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Impact d'une espèce ingénieure de l'écosystème et son utilisation en restauration écologique : Le cas de *Messor barbarus* (L.) dans les pelouses méditerranéennes

Tania de Almeida

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THÈSE DE DOCTORAT D'AVIGNON UNIVERSITÉ

École Doctorale N° 536
Agrosciences & Sciences

Spécialité / Discipline de doctorat :
Biologie

Institut Méditerranéen de Biodiversité et d'Ecologie
marine et continentale

Présentée par
Tania De Almeida

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Soutenance publique prévue le 11/12/2020 devant le jury composé de :

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Mme Estelle Forey, Maître de Conférences HDR, ECODIV, Université de Rouen Normandie
Examinatrice

Mme Apolline Auclerc, Maître de Conférences, LES, Université de Lorraine, ENSAIA **Examinatrice**

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*Ne te contente pas du « qu'est-ce que c'est »,
mais essaie de savoir le « pourquoi » et le « comment ».*

Robert Baden-Powell

AVANT-PROPOS

Cette thèse a été menée au sein de l'Institut Méditerranéen de Biodiversité et d'Ecologie marine et continentale (IMBE - UMR CNRS 7263 / IRD 127, équipe 4.1 « Ingénierie de la Restauration des Patrimoines Naturels et Culturels ») dans les locaux de l'IUT d'Avignon (Avignon Université). Elle a été co-dirigée par François Mesléard (Directeur de recherche Tour du Valat) et Thierry Dutoit (Directeur de recherche CNRS), et co-encadrée par Olivier Blight (Maître de conférences Avignon Université). Ces travaux ont été cofinancés par la Région PACA-Sud et par l'Institut de recherche Tour du Valat dédié à la conservation des zones humides méditerranéennes.

Le manuscrit comprend une introduction générale, trois chapitres sous forme d'articles scientifiques et une discussion générale. L'introduction générale, rédigée en français, permet de situer ces travaux dans une problématique de recherche plus large. Elle présente le cadre, les objectifs ainsi que les principales questions et hypothèses de la thèse.

Les trois chapitres, tous rédigés en anglais, abordent les différentes hypothèses qui structurent ce travail. Le premier et le deuxième chapitre ont été respectivement publiés dans les revues *Proceedings of the Royal Society B: Biological Sciences* (Volume 287, septembre 2020, 1935) et *Biological Conservation* (Volume 245, mai 2020, 108547). Le troisième chapitre constitue un article actuellement en cours de préparation pour la revue *Journal of Applied Ecology*. Une discussion générale synthétise et discute des principaux résultats et présente les perspectives de cette recherche.

La forme de ce manuscrit induit certaines redondances, notamment entre les paragraphes « Introduction » et « Matériels et méthodes » des deux premiers chapitres. Des transitions font le lien entre chaque chapitre et permettent de développer des notions clés qui n'auront pas été approfondies dans l'introduction générale. Les références bibliographiques ont toutes été reportées à la fin du manuscrit.

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INTRODUCTION GENERALE

I.1 Importance des relations abiotiques-biotiques dans le fonctionnement des écosystèmes

Un des objectifs majeurs des recherches menées en écologie fondamentale est de comprendre comment, au sein d'un écosystème, la distribution et l'abondance des espèces sont impactées par les interactions des organismes entre eux et avec leur environnement. Une grande partie des théories écologiques concernent le rôle des interactions entre les organismes sur la distribution et l'abondance des espèces, mettant l'accent sur les relations trophiques ou de compétition entre les organismes.

Au sein de l'écosystème, considéré comme une entité écologique composée d'un environnement et d'un ensemble d'espèces en relation entre-elles mais aussi avec leur environnement (e.g. air, eau, minéraux) sur une échelle spatiale définie (Tansley 1935), les relations entre organismes ne sont cependant pas les seules à modifier l'abondance et la distribution des espèces. Trois types d'interactions sont en effet possibles : des relations abiotiques-abiotiques (e.g. érosion, évaporation), des relations biotiques-biotiques (e.g. prédation, reproduction) et des relations abiotiques-biotiques (e.g. respiration, photosynthèse).

Le fait que certains organismes structurent fondamentalement leur écosystème de par leur activité physique est connu depuis longtemps (voir Buchman et al. 2007). Bien avant « l'écologie moderne », Morgan (1868) constate ainsi que la morphologie des cours d'eau est modifiée par les barrages des castors. Darwin (1881) et Shaler (1892) mentionnent quant à eux le rôle des vers de terre et autres invertébrés dans la structuration des sols. Il est alors reconnu que les modifications de l'environnement physique causées par les organismes sont susceptibles de jouer sur la distribution et l'abondance des organismes avec diverses conséquences sur le fonctionnement des écosystèmes. Néanmoins les généralisations font encore défaut et aucune définition claire permettant de distinguer ce processus des autres préalablement établis n'a été proposée (Naiman et al. 1988). Ce n'est qu'au cours des années 1990 que Jones et al. (1994) qualifient ces organismes, jouant un rôle dans la création, la modification et le maintien des habitats par le biais d'interactions non trophiques, d'espèces « ingénieures des écosystèmes ».

I.2 Les espèces ingénieuses des écosystèmes

I.2.1 Définitions

Les espèces ingénieuses des écosystèmes ont été définies par Jones et al. (1994) comme des « *organismes qui contrôlent directement ou indirectement la disponibilité en ressources d'autres organismes en causant des changements d'état physique du matériel biotique ou abiotique, en modifiant, maintenant et/ou créant des habitats* ». Ce concept a ensuite été précisé par Jones et al. (1997) avec l'ajout du terme « physique » afin de prendre en compte la possibilité pour les structures créées par un ingénieur des écosystèmes de constituer directement un habitat pour d'autres organismes. Les ingénieurs physiques des écosystèmes sont ainsi définis comme des « *organismes qui contrôlent directement ou indirectement la disponibilité des ressources pour d'autres organismes en provoquant des changements d'état physique du matériel biotique ou abiotique. Le processus d'ingénierie physique des écosystèmes par un organisme est la modification physique, le maintien ou la création d'habitats. Les effets écologiques de l'ingénieur sur d'autres espèces résultent du fait que les changements physiques contrôlent directement ou indirectement les ressources utilisées par ces autres espèces.* » (Jones et al. 1997). Cette définition, plus précise, est retenue pour la thèse.

Fondé sur cette nouvelle définition, un modèle conceptuel a été élaboré afin de comprendre et de prévoir les effets de l'ingénieur des écosystèmes sur son environnement (Jones et al. 2010). Il est fondé sur plusieurs relations de cause à effet reliant quatre composantes : l'ingénieur des écosystèmes, les changements d'état physique, abiotiques et biotiques associés (Figure I.1). Ce modèle permet de relier le processus d'ingénierie des écosystèmes et les changements abiotiques associés avec les conséquences biotiques dudit processus sur les autres espèces et l'ingénieur des écosystèmes. Les changements structurels et/ou abiotiques peuvent entraîner de nombreux types de réponses biotiques au niveau de l'organisme (e.g. la croissance et la reproduction), des espèces (e.g. les changements d'abondance et de distribution, les interactions interspécifiques comme la compétition) et de l'écosystème (e.g. les processus biogéochimiques et la productivité primaire). Les changements d'état physique induits par un ingénieur des écosystèmes contrôlent la disponibilité des ressources dont dépendent d'autres espèces ou l'ingénieur lui-même. Des rétroactions, positives ou négatives, de l'ingénieur peuvent alors exister lorsque les

changements d'état physique, abiotique et/ou biotiques affectent en retour l'activité et/ou la densité de l'ingénieur (Figure I.1). Ces rétroactions peuvent avoir lieu à la même échelle de temps que les effets de l'ingénieur ou à des échelles de temps différentes (Jones et al. 2010).

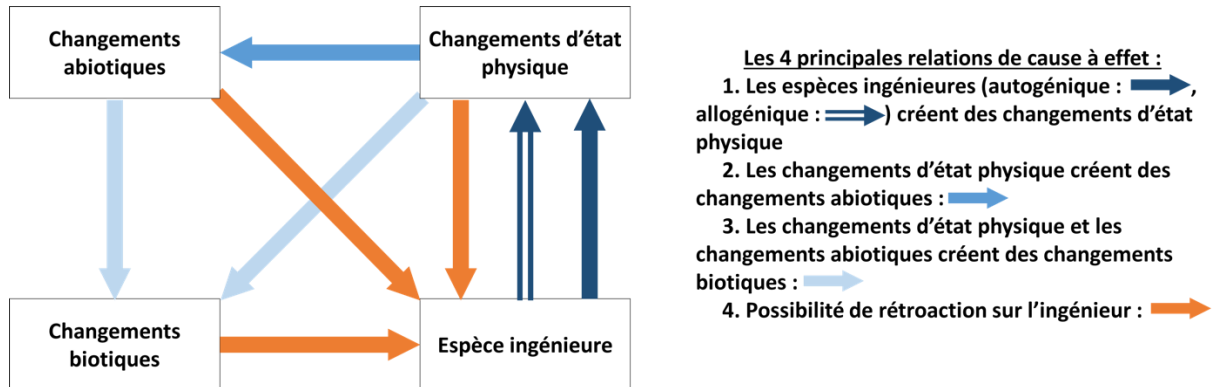


Figure I.1 : Concept d'ingénierie physique des écosystèmes effectuée par les ingénieurs des écosystèmes présentant les relations de cause à effet qui se produisent dans un système modifié par un ingénieur, d'après Jones et al. (2010).

I.2.2 Classification des ingénieurs des écosystèmes

Initialement, les espèces ingénieuses des écosystèmes ont été scindées en deux grandes classes : (1) les espèces autogéniques modifiant leur environnement par leur simple présence physique (tissus vivants et/ou mort) (e.g. les arbres) et (2) les espèces allogéniques modifiant leur environnement par transformation des matériaux vivants et/ou morts d'un état physique à un autre (e.g. les castors) (Jones et al. 1994). A partir de ces deux classes, les effets des ingénieurs des écosystèmes sur leur environnement ont été divisés en cinq catégories non exclusives (Figure I.2) (Jones et al. 1994). Dans ces différentes catégories, lorsque le matériel est transformé par la présence ou l'activité biologique de l'ingénieur, il est susceptible d'affecter, directement ou indirectement, le flux d'une ou plusieurs ressources. Les castors sont un exemple classique d'ingénieur allogénique (Figure I.2, cas 2b). En transformant un matériau vivant (arbre) en barrière physique (tronçons de bois morts), ceux-ci créent des retenues d'eau et génèrent d'importantes zones humides (Wright et al. 2002). Les arbres d'une forêt, en modifiant la disponibilité en eau et en nutriments des sols, la pénétration de la lumière ou encore la force du vent, seraient alors les équivalents autogéniques des castors (Figure I.2, cas 2a). La grande diversité d'exemples disponibles pour illustrer l'une ou l'autre de ces différentes catégories appuie l'hypothèse selon laquelle l'ingénierie des écosystèmes

est un processus majeur structurant l'ensemble des écosystèmes terrestres et aquatiques (Wright & Jones 2006; Meadows et al. 2012; Stokes et al. 2012).

