassins de carbone du sol pour 12 espèces herbacées différentes couramment utilisées dans la revégétalisation des talus.

Hypothèse 1 : Les caractéristiques liées à l'apport de C labile (taux d'allongement des racines, teneur en hémicellulose, biomasse racinaire) favorisent l'accumulation de C dans les bassins protégés de limon grossier et de limon fin + argile par activité microbienne. Les caractères racinaires liés à la récalcitrance (teneur élevée en lignine et en cellulose, rapport C:N élevé) favorisent l'accumulation de C dans le mélange de matière organique en particules (POM) grossier non protégé.

ii. Objectif 2 : Quel est l'effet de la sélection des espèces sur la séquestration du carbone dans différents bassins de carbone du sol ?

Hypothèse. 2 : Les espèces fixatrices de diazote (N_2) favorisent l'accumulation de C dans les bassins protégés de limon fin+argile car elles ont des caractéristiques plus liées à l'apport de C labile, tandis que les espèces non fixatrices de N_2 favorisent l'accumulation de C dans la fraction POM.

Chapitre III : Les destins du carbone du sol nouveau et ancien diffèrent dans le sol superficiel et le sous-sol nouvellement exposé et s'expliquent par les traits racinaires, des microbes et des particules du sol.

i. Objectif 1 : Quantifier les flux de nouveau C introduit par les plantes et de vieux C préexistant dans différents bassins de sol;

Hypothèse 1 : les fractions granulométriques du pétrole associées aux fractions de taille des particules du pétrole peuvent réguler les destins de l'ancien C et du nouveau C dans le processus de séquestration du C

ii. Objectif 2 : Rechercher l'effet synergique de la nouvelle entrée C et des changements de l'ancienne entrée C dans les différents bassins de carbone.

Hypothèse 2 : Le sort du nouveau C et de l'ancien C montrera des modèles indépendants.

iii. Objectif 3 : Étudier si les différents acteurs impliqués dans le stockage du carbone et l'influence que la plante et le sol ont sur eux peuvent expliquer les schémas des nouveaux flux de carbone et des anciens flux de carbone dans différents bassins de carbone du sol.

Hypothèse 3 : Nous faisons l'hypothèse que les traits racinaires liés à la composition chimique et à la récalcitrance entraîneront une nouvelle accumulation de C dans la POM, tandis que les traits liés à un apport élevé en C entraîneront le stockage dans des fractions protégées par consommation et dépôt microbiologiques. Je m'attends à ce que la stabilité des agrégats soit corrélée positivement avec la nouvelle accumulation de C total et dans le POM fin et les fractions grossières de limon en raison de la protection physique des agrégats. Nous nous attendons à ce que la teneur en N du sol soit positivement corrélée avec la nouvelle teneur en C. Nous pensons que la fraction fine dans le sol est corrélée positivement avec le nouveau stockage du carbone dans la fraction de limon fin + argile en raison des interactions organominérales, et que le nouveau stockage du carbone dans le limon fin + argile est plus élevé dans le sous-sol que dans le sol superficiel en raison des niveaux inférieurs de saturation en carbone du sol. Enfin, je m'attends à ce que l'activité, la diversité et l'abondance microbiennes soient fortement liées à la quantité de nouveau C déposé dans les fractions de limon grossier et de limon + argile protégées, et à la consommation et à la transformation du nouveau C dans les fractions grossières et fines non protégées du POM et du POM fin en raison des minéralisations des communautés microbiennes.

Chapitre IV : La qualité du sol détermine le 'priming effect' et les espèces végétales l'affinent : le rôle de la préférence du substrat et de la concurrence dans le sol superficiel et le sous-sol

i. Objectif 1 : Quantifier les changements dans le C et l'apport de nouveau C dans le sol pour déterminer les pertes de l'ancien C dans le sol superficiel et le sous-sol remontés à la surface et revégétalisé et le 'priming effect' de la revégétalisation avec des espèces fixant N_2 (*Medicago sativa*) et une espèce non fixant N_2 (*Lolium perenne*)

Hypothèse 1 : Notre hypothèse est que le sol superficiel aura des pertes plus élevées de vieux C en raison de la biomasse et de l'activité microbienne plus élevées. Cependant, en raison de la plus grande protection du vieux C dans le sous-sol et des changements des conditions environnementales dus à la revégétalisation, nous émettons l'hypothèse que le sous-sol aura des pertes de vieux C plus élevées que le sol nu, ce qui signifie un 'priming effect' positif plus élevé que le sol de surface.

ii. Objectif 2 : Quantifier le 'priming effect' dans différents bassins C liés aux fractions granulométriques du sol.