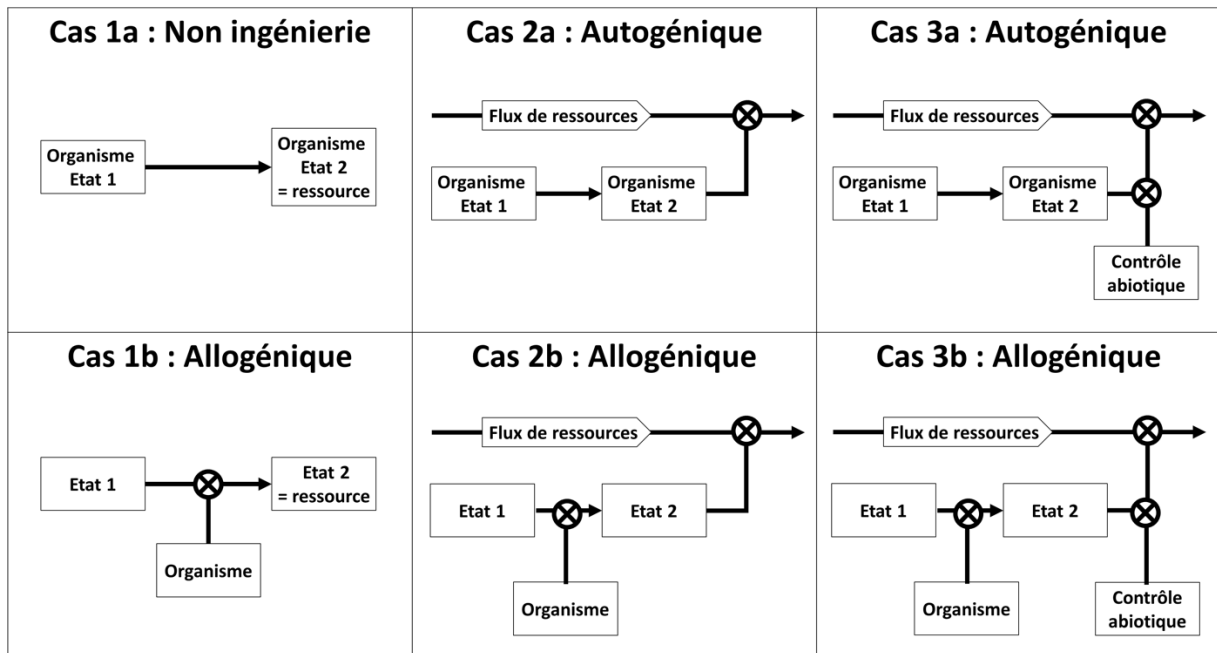


Figure 1.2 : Modèles conceptuels des effets des espèces ingénieries allogéniques et autogéniques sur l'environnement, d'après Jones et al. (1994). Les cercles barrés correspondent aux points d'altération. La mise à disposition directe d'une ressource par une espèce pour une autre n'est pas considérée comme de l'ingénierie (1a). Les espèces allogéniques transforment du matériel brut qui devient alors une ressource pour d'autres espèces (1b) ou qui est susceptible d'affecter le flux d'une ou plusieurs ressources, soit directement (2b) soit indirectement quand le matériel transformé affecte un paramètre abiotique majeur (3b). Le parallèle peut être fait avec les espèces autogéniques dont les tissus (vivants ou morts) représentent l'environnement (2a et 3a).

Considérant la façon dont les ingénieurs influencent les écosystèmes et la diversité des mécanismes et des trajectoires qui en résultent, Berke (2010) a proposé une nouvelle classification, utilisée dans cette thèse, en fonction de leur diversité fonctionnelle. Cette classification met l'accent sur la façon dont ils modulent la disponibilité des ressources pour d'autres organismes. Quatre classes fonctionnelles non exclusives d'ingénieurs des écosystèmes sont ainsi définies : les ingénieurs structurels, les bioturbateurs, les ingénieurs chimiques et les ingénieurs en éclairage. Les ingénieurs structurels correspondent aux organismes qui créent ou modifient des éléments structurels de l'habitat tels que les bivalves, les coraux, les castors, les termites ou encore les fourmis. Le terme bioturbateur fait référence aux organismes fouisseurs tels que crabes, les bivalves, les vers de terre et les fourmis. Certains organismes, capables de créer des gradients biogéochimiques, sont qualifiés d'ingénieurs chimiques, c'est notamment le cas de la plupart des plantes (aquatique ou terrestres), des oiseaux marins ou des ours, capables de contrôler la disponibilité des

nutriments marins dans les sols terrestres (Anderson & Polis 1999; Hilderbrand et al. 1999). Les organismes capables de modifier l'intensité, la pénétration et la diffusion de la lumière sont appelés ingénieurs en éclairage, comme le zooplancton, phytoplancton ou encore la plupart des plantes.

I.3 Enjeux actuels autour du concept d'ingénieurs des écosystèmes

Bien que le concept d'espèces ingénieuses des écosystèmes soit généralement accepté, il suscite plusieurs controverses. La plus commune porte sur son analogie au concept d'espèces clés de voûte. Paine (1995, 1966) définit bien ces dernières comme des espèces jouant un rôle disproportionné par rapport à leur abondance ou biomasse relative sur la structure et/ou le fonctionnement des communautés (occurrence, répartition et densité d'autres espèces) ou de son écosystème. Cependant ce rôle s'effectue souvent à travers des interactions trophiques or la définition des ingénieurs des écosystèmes exclue explicitement les interactions trophiques (Jones et al. 1997). Ces deux concepts soulignent donc des mécanismes différents. Le concept d'espèces clés de voûte repose sur les effets disproportionnés de ces espèces sur la structure et le fonctionnement des écosystèmes à travers des relations inter-spécifiques (concept axé sur les « résultats ») tandis que le concept d'espèces ingénieuses repose sur des changements d'états de l'écosystème et l'impact de ceux-ci sur les autres espèces (concept axé sur les « processus ») (Wilby 2002; Wright & Jones 2006). Néanmoins, les deux concepts ne sont pas exclusifs. Bien que ce ne soit pas le cas de toutes les espèces clés de voûte (e.g. les loutres de mer) (Jones et al. 1997), plusieurs peuvent être considérées comme espèces ingénieuses (e.g. les castors).

Certains auteurs soulignent également que tous les organismes modifient, d'une manière ou d'une autre, leur environnement et qu'ils sont donc tous susceptibles d'être considérés comme des ingénieurs des écosystèmes, rendant ainsi ce concept inutile (Reichman & Seabloom 2002). L'ubiquité de ce concept peut, *a contrario*, être considérée comme un point fort, dans la mesure où il s'applique largement à de nombreux organismes et pour de nombreux habitats. Dans certains cas, le travail de modification de l'environnement est partagé par toutes les espèces d'un système (e.g. diverses espèces de coraux créant des récifs), plutôt que le produit d'une seule espèce. Dans d'autres situations, la modification est principalement assurée par certaines espèces (e.g. les brise-vent des arbres forestiers).

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La quantification des impacts des ingénieurs des écosystèmes est un autre enjeu primordial de ce concept. En effet, les ingénieurs peuvent modifier leur environnement positivement et/ou négativement (Jones et al. 1997; Jones et al. 2010), que ce soit par exemple la biodiversité ou la productivité du milieu, en fonction du niveau d'organisation (e.g. une espèce, un groupe d'espèces tels que les producteurs primaires ou la richesse et l'abondance globales des espèces) (Streitberger et al. 2017) ainsi que de l'échelle temporelle et spatiale étudiée (Jones et al. 1997; Hastings et al. 2007). Afin de prendre en compte la variation des effets des ingénieurs des écosystèmes en fonction de l'échelle d'observation utilisée dans l'espace et le temps, Jones et al. (1997, 1994) proposent d'étudier six facteurs déterminant l'amplitude de l'impact d'une espèce ingénieure : (1) l'activité de chaque individu de l'espèce ingénieure, (2) la densité de la population, (3) la distribution spatiale (locale et régionale) de la population, (4) le temps de présence de la population sur le site, (5) la durabilité des impacts et constructions en absence de l'espèce ingénieure, (6) le nombre et le type de ressources directement et indirectement modifiés par les constructions, la manière dont ces ressources sont contrôlées et le nombre d'espèces dépendantes de ces ressources. Les facteurs 1 à 5 sont facilement quantifiables pour les espèces d'ingénieurs physiques alors que le facteur 6 est difficile à quantifier pour l'ensemble des espèces ingénieures. Ce dernier facteur paraît cependant essentiel pour comprendre l'impact d'une espèce sur son écosystème (Jones et al. 1997) et donc pour discriminer des espèces aux impacts « majeurs » des autres espèces ingénieures. Bien que ces six facteurs soient bien identifiés et cernés dès la première définition du concept d'ingénieur des écosystèmes, très peu d'études les ont pris en compte simultanément pour déterminer l'effet d'une ou plusieurs espèces ingénieures sur un site.

Identifier des ingénieurs clés dans le maintien ou le devenir d'un écosystème paraît nécessaire pour comprendre l'assemblage local des communautés et le fonctionnement de l'écosystème. Pour cela, il faut être capable de comprendre et de prévoir, notamment grâce aux six facteurs cités précédemment, quand, où et quels ingénieurs des écosystèmes auront des effets « importants » par rapport aux effets « mineurs » des autres espèces (Jones et al. 1994; Jones et al. 1997; Wright & Jones 2006; Badano et al. 2010). Ces connaissances fondamentales sont également déterminantes dans des contextes appliqués. Les espèces ingénieures, par leur rôle à la fois sur les compartiments abiotiques et biotiques de l'écosystème, apparaissent en effet des éléments (outils) privilégiés à utiliser en restauration

écologique, particulièrement en ingénierie écologique (voir « Transition vers le chapitre 2 » pages 57-60 pour plus d'informations) (Byers et al. 2006; Wright & Jones 2006; Hastings et al. 2007; Jones et al. 2010). Ces organismes sont capables de contribuer à la résilience de l'écosystème suite à une perturbation (Peterson et al. 1998). En effet, après une perturbation, ils peuvent accélérer la restauration écologique spontanée en rétablissant des fonctions de l'écosystème et faciliter le rétablissement d'autres espèces dans le milieu (Manning et al. 2015). Leur utilisation pourrait ainsi augmenter la probabilité de réussite de la restauration d'un milieu tout en réduisant les coûts et les efforts humains nécessaires (e.g. génie civil) (Byers et al. 2006). Les interactions du milieu étant complexes et souvent difficiles à appréhender, il est nécessaire d'acquérir le maximum de connaissances à la fois sur l'écosystèmes à restaurer (e.g. fonctionnement, structure) ainsi que sur l'espèce ingénieure considérée (e.g. biologie, écologie, interactions avec les autres espèces) afin d'éviter les potentielles conséquences négatives de l'utilisation d'une espèce ingénieure (Nellis & Everard 1983; voir par exemple Elliott et al. 1996).

I.4 Des exemples d'espèces ingénieures

I.4.1 Le castor communément admis comme espèce ingénieure

De nombreuses espèces animales modifiant leur environnement pour le rendre plus vivable, pour faciliter les recherches de nourriture ou encore pour se protéger des prédateurs, sont qualifiées d'ingénieures des écosystèmes. Le castor, déjà mentionné, est souvent considéré comme l'archétype d'une espèce ingénieure des écosystèmes allogénique. En construisant des barrages sur les cours d'eau, celui-ci crée des retenues d'eau dont les effets sont considérables sur la structure des communautés et le fonctionnement des écosystèmes au sein de ces retenues et des cours d'eau associés (Naiman et al. 1988). Ses activités modifient l'hydrologie (Smith et al. 1991), le cycle et la disponibilité des nutriments (Johnston & Naiman 1990; Naiman et al. 1991) ou encore les cycles biogéochimiques (Naiman et al. 1994) affectant alors directement ou indirectement la faune dont les invertébrés des cours d'eau, les poissons, les amphibiens, les oiseaux et les mammifères (Rosell et al. 2005) mais aussi la flore (Wright et al. 2002; Bartel et al. 2010). Le castor enrichit le milieu et modifie directement ou indirectement la végétation ligneuse riveraine (e.g. abattage, inondation). Lorsque les structures construites sont abandonnées par celui-ci, jusqu'à 8 à 10 ans après le début de l'occupation (Remillard et al. 1987), les barrages se rompent et engendrent des

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prairies étendues, au niveau et autour de l'ancienne retenue d'eau, qui peuvent persister pendant plus de 50 ans (Wright et al. 2002). Contrairement aux forêts riveraines voisines, les prairies issues de l'activité des castors possèdent une forte luminosité et des niveaux d'azote et d'humidité du sol élevés (Naiman et al. 1994), ce qui affecte la succession forestière et augmente l'hétérogénéité de l'écosystème (Johnston & Naiman 1990; Wright et al. 2002). De par l'ensemble des fonctions assurées dans son milieu, le castor peut ainsi être considéré comme une espèce particulièrement intéressante pour la restauration des zones humides et des cours d'eau (Pollock et al. 2014; Law et al. 2016; Law et al. 2017; Puttock et al. 2017). Law et al. (2017) ont d'ailleurs démontré son utilité dans le cadre de restauration de zones humides dégradées par l'agriculture. Douze ans après leur réintroduction, les castors ont notamment permis d'augmenter la richesse moyenne et l'hétérogénéité de la végétation ainsi que le recouvrement des espèces associées aux conditions de forte humidité et luminosité.

1.4.2 Les vers de terre et les termites

Les vers de terre et les termites représentent une part importante de la biomasse des invertébrés du sol. Ils sont parmi les groupes d'ingénieurs du sol les plus étudiés (Jouquet et al. 2014). Les termites et les vers de terre, en plus d'être des détritivores, agissent aussi sur la disponibilité en ressources d'autres espèces par la création de biopores ou d'agrégats. Ils participent à la plupart des fonctions clés du sol telles que la décomposition des résidus organiques à la surface, le cycle des nutriments, l'infiltration et le stockage de l'eau ou encore l'érosion (Lavelle & Spain 2001). Leurs actions au niveau du sol augmentent l'activité microbienne dans celui-ci, accélérant la minéralisation et la libération des nutriments. Leur activité favorise ainsi la croissance des plantes en améliorant la distribution des racines dans le sol et donc l'accès aux nutriments (e.g. phosphore and nitrates) absorbés par les racines (Blouin et al. 2013; Ojha & Devkota 2014). L'ensemble des fonctions effectuées par ces organismes en fait des ingénieurs des écosystèmes d'intérêt majeur dans le cadre de projets de restaurations écologiques. Ils ont été utilisés plusieurs fois, en particulier les vers, pour restaurer des propriétés du sol (Jouquet et al. 2014) telles que la structure des agrégats et la porosité (Fraser et al. 2003; Marashi & Scullion 2003; Pardeshi & Prusty 2010), la fertilité (Fraser et al. 2003) ou la réhabilitation suite à une contamination (Lukkari et al. 2006; Sizmur et al. 2011). Ces organismes ont cependant été peu employés pour restaurer les compartiments biotiques des écosystèmes (Roubíčková et al. 2009; Mudrák et al. 2012). Pour

Forey et al. (2018), l'inoculation de vers de terre pourrait être utilisée afin d'augmenter la productivité des plantes, mais elle pourrait aussi nuire à la biodiversité des sols, par la diminution d'abondance et de diversité de collemboles, et la modification des interactions de surface.

D'autres organismes du sol, moins étudiés que ces deux taxons, sont également susceptibles de jouer un rôle important dans la régulation des fonctions des écosystèmes dans certains environnements (Jouquet et al. 2014). C'est le cas des scarabées bousiers (Brown et al. 2010), des mille-pattes (Toyota et al. 2006), mais surtout des fourmis (Wills & Landis 2018).

I.5 Les fourmis : des espèces ingénieures structurelles et bioturbatrices

Les fourmis, avec environ 16 460 espèces décrites aujourd'hui (Bolton 2020), représentent le groupe d'insectes sociaux le plus diversifié. Elles sont parmi les organismes les plus abondants en milieu terrestre (Hölldobler & Wilson 1990). Leur biomasse est élevée et souvent considérée comme équivalente à la biomasse humaine (Hölldobler & Wilson 1990). Ces organismes sont considérés comme des acteurs clés de leur environnement. Elles ont la capacité de monopoliser l'espace et les ressources et donc d'influencer d'autres espèces dans les zones qu'elles occupent (Andersen, 1992). Elles affectent les compartiments de l'écosystème de surface (e.g. plantes, invertébrés) comme souterrains (e.g. sol, invertébrés) (Figure I.3). Certaines espèces jouent un rôle significatif en tant qu'ingénieurs du sol, prédateurs, recycleurs de nutriments et régulateurs de la croissance et de la reproduction des plantes (Folgarait 1998; Zelikova et al. 2011; Del Toro et al. 2012). Leurs nids constituent des zones où l'hétérogénéité du sol et celle des communautés (animales et végétales) sont les plus élevées. Après l'abandon du nid ou mort de la colonie, ces « hotspots » plus riches ont la capacité de perdurer plusieurs années (Lobry de Bruyn 1999; Kristiansen & Amelung 2001; Kristiansen et al. 2001; Lane & BassiriRad 2005) et d'être colonisés par d'autres espèces, notamment végétales (McGinley et al. 1994).

Bien que les rôles multiples joués par les fourmis au sein de leurs écosystèmes soient bien documentés, nous ne possédons jusqu'à présent que des visions partielles de leurs effets. Les études portant sur ce sujet ne s'attardent en effet que sur un ou deux compartiments et n'offrent pas de vision globale. Une vision globale du rôle des fourmis dans leurs écosystèmes permettrait d'acquérir plus de connaissances pour leur utilisation lors de projet de restauration écologique. Actuellement, ces organismes sont utilisés en bioindication (Casimiro

et al. 2019) notamment afin de déterminer si la restauration d'un milieu est un succès ou non mais, malgré un potentiel intéressant, ils restent sous exploités comme moyens de restauration. A notre connaissance les fourmis n'ont ainsi été utilisées qu'une seule fois pour restaurer un écosystème (voir Bulot 2014 pour plus d'informations).

Si l'on considère les six facteurs déterminés par Jones et al. (1997, 1994), une étude multi-compartiments apparait nécessaire pour appréhender au mieux l'amplitude de l'impact d'une espèce ingénieure.

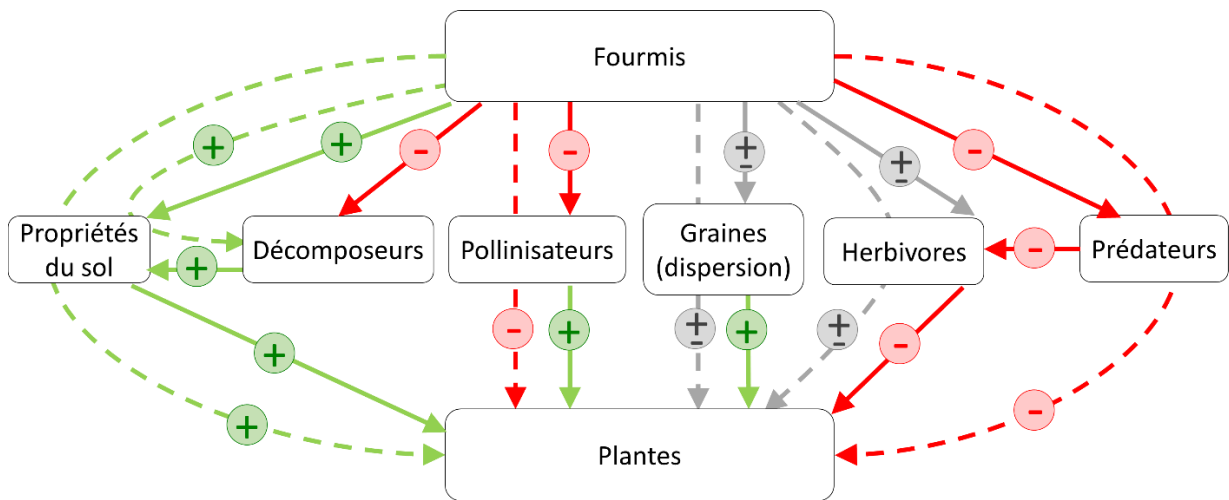


Figure 1.3 : Impacts directs (lignes continues) et indirects (lignes discontinues) des fourmis sur les compartiments abiotiques et biotiques. En rouge les interactions négatives, en vert les interactions positives et en gris les interactions pouvant être à la fois positives et négatives (traduite d'après Wills & Landis 2018).