Hypothèse 2 : Étant donné la protection plus élevée de C dans la fraction plus fine du sol (fractions limon et limon + argile), nous supposons que le 'priming effect' se produira dans les fractions de matière organique particulaire non protégée (POM et POM fin).

iii. Objectif 3 : Étudier l'évolution dans le temps des sources de C respiré dans le système (représentées par l'abondance du ^{13}C) et ses corrélations avec les nouvelles pertes de C, les nouvelles entrées de C et le priming effect.

Hypothèse 3 : La source de respiration dans le système sol-plante se tournera davantage vers les intrants végétaux marqués au fil du temps, avec le développement des plantes. Les nouveaux apports de C seront positivement corrélés avec l'abondance du ^{13}C dans le CO_2 respiré (A13C). Cependant, je m'attends à des comportements différents dans les deux sols en ce qui concerne les pertes de nouveau C. Dans le sol superficiel , je suggère que l'A13C sera corrélé négativement avec les pertes de nouveau C, en raison d'un apport élevé de nouveau C dans le système et de l'utilisation accrue de nouveau C comme substrat pour la croissance microbienne (reflétée par un A13C supérieur). Dans le sous-sol, je fais l'hypothèse d'une corrélation positive entre l'A13C et les pertes de nouveau C, puisqu'un faible apport de nouveau C augmentera l'activité microbienne qui, ne pouvant satisfaire leurs besoins énergétiques principalement à partir de ces sources labiles, exploitera le nouveau C plus efficacement. De la même façon, le ‘priming effect’ sera corrélé négativement à A13C dans le sol arable, tout en étant corrélé positivement dans le sous-sol.

APPROCHE ET CONCEPTION EXPERIMENTALE

Pour atteindre ces objectifs, deux expériences ont été conçues et réalisées dans le cadre de ce projet de recherche.

Dans la première expérience, 12 espèces herbacées différentes ont été cultivées en monoculture dans 72 boîtes de culture (six répétitions par espèce). Sur ces six répétitions, la moitié a été utilisée pour l'échantillonnage du sol, tandis que l'autre moitié a été cultivée dans des boites munies de fenêtres en PVC utilisées pour observer la croissance des racines. Toutes les deux semaines, chaque fenêtre de racines a été photographiée pour évaluer le taux d'allongement des racines et les caractéristiques des racines. Après 10 mois, des carottes de sol ont été prélevées pour évaluer 1) les caractéristiques architecturales des racines, 2) la composition chimique des racines, 3) le carbone du sol dans quatre fractions de sol différentes (POM <200 µm ; POM fin 50-200 µm, limon 20-50 µm, limon + argile <20 µm), 4) la respiration microbienne induite (SIR) du substrat comme indicateur d'activité microbiologique.

Dans la deuxième expérience, deux des 12 espèces présentant des tendances aux extrémités opposées du spectre économique racinaire (*Lolium perenne* et *Medicago sativa*) ont été sélectionnées et cultivées en monoculture en pots. Les pots ont été cultivés dans des microcosmes avec des conditions environnementales constantes et du CO_2 atmosphérique constamment enrichi en ^{13}C pendant 183 jours sur deux types de sol. Les deux types de sol, soit le sol superficiel (0-30 cm) et le sous-sol (110-140 cm), ont été extraits du même profil de sol à Pisciotta (SA), en Italie. Les sols étaient argileux et présentaient des caractéristiques contrastées (teneur en azote, stabilité des agrégats, biomasse et activité microbienne). De plus, dans le sol superficiel , la teneur en argile était légèrement inférieure à celle du sous-sol (-8 %) et la teneur en C nettement supérieure (sol superficiel 12 mgC g^{-1} sol ; sous-sol 6 mgC g^{-}1 sol), ce qui entraîne un niveau de saturation en C supérieur. Les plantes et les sols ont été croisés et six pots répliqués ont été semés. Par ailleurs, six pots témoins nus (non semés) ont été mis en place pour chaque sol. Toutes les deux semaines, la respiration du sol était échantillonnée pour évaluer ^{13}C% de CO_2 respiré et le CO_2 dérivé de la plante, et après six mois, les pots étaient collectés et le sol échantillonné pour une gamme de différentes caractéristiques du sol, des racines et des caractéristiques microbiologiques.