1.5.1 Impacts sur le sol

Lors de la construction et de la maintenance du nid, les fourmis rapportent des couches inférieures de sol à la surface mélangeant ainsi, via leur activité de bioturbation, les différentes couches de sol (Cammeraat et al. 2002; Dostál et al. 2005). Cette modification de la structure du sol ainsi que la création de macropores peuvent favoriser l'infiltration de l'eau en réduisant la densité apparente du sol (Lobry de Bruyn & Conacher 1990; Cammeraat & Risch 2008). Les nids peuvent alors constituer des zones de ruissellement, modifiant la distribution des nutriments et de l'eau dans le sol et créant ainsi des îlots de fertilité et d'humidité élevée (Lobry de Bruyn & Conacher 1990; Eldridge 1994; Cammeraat et al. 2002). Les activités de bioturbation, de fourragement et de dépôt de déchets à l'entrée du nid par les fourmis, peuvent également modifier directement ou indirectement la quantité de nutriments dans le sol (Frouz et al. 1997; Dostál et al. 2005; Wagner & Jones 2006; Griffiths et al. 2018). Souvent

disproportionné par rapport à leur biomasse, l'impact des fourmis sur le sol peut perdurer au-delà de 20 ans après l'abandon du nid en fonction du milieu et de l'espèce considérée (Lobry de Bruyn 1999; Kristiansen & Amelung 2001; Kristiansen et al. 2001).

1.5.2 Impacts sur la faune

Les fourmis modifient directement ou indirectement l'abondance, la diversité voire le comportement des autres arthropodes présents au sein de l'écosystème (Wills & Landis 2018). Elles peuvent réduire directement les populations d'autres organismes par prédation ou compétition (Styrsky & Eubanks 2007; Sanders et al. 2011). La présence des fourmis peut également provoquer des changements sur le comportement, le développement ou la croissance des proies potentielles (Cembrowski et al. 2014; Mestre et al. 2014). L'accumulation de litière et de nutriments, affectant à la fois la disponibilité des ressources et les conditions microclimatiques dans le sol, favorise l'abondance et la diversité de faune du sol (micro-organismes, nématodes et/ou micro-arthropodes) dans les nids (Wagner et al. 1997; Boulton et al. 2003; Boulton & Amberman 2006).

Des relations mutualistes entre fourmis et autres organismes existent, notamment avec les plantes ou certains pucerons. Ces derniers, pour bénéficier de la protection des fourmis, mettent à disposition leur miellat, qui est une ressource en glucide pour les fourmis. Cette relation peut avoir des effets directs et/ou indirects positifs et/ou négatifs sur le compartiment biotique. En protégeant les pucerons, les fourmis favorisent leur établissement avec pour conséquence potentielle un impact négatif sur les plantes. Dans la plupart des cas, les interactions de mutualisme impliquant les fourmis sont cependant bénéfiques pour les plantes (Styrsky & Eubanks 2007). C'est notamment le cas lorsque les fourmis diminuent la prédation de la plante par d'autres herbivores (Ando & Ohgushi 2008; Pringle et al. 2011). Le ratio coût-bénéfice de la présence de pucerons est alors avantageux pour la plante (Pringle et al. 2011). En réduisant la présence d'herbivores, les fourmis peuvent aussi avoir un effet indirect négatif sur les prédateurs (Styrsky & Eubanks 2007).

1.5.3 Impacts sur la végétation

Le changement des propriétés physico-chimiques du sol provoqué par les fourmis peut modifier le développement des parties endogées et épigées des plantes en offrant de meilleures conditions pour leur germination, croissance et survie (Dean & Yeaton 1993; Eldridge 1994).

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Les fourmis peuvent également jouer un rôle important dans la dynamique des communautés végétales en agissant soit comme agents de dispersion des graines, soit comme prédateurs de graines, ou les deux (Levey & Byrne 1993; Retana et al. 2004; Giladi 2006; Arnan, Retana, et al. 2010; Arnan et al. 2012). Les fourmis dispersent les graines par deux mécanismes principaux. Le premier est la myrmécochorie, ou dispersion des graines par l'intermédiaire de l'élaosome, un appendice riche en lipides qui attire principalement les fourmis non granivores et « récompense » la dispersion des graines (Bronstein et al. 2006; Giladi 2006). Cette première relation est considérée comme une relation mutualiste (Bronstein et al. 2006). On considère qu'au total, au moins 11 000 espèces d'angiospermes appartenant à 77 familles différentes sont dispersées par myrmécochorie (Lengyel et al. 2009). Le second mécanisme de dispersion, la dizoochorie, est effectué par des espèces granivores. Il n'est pas influencé par une structure particulière des graines (Retana et al. 2004; Vander Wall et al. 2005; Arnan, Rodrigo, et al. 2010; Arnan et al. 2011). Dans ce cas, les graines peuvent être perdues, abandonnées en chemin ou déposées à l'entrée du nid sur le dépotoir. Cette dispersion peut, d'une certaine manière, compenser la prédation exercée sur les graines (Arnan et al. 2011; Arnan et al. 2012). Dans les deux cas, les graines conservent leurs capacités de germination.

Les fourmis, et notamment les fourmis granivores, ont donc des effets directs et indirects importants sur les communautés végétales. C'est notamment le cas pour les écosystèmes herbacés méditerranéens où les fourmis du genre *Messor* sont les espèces de fourmis granivores les plus abondantes (Cerde & Retana 1994; Azcárate & Peco 2007).

1.6 *Messor barbarus* potentielle espèce ingénieure des pelouses méditerranéennes

1.6.1 Choix de l'espèce *M. barbarus* comme modèle d'étude

Messor barbarus (Linnaeus 1767) (Hymenoptera: Formicidae) est une fourmi granivore (Heredia & Detrain 2000) commune dans les prairies méditerranéennes du sud de l'Europe et du nord de l'Afrique (Lebas et al. 2016). Cette espèce a déjà été présentée comme une potentielle espèce ingénieure des écosystèmes (Bulot 2014).

Parmi les six facteurs essentiels pour comprendre l'impact d'une espèce sur son écosystème (Jones et al. 1997), les quatre premiers apparaissent assez facilement quantifiables pour cette espèce. En effet, son rôle dans la modification des propriétés physico-chimiques du sol des pelouses méditerranéennes a déjà été étudié (facteur 1) (Azcárate &

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Peco 2007); sa densité de population peut être estimée en comptant le nombre de nids (facteur 2) ; sa distribution spatiale est bien connue (facteur 3) (Lebas et al. 2016) et son temps de présence est déterminé sur certains sites (facteur 4). La durabilité de ses impacts et de ses constructions en son absence (facteur 5) n'a pas encore été quantifiée mais il a été démontré pour d'autres espèces que l'impact des fourmis sur le sol peut perdurer jusqu'à plus de 20 ans après l'abandon du nid (Lobry de Bruyn 1999; Kristiansen & Amelung 2001; Kristiansen et al. 2001). Ce facteur aurait pu faire l'objet d'une étude complémentaire mais ne pouvait hélas pas rentrer dans le cadre de cette thèse au regard de la durée estimée de suivi. Le dernier facteur, comme pour de nombreuses autres espèces ingénieures, n'a pas encore été étudié pour *M. barbarus*. Ce point semble cependant essentiel à quantifier pour comprendre dans sa globalité le rôle d'une espèce ingénieure dans son écosystème au travers des différentes relations directes et indirectes induites par son activité.

Messor barbarus semble alors un bon modèle pour l'étude de l'impact d'un ingénieur des écosystèmes sur son environnement et pouvoir ainsi quantifier le sixième facteur déterminé par Jones et al. (1997), souvent négligé.

1.6.2 Biologie et écologie

Messor barbarus est une espèce polymorphe. Elle vit en colonies monogynes composées de 5 500 à 21 000 ouvrières (Cerdan 1989) qui atteignent une taille stable en cinq années d'existence. Son vol nuptial a lieu au début de l'automne lorsque les conditions environnementales sont favorables, la température est élevée (> 20°C), le ciel dégagé avec peu ou pas de vent et lorsqu'il y a eu de fortes précipitations la veille du vol (Cerdan 1989; Gómez & Abril 2012). Après l'accouplement en vol, les individus tombent au sol ; les femelles fécondées s'arrachent alors les ailes et cherchent et/ou creusent un lieu pour passer l'hiver. La reine peut vivre jusqu'à 15-20 ans (en laboratoire), après quoi la colonie disparaît (Baraibar et al. 2011).

Le nid est composé d'un réseau dense de galeries et de chambres interconnectées qui peuvent atteindre plusieurs mètres de profondeur (Cerdan 1989) et s'étendre sur plusieurs mètres carrés, au moins 6 m² (obs. pers.). Il est principalement entretenu par les ouvrières qui utilisent leurs mandibules pour transporter les particules de sol en dehors du nid (Oliveras et al. 2005). Elle forme en surface un réseau arborescent de pistes permanentes et temporaires à partir du nid dont la longueur totale peut dépasser 80 m (Detrain et al. 1996).

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Messor barbarus a principalement été étudiée pour son rôle sur les communautés végétales. Elle joue notamment un rôle positif sur le transport, le stockage et la redistribution de graines (Detrain et al. 1996; Detrain et al. 2000; Detrain & Tasse 2000; Azcárate et al. 2005; Azcárate & Peco 2006; Sánchez et al. 2006; Azcárate & Peco 2007). Elle est ainsi considérée comme la principale espèce prédatrice de graines dans les écosystèmes herbacés méditerranéens (Detrain & Pasteels 2000; Azcárate & Peco 2003) jouant un rôle important dans leur dispersion. Quelques études ont été également effectuées sur son impact sur le sol. Les propriétés physico-chimiques du sol peuvent différer entre les nids et les zones alentours sans nids, avec notamment une augmentation de la quantité de limons et d'azote totale au sein des nids (Azcárate & Peco 2007). Aucune étude n'a jusqu'à présent considéré son impact sur les communautés de faune. Cependant, des études sur *Messor andrei* montrent que cette espèce accroît la richesse et l'abondance des nématodes, microarthropodes, bactéries et ciliés (Boulton & Amberman 2006). Ces deux espèces étant biologiquement proches, l'hypothèse d'un impact similaire de *M. barbarus* sur les communautés de faune peut être proposée.

1.6.3 Habitat : les pelouses méditerranéennes

Le bassin méditerranéen est considéré comme l'un des 35 points chauds de biodiversité (Myers et al. 2000) auquel contribuent, notamment par leur espèces endémiques, les nombreuses pelouses méditerranéennes (Médail & Quézel 1997; Myers et al. 2000).