PRINCIPAUX RESULTATS

Au chapitre II, nous n'avons observé aucun effet significatif des espèces sur l'accumulation de C dans les différents gisements de C associés aux fractions du sol. Cependant, lorsque nous avons observé l'effet de la famille, les espèces de Fabaceae fixatrices de N_2 ont accumulé plus de C dans la fraction de limon fin protégée, tandis que les espèces de Poaceae non fixatrices de N_2 dans la fraction POM. Les caractéristiques des racines différaient significativement entre les deux familles, les Poaceae ayant des tissus plus récalcitrants (lignine et cellulose élevées, et rapport C:N élevé), une biomasse racinaire plus faible et un taux d'allongement des racines plus faible. Les espèces de Fabaceae présentaient des tissus plus labiles (hémicellulose élevée et faible rapport C:N), une biomasse plus élevée et un taux d'élongation des racines plus élevé. Les espèces de Fabaceae ont également augmenté l'activité microbienne. Grâce à l'analyse en composantes principales et aux corrélations de Pearson, nous avons montré l'effet d'un apport élevé de C labile (typique des espèces acquisitrices à croissance rapide) entraînant une accumulation plus élevée dans la fraction de limon protégée. Les espèces conservatrices à croissance lente, à l'autre extrémité du spectre économique racinaire, augmentent l'accumulation de C dans la POM non protégée. Cette différence dans les stratégies d'accumulation de C a confirmé la corrélation entre le spectre économique racinaire et le stockage de C dans différents basin de C. Cette différence était due à l'effet de l'espèce sur l'activité microbienne. Une activité microbienne élevée chez les espèces de Fabaceae a favorisé la minéralisation de l'intrant C et son entombage dans la fraction limoneuse, tandis que l'activité microbienne plus faible chez les espèces de Poaceae a diminué la décomposition et la minéralisation du C introduit par rotation des racines et augmenté sa stabilité et son accumulation dans la fraction POM.

Dans le chapitre III, nous avons montré comment l'apport de C dérivé de nouvelles plantes et les pertes de C préexistant étaient en synergie, avec un apport plus élevé de nouveau C diminuant les pertes d’ancien C. Les espèces plantées en surface ont considérablement augmenté l'apport de nouveau C dans le sol et diminué le rendement du C ancien. En particulier, *M. sativa* avait un apport plus élevé et des pertes plus faibles que *L. perenne*. L'apport de nouveau C s’est principalement fait au niveau des fractions POM et limons fins+argile dans les sols. Dans le sol superficiel , l'ancien C a diminué dans tous les gisements sauf dans la fraction limons fins+argile, où il s'est accumulé. Dans le sous-sol, l'ancien C a diminué dans tous les gisements sauf dans le POM, où la diminution n'a pas été significative. Les différentes caractéristiques des racines, des microbes et du sol étaient mieux corrélées avec l'apport de nouveau C dans les fractions que les changements de l'ancien C. Les anciennes pertes de C semblaient plus liées au choix du sol et " intrinsèques " au système du sol. Cette entrée de nouveau C était principalement corrélée positivement avec la production de biomasse racinaire, tandis que le rapport C:N était corrélé négativement avec la nouvelle entrée de C dans les fractions POM et limons fins+argile. Les caractères racinaires sont mal corrélés aux variations de quantité de l'ancien C. Les caractéristiques microbiologiques ont été le principal facteur à l'origine des nouveaux apports de C, corrélés positivement à l'augmentation du nouveau C dans chaque fraction. Ils étaient aussi positivement corrélés avec l'ancienne accumulation de C dans la fraction limons fins+argile. En ce qui concerne les caractéristiques du sol, la stabilité des agrégats et la teneur en N étaient en synergie et en corrélation positive avec les nouveaux apports de C dans le système et l'accumulation des anciens C dans la fraction limons fins+argile. La fraction fine du sol (<20µm) a été corrélée négativement avec la nouvelle entrée de C et, étonnamment, l'ancienne accumulation de C dans la fraction limons fins+argile. Ces résultats ont clairement montré comment le type de sol constitue le facteur pricipal influant sur le stockage et le cycle du carbone dans le sol car la fertilité et l'activité microbienne du sol constituent le moteur de la séquestration du carbone. Les espèces végétales ont un effet secondaire sur le stockage et le cycle du carbone dans le sol . *M. sativa* est l’espèce la plus influente parmi les 12 étudiées et agit en augmentant l'apport de nouveau C grâce à une production plus élevée de tissus labiles et une activité microbienne accrue. Une faible saturation en C du sol ne semble pas avoir d'influence positive sur le stockage du C dans la fraction limons fins+argile. Cependant, lorsque l'apport de nouveau C dans la fraction limons fins+argile est normalisé par la biomasse racinaire (pour estimer l'apport de nouveau C par g de racine), le sous-sol a un rendement de stockage C supérieur à celui de la terre végétale, et *L. perenne* a un apport supérieur par g de biomasse produite. Une saturation plus faible en C pourrait donc avoir un effet positif sur le stockage du C dans le sol, mais cet effet est atténué par la fertilité du sol (qui détermine la production de biomasse et l'apport de C dans le système) et l'activité microbienne (qui transforme l'apport de C et le transfère dans la fraction limons fins+argile par métabolisme microbien). Dans ce chapitre, nous montrons clairement la puissance du couplage des techniques de marquage isotopique avec le fractionnement du sol pour décrire efficacement les changements de C dans le sol et étudier leurs corrélations avec les différents acteurs impliqués.