Les communautés herbacées du nord-ouest du bassin, caractérisé par des étés chauds et secs et des hivers frais et humides (Grove & Rackham 2003), sont dominées par des poacées. Elles constituent des écosystèmes semi-naturels dépendant classiquement de trois facteurs : le climat, les conditions édaphiques et l'impact anthropique. Ces écosystèmes sont en grande partie maintenus par le pâturage ou les feux (San Miguel 2008). Les espèces végétales présentes montrent ainsi une forte adaptation à la sécheresse estivale, aux précipitations et à leur imprévisibilité, au pâturage extensif ainsi qu'aux régimes de feux naturels ou pastoraux (Blondel et al. 2010).

Les pelouses méditerranéennes, pour la plupart riches en espèces végétales mais aussi animales inféodées ou utilisatrices, font donc l'objet d'une protection par l'Union Européenne (San Miguel, 2008; European Union Habitats Directive, 1992). Cependant, leur superficie a drastiquement régressé ces dernières décennies, notamment en raison de changements d'affectation ou d'abandons des terres. Les pelouses du nord-ouest du bassin méditerranéen

recouvrent actuellement environ 26 000 km² (“EUNIS - European Nature Information System” 2020).

I.7 Hypothèses, objectifs et organisation principale de la thèse

Ce projet de thèse s’insère dans une réflexion générale sur le concept d’ingénieur des écosystèmes. En effet, jusqu’à présent, la plupart des études menées sur les ingénieurs des écosystèmes se sont focalisées sur le rôle d’ingénieur pour un voire deux compartiments. Comme vu précédemment, selon Jones et al. (1997, 1994) six facteurs doivent nécessairement être considérés pour appréhender globalement la variation des effets des ingénieurs à différentes échelles, dans l’espace comme dans le temps. Ce constat implique de prendre en compte le nombre et le type de ressources directement et indirectement modifiées par leurs actions, mais aussi la manière dont ces ressources sont contrôlées et le nombre d’espèces dépendantes de ces ressources.

Cette thèse a ainsi pour objectif de considérer dans sa globalité le rôle d’un ingénieur des écosystèmes, *M. barbarus*, au sein de son environnement, les pelouses méditerranéennes, en étudiant plusieurs compartiments mais aussi plusieurs niveaux de modification des compartiments biotiques (présence-absence, abondance, interactions, traits fonctionnels). Cette étude multi-compartiments vise à accroître les connaissances théoriques sur l’impact à différents niveaux d’une espèce ingénieure ainsi qu’à développer des connaissances techniques permettant d’utiliser l’espèce dans des opérations de conservation et/ou restauration.

Le deuxième objectif de ce projet est ainsi d’évaluer la possibilité d’utiliser cette espèce en restauration écologique et de généraliser son utilisation ainsi que celle des autres fourmis, pour faciliter les projets de restauration futurs. Par leur action déterminante sur leur écosystème, les espèces ingénieures représentent un outil prometteur et durable mais encore peu utilisé en gestion ou restauration « de la nature par la nature » (Dutoit 2014). Faciliter la dispersion d’une espèce ingénieure ou l’introduire sur un site dégradé pourrait permettre d’accélérer la restauration du site, la structure abiotique, la composition spécifique mais aussi de nombreuses fonctions.

Cette thèse est structurée en trois axes. Le premier concerne l’impact multi-compartiments, direct et indirect, de *M. barbarus* sur son écosystème (Chapitre 1). Le second porte sur le rôle potentiel de cette espèce ingénieure dans un cas appliqué de restauration

écologique (Chapitre 2). Le troisième axe porte sur l'utilisation plus générale des ingénieurs, particulièrement des fourmis, en restauration écologique par la mise en place d'une méthodologie appliquée basée sur les traits (Chapitre 3).

Trois principales questions correspondant aux trois chapitres sont ainsi posées :

1) Quels sont les impacts directs et indirects de *M. barbarus*, au sein d'une pelouse méditerranéenne semi-naturelle, sur les propriétés physico-chimiques du sol, les communautés végétales, les communautés d'invertébrés épigés et endogés ainsi que sur les relations trophiques et non trophiques entre ces compartiments ?

Afin d'évaluer si *M. barbarus* agit comme une espèce ingénieure des écosystèmes au sein des pelouses méditerranéennes nous avons donc réalisé une étude multi-compartiments (sol, végétation et faune) de son rôle au sein de son écosystème considéré en bon état de conservation (Chapitre 1). Nous avons ainsi émis les hypothèses que cette espèce : (1) modifie la texture du sol en redistribuant les particules de sol et augmente les nutriments du sol par l'accumulation de matières végétales ; (2) ce qui à son tour affecte la communauté végétale (augmentation de la biomasse et de la hauteur des plantes) et l'état physiologique des plantes (e.g. indices d'anthocyanes, chlorophylles et azotés des feuilles); (3) augmente l'abondance et la présence de la faune de surface et souterraine tout en modifiant la structure de leur communauté ; (4) modifie, par ses impacts directs et indirects sur toutes ces composantes, les relations trophiques et non trophiques au sein de l'écosystème.

2) Quel rôle joue *Messor barbarus* dans la restauration des propriétés physico-chimiques du sol, de la banque de graines et de la communauté végétale de la pelouse dégradée ?

Après avoir évalué le rôle d'ingénieur de *M. barbarus* au sein des pelouses méditerranéennes, nous avons cherché à évaluer dans quelle mesure celle-ci peut être utilisée en ingénierie écologique (Chapitre 2). Neuf ans après une fuite d'hydrocarbures et sept ans après la restauration du sol qui a suivi, nous avons ainsi analysé sa contribution dans la restauration des propriétés physico-chimiques du sol et les communautés végétales de la Plaine de la Crau (France). Nous avons formulé les hypothèses suivantes : (1) son activité de création et maintenance du nid contribue à un changement de la texture du sol; (2) la mise en

place d'un dépotoir permet à la fois l'enrichissement du sol avec une augmentation d'apport organique, un changement des propriétés chimiques mais aussi un changement de la banque de graines du sol (communauté et densité); (3) l'enrichissement du sol ainsi qu'une augmentation de la densité de graines entraîne un changement des communautés végétales *in situ*.

3) Quel est le potentiel d'utilisation des fourmis en écologie de la restauration ? Quels traits des fourmis doivent être considérés pour la mise en place d'une méthodologie de leur utilisation en ingénierie écologique destinées aux gestionnaires ?

Bien que certaines fourmis puissent être considérées comme des ingénieures des écosystèmes (Chapitre 1) et permettent d'accélérer la restauration des propriétés physico-chimiques du sol et des communautés végétales (Chapitre 2), elles sont actuellement très peu utilisées en ingénierie écologique. La plupart des gestionnaires ne sont pas myrmécologues et peuvent donc rencontrer des difficultés pour identifier de bonnes espèces candidates pour la restauration de leurs sites. Dans un premier temps, nous avons évalué le potentiel des fourmis en écologie de la restauration puis nous avons listé l'ensemble des traits connus pour affecter les compartiments abiotiques et biotiques et/ou pertinents pour effectuer un suivi du succès de restauration. Afin de faciliter la phase de sélection des fourmis dans un projet de restauration, nous proposons une méthodologie basée sur les traits pour effectuer une première sélection des espèces potentiellement utilisables en fonction des objectifs fixés.

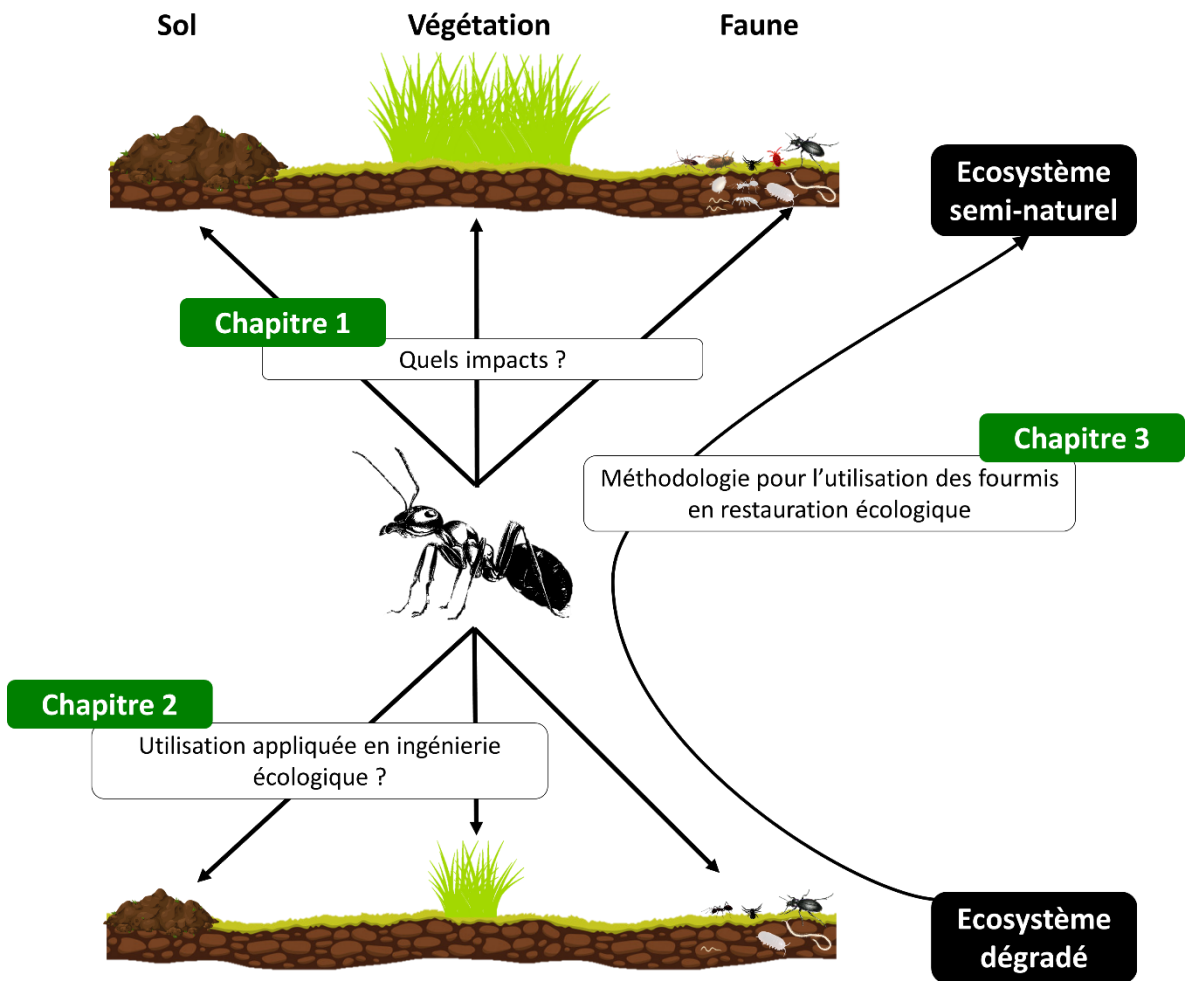


Figure I.4 : Représentation schématique de l'organisation générale de la thèse.