Enfin, dans le Chapitre IV, nous montrons une fois de plus comment le sol est l'élément principal qui façonne le 'priming effect', avec des pertes de carbone plus élevées dans le sol superficiel que dans le sous-sol en raison de la biomasse et de l'activité microbienne accrues, mais un 'priming effect' négatif dans le sol superficiel et positif dans le sous-sol. L'augmentation de l'apport de nouveau C dans le sol favorise le passage de la préférence pour le substrat des plantes de l'ancien C préexistant à l'apport de nouveau C. Ceci peut être observé dans les résultats de respiration du sol : dans le sol superficiel , l'augmentation de la signature du ^{13}C au cours des six mois est supérieure à celle de le sol superficiel , atteignant une quantité plus élevée de CO_2 provenant de la minéralisation du nouveau C introduit par rapport à l'ancien C préexistant dans le sol sol. La quantité totale d'ancien C consommée dans un sol végétalisé diminue par rapport à un sol nu, ce qui entraîne un 'priming effect' négatif. Dans le sous-sol, l'apport de nouveau C n'est pas assez élevé pour permettre le changement de préférence du substrat, et les communautés microbiennes continuent d'utiliser l'ancien C préexistant pour l'acquisition des nutriments. Dans le sous-sol, nous pouvons observer un effet significatif des espèces, *M. sativa* ayant un 'priming effect' positif plus élevé que *L. perenne*. Ceci peut s'expliquer par la concurrence entre les communautés microbiennes et les plantes pour l'acquisition d'azote. L'absorption racinaire par *L. perenne* concurrence les communautés microbiennes pour l'acquisition d'azote et réduit leur activité, ce qui réduit globalement leur efficacité à consommer l'ancien C et entraîne un faible 'priming effect' (pas significativement différent du sol nu). *M. sativa* étant une espèce fixatrice de N_2, elle ne concurrence pas les microorganismes pour le N. L'augmentation de l'intrant de C labile augmente en fait la biomasse et l'activité microbiennes et l'extraction de l'ancien C pour l'exploitation des ressources. Pour cette raison, *M. sativa a* un 'priming effect' positif plus élevé. Dans ce chapitre, nous réconcilions les théories de la préférence pour le substrat et celles de la concurrence, qui déterminent le 'priming effect' et dépendent de la fertilité du sol et, ensuite, des espèces végétales. Le 'priming effect' de la fertilité du sol se manifeste par la préférence du substrat, le sol fertile permettant aux communautés microbiennes de changer de substrat et ayant pour résultat un 'priming effect' négatif. Les sols pauvres ne permettent pas le changement de substrat et donnent lieu à un 'priming effect’ positif, dont l'ampleur est déterminée par l'absence de concurrence microbienne pour l'azote par les plantes. Nous avons également observé que le ‘priming effect’ dans le sous-sol était plus élevé dans les fractions limon et limon fin + argile, remettant en question la stabilité effective de ces fractions.

CONCLUSION ET APPLICATION PRATIQUE

Avec cette recherche, nous avons mis en évidence comment les espèces fixant N_2 sont plus efficaces pour la séquestration du C grâce à un apport plus élevé de C labile qui augmente le stockage total du C, plus particulièrement dans les bassins de C stables limon et limon + argile. L'apport plus élevé dans les bassins protégés limon et limon + argile est lié aux caractéristiques racinaires liées à la labilité (en particulier les caractéristiques chimiques des racines) qui augmentent l'activité microbiologique. Dans cette perspective, l'étude du spectre économique racinaire est un outil prometteur pour établir un lien entre les traits racinaires et la séquestration du carbone. La symbiose avec la bactérie *Rhizobium* joue également un rôle important en augmentant la production et le dépôt d'exopolysaccharides dans les fractions fines du sol.

Le sol est le principal facteur qui influe sur le stockage du C, et l'analyse des basins de carbone liés aux fractions du sol couplé à l'expérience de l'étiquetage isotopique est une méthodologie puissante pour démêler les mécanismes du cycle C. Le sol superficiel a un apport plus élevé en C en raison d'une fertilité plus élevée et d'une activité microbienne plus élevée, ce qui augmente le dépôt de C dans la fraction protégée de limon et de limon + argile. Le sol superficiel a également moins de pertes de carbone ancien grâce au passage de la consommation préférentielle de substrat de l'ancien C vers le nouveau C des communautés microbiologiques. Globalement, l'effet de la saturation en C sur le stockage du C dans la fraction limon + argile semble être soumis à la qualité du sol en termes de teneur en N et d'activité microbiologique. Cependant, lorsque ces exigences sont satisfaites, il peut stocker C plus efficacement, comme le suggère la quantité plus élevée de C déplacée dans les fractions de limon + argile par g de racine dans le sous-sol (faible saturation en C) par rapport à la terre végétale (saturation en C élevée).

Le sol est également le principal moteur de le ‘priming effect’, le sol superficiel présentant un ‘priming effect’ négatif en raison du passage des communautés microbiologiques de l'ancien C au nouveau C. Dans le sous-sol, le ‘priming effect’ est positif et la concurrence détermine son ampleur : *L. perenne* diminue le ‘priming effect’ positif (presque aucun ‘priming effect’) grâce à la compétition pour l'azote qui inhibe l'activité microbiologique. M. sativa, d'autre part, augmente l'azote du sol grâce à sa capacité de fixation de l'azote et augmente l'activité microbiologique, ce qui augmente globalement le 'priming effect' positif.

Une de nos principales indications pratiques est de ne pas considérer le potentiel de stockage du C du sol uniquement du point de vue de la minéralogie, de la teneur en argile ou de la saturation en C, mais de faire attention à la santé du sol. Plus spécifiquement, pour évaluer ses niveaux de fertilité (teneur en N), la stabilité des agrégats (MWD) et le développement des communautés microbiennes (évaluation de leur biomasse et/ou activité). La diversité microbienne pourrait également être un indicateur important. Ces indicateurs sont liés à un apport plus élevé de C dans le sol par le biais d'une production accrue de biomasse, d'un transfert vers un bassin limons fins+argile protégé et d'un 'priming effect' négatif dû à un changement d'utilisation du substrat.

L'utilisation de le sol superficiel fertile augmente l'accumulation de carbone par rapport à un sous-sol pauvre et il est donc souhaitable pour la revégétalisation des sols géotechniques. Lors de la revégétalisation de le sol superficiel fertile, les espèces à croissance rapide qui fixent l'azote (c.-à-d. les légumineuses) avec un apport élevé de C labile sont plus efficaces pour stocker le C dans un bassin protégé de limons et de limons fins+argile par un apport racinaire et un renouvellement microbien *in vivo* plus élevés. De plus, la revégétalisation de le sol superficiel a un 'priming effect' négatif, ce qui augmente la stabilité du C préexistant.

Cependant, l'utilisation de terre végétale n'est pas toujours possible. Certaines conditions particulières peuvent nécessiter la revégétalisation du sous-sol ; par exemple, en cas d'indisponibilité de le sol superficiel fertile, l'impact écologique de l'enlèvement de le sol superficiel fertile d'une zone différente, ou en raison de vastes zones excavées qui seraient trop coûteuses économiquement et écologiquement pour être couvertes de terre végétale fertile (comme de vastes carrières). Dans ce cas, nous conseillons de:

1) Fertiliser le sol pour augmenter la production de biomasse et le stockage du C.

2) Ensemencer avec des communautés microbiennes.

De plus, la saturation en C basse n'augmente pas le stockage du C protégé dans le sous-sol dans notre expérience, mais elle est encore prometteuse pour le stockage potentiel du C si la fertilité et les exigences microbiennes sont respectées. Si la fertilisation et l'inoculation microbienne sont impossibles, nous suggérons d'éviter l'utilisation d'espèces fixant l'azote, car l'augmentation de la biomasse microbienne liée à ces espèces entraînerait une minéralisation plus importante de l'ancien C.