TRANSITION VERS LE CHAPITRE 1

Les études sur l'ingénierie des écosystèmes impliquent deux hypothèses distinctes mais non exclusives : (1) de par son activité, une espèce ingénieure influence un ensemble de conditions abiotiques et (2) ces conditions altérées influencent à leur tour un ensemble de traits biologiques des autres organismes (Hastings et al. 2007). En altérant la composition spécifique de la communauté (animales et/ou végétales), l'ingénieur des écosystèmes agit alors comme un filtre écologique (Meyer 2008).

La théorie des filtres conceptualise l'assemblage des communautés, les processus déterminant la présence ou non des espèces dans un milieu donné à partir d'un pool d'espèces régionales jusqu'à un pool d'espèces locales (Diaz et al. 1998; Grime 1998; Belyea & Lancaster 1999; Lortie et al. 2004). Initialement conçue pour des communautés végétales elle peut également s'appliquer à des communautés animales. La structure des communautés locales y est régie par les trois grands filtres écologiques : la dispersion, les conditions abiotiques et les interactions biotiques (Figure T1.1). Le premier filtre correspond à la capacité d'une espèce à coloniser un milieu soit à partir d'un pool d'espèces externes (dispersion spatiale via par exemple une pluie de graines), soit à partir d'un pool d'espèces internes (dispersion temporelle via par exemple une banque de graines). Ce filtre est parfois désigné comme filtre historique, dans la mesure où il implique également des processus à longs termes tels que la spéciation et l'extinction s'opérant à des échelles de temps évolutives (MacArthur & Wilson 2001). La présence des espèces n'est pas liée à leur capacité à tolérer un milieu mais à des processus aléatoires, il est donc également qualifié parfois de filtre stochastique, en opposition aux deux autres filtres dits déterministes (Zobel 1997; Lortie et al. 2004). Le second filtre définit la niche écologique fondamentale d'une espèce, c'est-à-dire la gamme des conditions environnementales (e.g. les conditions climatiques, édaphiques, topographiques ou certaines perturbations) dans laquelle une espèce peut vivre. Le troisième filtre correspond aux interactions (positives et négatives), intra- et inter-spécifiques entre organismes vivant au sein d'une même communauté. Les communautés et les écosystèmes étant dynamiques (De Leo & Levin 1997; Fattorini & Halle 2004), les trois filtres le sont également (Fattorini & Halle 2004). Leurs effets peuvent agir isolément ou conjointement et ainsi varier dans l'espace et le temps (Keddy 1992).

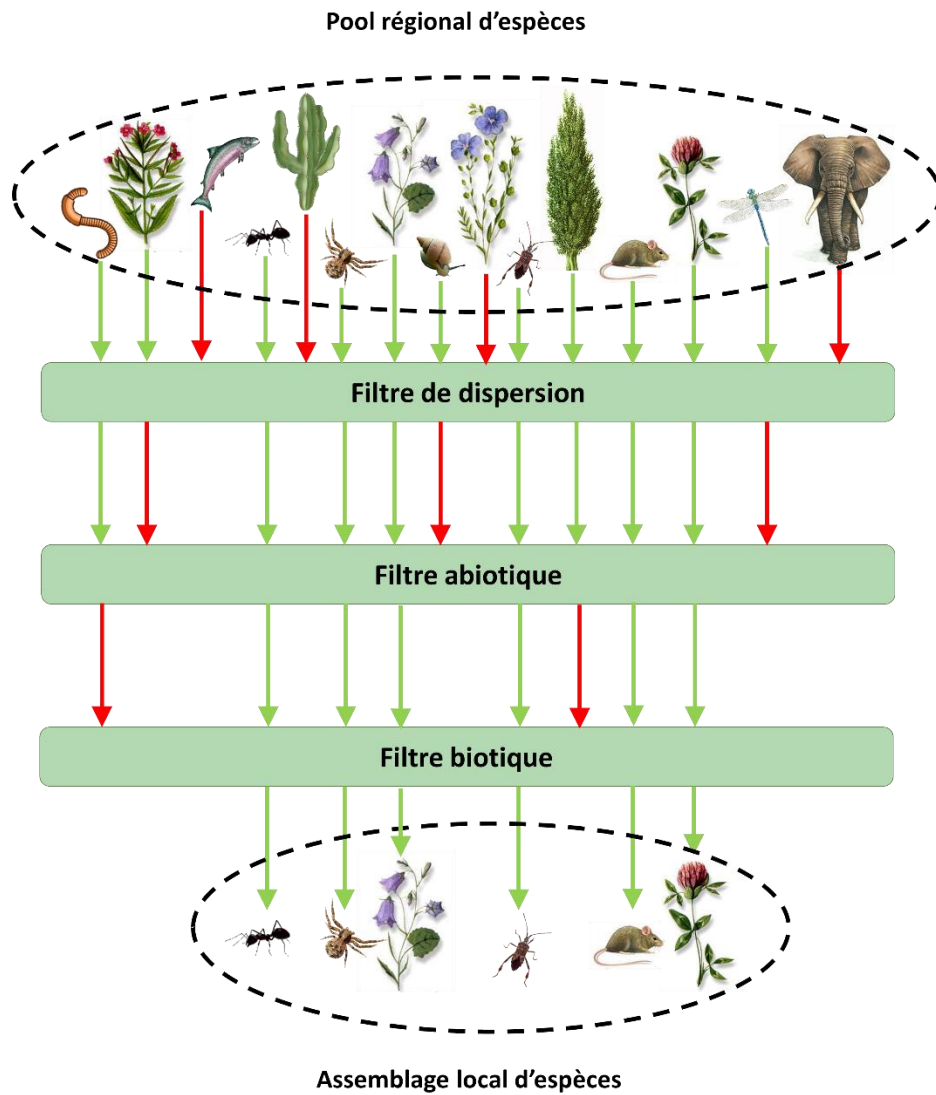


Figure T1.1 : Théorie des filtres (Diaz et al. 1998; Grime 1998; Belyea & Lancaster 1999; Lortie et al. 2004). A partir d'un pool régional d'espèces, les espèces devront traverser successivement les filtres de dispersion, abiotique et biotique afin de former l'assemblage local d'espèces. Les flèches vertes indiquent le franchissement d'un filtre, à l'inverse les flèches rouges correspondent aux espèces ne pouvant franchir un filtre. Pour faciliter la représentation, l'échelle de taille des organismes n'est pas respectée.

Transition vers le chapitre 1

Ce premier chapitre présente une étude multi-compartiments visant à évaluer la nature et la magnitude des effets d'une espèce ingénieure, *M. barbarus*, sur son écosystème. En considérant son action sur les filtres abiotiques et biotiques, nous mesurons l'impact de cette espèce sur les propriétés physico-chimiques du sol, les communautés de plantes et d'invertébrés, et les relations trophiques et non trophiques entre espèces et entre espèces et habitat (Figure T1.2).

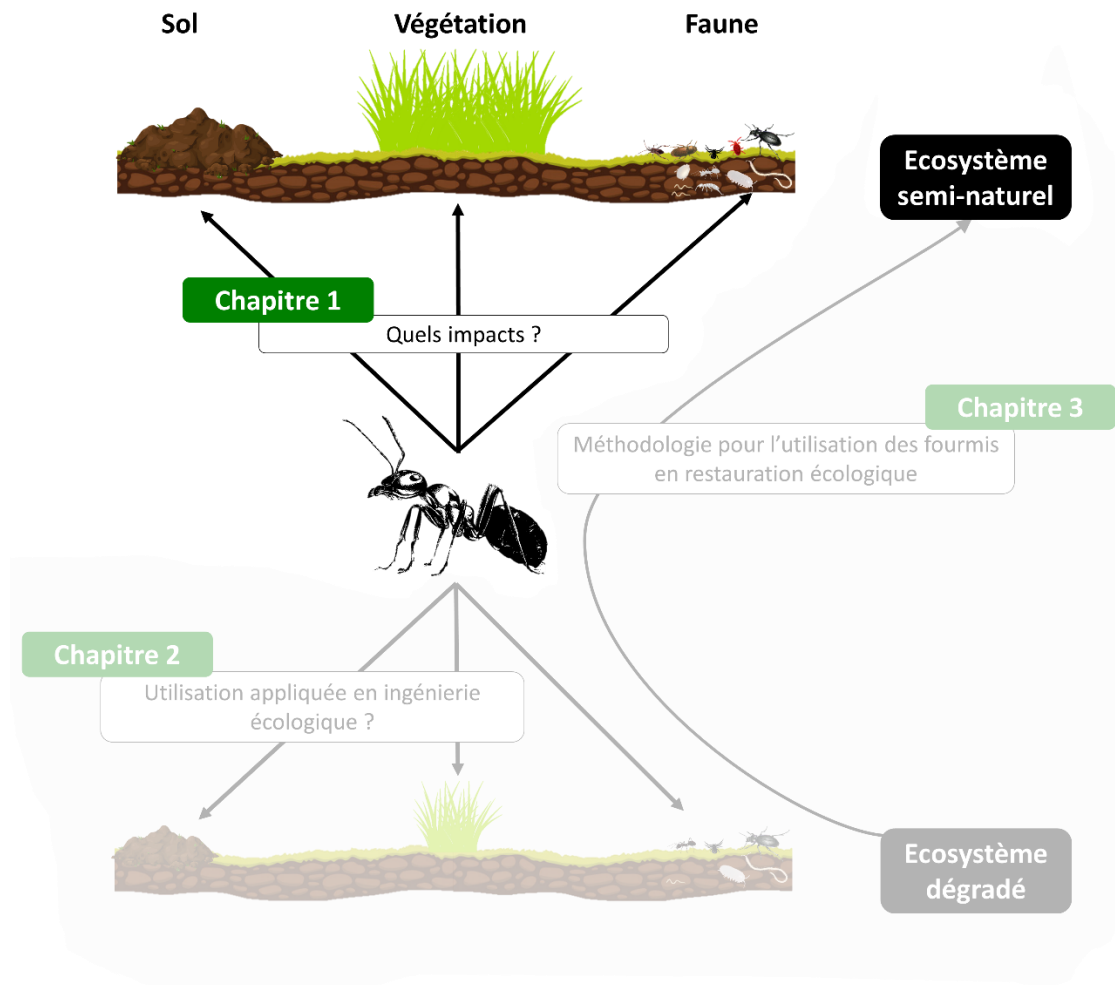


Figure T1.2 : Localisation du chapitre 1 dans l'organisation générale de la thèse

CHAPITRE 1

Above- and below-ground effects of an ecosystem engineer ant in Mediterranean dry grasslands

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Abstract

Within a local assemblage, ecosystem engineers can have major impacts on population dynamics, community composition and ecosystem functions by transforming or creating new habitats. They act as an ecological filter altering community composition through a set of environmental variables. The impact of ants on their environment has been widely studied, but their multi-component effects (both trophic and non-trophic) have been rarely addressed. We investigated the roles of *Messor barbarus*, one of the commonest harvester ant species in south-western European Mediterranean grasslands. We analysed soil physico-chemical parameters, above-ground vegetation (e.g. species richness, plant community, micro-local heterogeneity, plant biomass) and above- and below-ground fauna (macrofauna, Collembola, Acari and nematodes). A clear and strong local impact of *M. barbarus* on soil, vegetation and fauna compartments emerges. The environmental filter is altered by modifications to soil physico-chemical properties, and the biotic filter by changes to plant communities and altered above- and below-ground fauna abundance, occurrence and community structure. The engineering activity of *M. barbarus* affects not only these separate ecosystem components, but also the trophic and non-trophic relationships between them. By altering ecological filters at a local scale, *M. barbarus* creates habitat heterogeneity that may in turn increase ecological niches in these highly diverse ecosystems.

Keywords: *Messor barbarus*, soil, plant communities, fauna, trophic and non-trophic relationships.

1.1 Introduction

One of ecology's major goals is to understand how organisms' interactions with each other and their environment impact species distribution and abundance. Some species, known as ecosystem engineers, are able to modify their community assembly. These engineers have major impacts on population dynamics, community composition and ecosystem functions (Wright & Jones 2006; Kleinhesselink et al. 2014) through environmental changes caused either by their physical presence or by their actions or interactions, transforming or creating new habitats (Jones et al. 1997). The ecosystem engineer concept has, however, been the subject of debate, with some scholars arguing that all organisms engineer their environments to some degree (Reichman & Seabloom 2002). It is therefore important to determine the nature and the magnitude of ecosystem engineers' impacts on local communities and their habitat.

Two distinct but linked hypotheses underpin studies on ecosystem engineers, (1) they affect a set of environmental conditions and, (2) by modifying these conditions they act as an ecological filter altering community composition (Meyer 2008) by promoting a set of the other organisms' biological traits (Hastings et al. 2007). The ecological filter theory provides a conceptual framework for understanding the processes that determine which and how many species live in a particular locality. This theory explains local community composition and structure by a set of discrete and sequential mechanisms that constrain a larger species pool of potential residents to the subset that occurs within a community, through three filters (Lortie et al. 2004). First, only a subset of the regional pool is available to colonise particular sites, depending both on the landscape matrix and on each species' dispersal ability and population size (Herault & Thoen 2009). Second, only some species have the physiological capacity to withstand the prevailing environmental conditions. Finally, only some species are able to withstand certain biotic interactions that may depend, for example, on the availability of food resources (Gámez-Virués et al. 2015) or the presence of natural enemies (Skelton et al. 2017).

The ecological filter theory, which has been tested in a wide range of marine and terrestrial ecosystems (e.g. Lortie et al. 2004; Bremner 2008), is particularly relevant to disentangle mechanistic and stochastic processes in the organisation of local communities (Poff 1997). In terrestrial ecosystems, ants have been identified as ecosystem engineers that

are able to act on all three filters. They affect plant communities by altering their dispersal capacities and modifying microenvironmental conditions (Lengyel et al. 2010). By their structural engineering activities during nest construction and maintenance, ants may influence soil physico-chemical properties. Moreover, modifying the habitat structure may alter non-trophic relationships by creating habitat heterogeneity (e.g. Lane & BassiriRad 2005). Ants are also a key component of local trophic relations (Moya-Laraño & Wise 2007). Their accumulation of organic material (e.g. seeds, plant tissues and insect carcasses accumulated on their refuse piles) increases the density of microbial and faunal decomposers (Boulton & Amberman 2006) such as collembolans, with a cascading effect on their predators (Schuch et al. 2008). In addition, as predators, ants may directly or indirectly impact the abundance and diversity of other arthropods within an ecosystem (Sanders et al. 2011).

Trophic and engineering functions are rarely considered in combination (Coggan et al. 2018), and very few planned observations of engineer interactions to date even consider both functions (Sanders & Van Veen 2011). Yet the engineering concept can contribute to theories of species coexistence. If engineers influence a set of environmental conditions, and if species selection and adaptation follow, then engineering should markedly enhance opportunities for niche differentiation, diversification and coexistence at the same or at multiple trophic levels (Laland et al. 1999). While the impact of ants on their environment has been widely studied, only a few studies have addressed the multi-component effects (both trophic and non-trophic) of an ant species on its ecosystem (Sanders & Van Veen 2011).

In south-western European grasslands, the native granivorous ant *Messor barbarus* is known to redistribute seeds and to change soil physico-chemical properties (Azcarate & Peco 2007) (see supplementary material, Method S1.1). We recently demonstrated its capacity to improve the physical and chemical properties of a degraded soil in the Plain of la Crau, as well as its positive impact on plant communities and soil seed banks (De Almeida, Blight, et al. 2020). However, a global study that includes fauna and trophic and non-trophic relationships is necessary to obtain a comprehensive understanding of their role as engineers. This study assessed whether *M. barbarus* acts as an ecosystem engineer driving community assembly through trophic and non-trophic interactions. We conducted this study in xerophytic meadows of the Camargue delta regional Nature Reserve (South of France). We hypothesised that *M. barbarus*: (1) modifies soil texture by redistributing soil particles, and increases soil nutrients through the accumulation of plant materials; (2) which in turn affect the plant

community (e.g. increase in plant biomass and height) and physiological status; (3) and increases above- and below-ground fauna abundance, occurrence and change their community structure; and finally, (4) by its direct and indirect impacts on all these components, changes trophic and non-trophic relationships.

1.2 Materials & methods

1.2.1 Study area

The study was carried out at the Domaine de la Tour du Valat in the Rhône delta, southern France (43°29' N, 4°40' E). This reserve is composed of almost 2 700 ha of xero- and halophytic mixed meadows with patches of saline steppes and temporary marshes managed by traditional extensive cattle grazing (Mesléard et al. 2017). Xerophytic meadows are a priority habitat (code 6220, European Union Habitats Directive, 1992). They are characterised by a high proportion of annual plant species including those characteristic of the Mediterranean region, such as *Brachypodium distachyon* (L.) P.Beauv, *Euphorbia exigua* L., *Plantago lagopus* L., *Trifolium scabrum* L., *Trifolium suffocatum* L., *Filago pygmaea* L. and *Catapodium rigidum* (L.) C.E. Hubb. (Bensettiti et al. 2005).

The climate is Mediterranean with cold winters and warm dry summers; precipitation (average: 600 mm year⁻¹) occurs mainly during autumn and, to a lesser extent, spring (Mesléard et al. 2017).

1.2.2 Experimental design

We randomly selected thirty of the largest *M. barbarus* active nests scattered over 10 randomly selected xerophytic meadows over an area of 262 ha extensively grazed by bulls (0.1 livestock units). The selected nests were class 4 on the Blanco-Moreno et al. (2014) five-level scale (no class 5 were observed). This scale is based on a combination of factors: surface area occupied by the colony and number of entrances (class 4: nests covering 2–4 m² with 3–4 entrances). To ensure that we had applied this classification scheme accurately we measured the relevant parameters for each candidate nest. On each meadow, we selected the same number of ant-free patches as ant patches of 4 m² with no sign of ant activity (i.e. refuse piles, nests or tracks). They were located at a minimum distance of 5 m from a nest to avoid any border effect of colony activities (Cerdan 1989).

Messor barbarus nest location is unpredictable, known to be strongly influenced by proximity to long-established colonies (Blanco-Moreno et al. 2014) rather than by soil properties (e.g. organic carbon (C), soil strength or aggregate distribution) (Baraibar et al. 2011), and distribution can be either regular or random (Blanco-Moreno et al. 2014). Moreover, after their nuptial flight in autumn, mated queens land randomly. For the purposes of this study, therefore, differences in ecological variables between ant patches and ant-free patches are considered to result from the engineering activity of *M. barbarus*.

1.2.3 Soil physico-chemical variables

We collected soil samples on the nest green belt (dense vegetation belt located on the refuse pile from the previous year) and randomly in the 4 m² ant-free patches by throwing the soil sample container. When it landed on bare soil area, which represented in average 18% of the patch, we threw it again. We sampled the top few centimetres of soil (about 200 g) (De Almeida, Blight, et al. 2020) both in ant and ant-free patches in June 2018. To quantify both nitrate (N-NO₃) and ammonium (N-NH₄), about 50 g of soil was frozen and stored until analysis. The rest of the soil sample was air-dried and sieved (2 mm). Then, using standard international methods (Pipet method - NF X 316107) (Baize 2018), we assessed: particle-size distribution without prior decarbonisation (clay (< 0.002 mm), fine silt (0.002–0.02 mm), coarse silt (0.02–0.05 mm), fine sand (0.05–0.2 mm), coarse sand (0.2–2 mm)) and chemical properties (organic C, total nitrogen (total N), available phosphorus (available P) (Olsen et al. 1954), calcium oxide (CaO), magnesium oxide (MgO), potassium oxide (K₂O), sodium oxide (Na₂O), pH_{KCl}, Cation Exchange Capacity (CEC), C:N ratio, total organic matter (TOM)).

1.2.4 Plant community

We sampled plant communities in 2 m x 2 m (4 m²) quadrats placed on the thirty selected nests (ant patches) and thirty ant-free patches in May 2018. The abundance of each plant species in each quadrat was defined according to a modified Braun-Blanquet scale (Braun-Blanquet et al. 1952) as follows: 0.2 = represented by a single individual in the 4 m² quadrat, 1 = covering < 5 %, 2 = covering between 5 % and 25 %, 3 = covering between 26 % and 50 %, 4 = covering between 51 % and 75 % and finally 5 = covering > 75%. In each quadrat, we also estimated both the percentage of bare soil based on canopy cover, and the vegetation height.

To assess the heterogeneity of the micro-local plant composition generated by ant activities around the nest (e.g. soil disturbed and deposited at the top of ant hills, refuse piles scattered around or ant “trails”) we placed three quadrats of 10 cm x 10 cm (0.01 m²) in each 4 m² quadrat. In the ant patches, they were placed as follows: one in the “green belt”, one in the “bioturbated soil” (top of ant nest where soil is heavily disturbed) and one in an area showing neither of the two previous traces of ant activity. In ant-free patches, the 0.01 m² quadrats were placed randomly. In each 0.01 m² quadrat, we counted all plants in May 2018, when most seedlings can be identified (Mamarot 2002). Then for each of the 60 patches, a micro-local vegetation heterogeneity index based on the average of three Bray-Curtis index distances – a dissimilarity index varying between 0 and 1: 0 for similar communities and 1 for distinct communities – was calculated between the three 0.01 m² quadrats.

We measured aboveground dry biomass by cutting the vegetation to ground level, then placing a 50 cm x 50 cm (0.25 m²) quadrat per 4 m² quadrat in the “green belt” of ant patches and randomly in ant-free patches. The measurements were taken in May 2019 during the peak in plant productivity. Then, each sample was oven-dried at 40 °C up to constant weight.

Plant community physiological status was assessed in May 2019 with a hand-held multi-parameter optical sensor Multiplex ResearchTM (Force-A, Orsay, France). This fluorometer uses fluorescence technology with multiple excitation (UV, blue, green and red) and detection wavelengths (yellow, red and far-red) to measure constitutive and induced leaf epidermal anthocyanins, chlorophylls and nitrogen balance indices (see supplementary material, Method S1.2) (Cerovic et al. 2008; Agati et al. 2011). Anthocyanins and Flavonols are plant secondary compounds that can be affected by stress and thus reveal a physiological dysfunction. Without N limitation, a plant promotes its primary metabolism and synthesizes proteins (nitrogenous molecules) containing chlorophyll, and few flavonols (carbon compounds). The nitrogen balance index which corresponds to the Chlorophyll/Flavonols ratio is a useful indicator of N deficiency. We took 10 measurements on the “green belt” in ant patches and at randomly selected points in ant-free patches.

1.2.5 Above- and below-ground fauna

Fauna was sampled in spring 2018. Macrofauna was hand sorted from soil monoliths (25 x 25 x 25 cm) that were placed to maximize the cover of the green belt in the ant patches.

In the ant-free patches, soil monoliths were placed randomly. Invertebrates were stored in 70% ethyl alcohol and then identified to order level and counted. Rare taxa - fewer than 5 individuals - were not considered.

Mesofauna was collected using two core-samples (5 cm diameter) from the upper 7 cm of soil from the “green belt” of ant patches and randomly from ant-free patches by throwing the sample container. When it landed on bare soil area, we threw it again. Collembola and Acari were extracted over a period of seven days using a modified high-gradient canister method (Macfadyen 1961) and stored in 70% ethyl alcohol. Collembola taxa were assigned to life history groups (epedaphic, hemidaphic and euedaphic) according to Gisin (1943). Acari were divided into three suborders reflecting their trophic level: Oribatida, Gamasida and Actinedida.

Microfauna was sampled using the same protocol as for mesofauna. Nematodes were extracted over two days from moist soil using the Baermann funnel method (Barker 1985) and a first count of live specimens performed in the subsequent days. They were then fixed in 4% formalin solution, mounted on glass slides under a microscope, and the first 200 individuals encountered were divided into trophic groups following Yeates *et al.* (1993). Nematodes that could not be assigned to a trophic group with certainty were classified in the group with the most similar morphological feeding structure.

1.2.6 Soil respiration

In each patch, *in situ* soil C effluxes ($\text{g CO}_2 \text{ m}^{-1} \text{ h}^{-1}$) (release of CO_2 from soils and plants due to production of CO_2 by leaves, roots, soil organisms and chemical oxidation of C compounds) were recorded in May 2019 with a portable infrared gas analyser (IRGA, EGM-4) connected to a closed soil respiration chamber (SRC-1) (PP Systems, Massachusetts, United States) before removal of aboveground vegetation. To prevent leakage of CO_2 into the air when placing the chamber on the grass, a PVC tube (10 cm diameter and 11 cm in height) was sunk 1 cm deep in the soil prior to measurement (see supplementary material, Method S1.3).

1.2.7 Statistical analyses

To test the effect of ants (fixed-effect predictor variable) on soil physico-chemical properties, soil respiration, plant community parameters and fauna abundance and occurrence, linear mixed-effect models (LMMs) or generalised linear mixed models (GLMMs) were selected according to the distribution of model residuals. Chlorophyll index, anthocyanin

index, nitrogen balance index and plant species richness were fitted with a Gaussian distribution. All soil physico-chemical parameters, other plant community parameters and soil respiration analyses were fitted with a gamma distribution (R package “lme4”, (Bates et al. 2015)). Both mesofauna and microfauna abundance were fitted with a negative binomial distribution (R package “MASS”, (Ripley 2011)), while macrofauna abundance was fitted with a zero-inflated generalised linear mixed model (R package “glmmTMB” with family “nbinom2”, (Brooks et al. 2017)) and macrofauna occurrence with a binomial distribution (in $\text{g}\cdot\text{m}^{-2}$ and $\text{ind}\cdot\text{m}^{-2}$ respectively for plant biomass and fauna abundance). To assess differences in total abundance among the three trophic levels (decomposers, primary consumers, secondary consumers) and the ratio between them in ant and ant free-patches, GLMMs were selected respectively with a negative binomial distribution and a Gaussian distribution. The identity number of the xerophytic meadows was used as a random factor in all models.

Effect sizes were estimated using Cohen’s d index with 95% confidence intervals for each variable normally distributed (Cohen 1992) (R package “effsize”, (Torchiano 2019)). The formula was adapted for non-normal data (Fritz et al. 2012). The magnitude of the effects was assessed on the following scale: $|d| < 0.2$ "negligible", $|d| < 0.5$ "small", $|d| < 0.8$ "medium", $|d| > 0.8$ "large". A positive Cohen’s d indicates a higher response variable value in ant patches than in ant-free patches.

Changes in plant community composition were visualised via Non-metric Multidimensional Scaling (NMDS) based on the Bray-Curtis dissimilarity index to ordinate the characteristics of plant communities (composition and abundance). Differences in plant community composition were tested by permutational multivariate analysis (PERMANOVA) using the Adonis function (R package “vegan”, (Oksanen et al. 2016)).

Path analyses (PA) were performed to evaluate the impact of ant presence on soil physico-chemical and vegetation parameters and trophic webs in both above- and below-ground compartments (R package “lavaan”, (Rosseel 2012)). Path analysis is specific structural equation modelling (SEM) used to represent causal networks between several measured variables and to test model data consistency (Grace 2006). Because no statistically valid model linking the above- and below-ground compartments was obtained, we developed two conceptual models biologically relevant to above- and below-ground compartments (see supplementary material, Method S1.4). For the above-ground compartment, we assessed the causal relationships between N-NH_4 , clay content, vegetation heterogeneity and height, bare

soil percentage, plant biomass and above-ground invertebrate abundances (epedaphic collembolans, Hemiptera, Araneae, plant feeders and Coleoptera predators) in both ant and ant-free patches. For the soil compartment, we assessed the causal relationships between vegetation height, plant biomass, clay content, TOM and soil invertebrate abundances (microfauna primary and secondary consumers, mesofauna primary and secondary consumers, earthworms, macrofauna detritivore and Geophilomorpha) in both ant and ant-free patches. The full models were simplified by stepwise exclusion of non-significant variables until a minimum adequate model was reached. The adequacy of each model was determined by non-significant differences between the predicted and observed covariance matrices (chi-squared tests, $P > 0.05$), low root mean squared error of approximation index (RMSEA < 0.1) and high comparative fit index (CFI > 0.90) (Grace 2006).

All statistical analyses were performed using R software version 3.6.1 (R Development Core Team, 2011).

1.3 Results

1.3.1 Soil physico-chemical parameters

Clay, fine silt and coarse silt content were respectively 25 %, 30 % and 64 % higher in ant-free patches than in ant patches, with a large effect from ants on coarse silt. Coarse sand was 17 % higher in ant patches (see supplementary material, Table S1.1 and Figure S1.1).

Available P and N-NH₄ content were respectively 51 % and 39 % higher in ant patches than in ant-free patches, with a large effect. In contrast, C:N ratio and CaO, K₂O, MgO, Na₂O, and total N content were higher in ant-free patches than in ant patches. No other significant differences were found (see supplementary material, Table S1.1 and Figure S1.1).

1.3.2 Plant community

At the 0.01 m² scale, vegetation heterogeneity was 14 % higher (medium effect) in ant patches (Table 1.1 and Figure 1.1). At the 0.25 m² scale, plant biomass was 2.5 times higher (large effect) in ant patches (Table 1.1; Figure 1.1). At the 4 m² scale, vegetation height was 30 % higher in ant patches. We found no significant difference in plant community composition ($R^2 = 0.02$, Pseudo-F = 1.01; $P = 0.43$) and plant species richness (Table 1.1) between ant and ant-free patches.

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The chlorophyll index was 10 % higher in ant patches, while the anthocyanin index was 29 % higher in ant-free patches (Table 1.1).

Table 1.1: Ant effects on plant community variables. Values are means \pm standard errors; n= 30. Z or t values and associated P values were obtained from LMM or GLMM with gamma distribution. Bold values indicate significant differences between ant patches and ant-free patches.

	Z or t value	P value	Ant patches	Ant-free patches
Vegetation heterogeneity	-2.30	0.02	0.75 \pm 0.02	0.66 \pm 0.03
Plant biomass (g/0.25m ²)	-9.12	< 0.001	154.2 \pm 12.3	63.4 \pm 5.3
Vegetation height (cm)	-2.83	0.005	9.2 \pm 0.7	7.1 \pm 0.5
Species richness	-1.53	0.13	38.5 \pm 1.0	36.6 \pm 1.0
Nitrogen Balance Index	-1.49	0.14	0.19 \pm 0.01	0.18 \pm 0.01
Anthocyanin index	2.28	0.03	0.07 \pm 0.01	0.09 \pm 0.01
Chlorophyll index	-2.80	0.01	1.52 \pm 0.04	1.38 \pm 0.03
Bare soil percentage (%)	-0.03	0.98	18.5 \pm 2.6	17.8 \pm 1.9

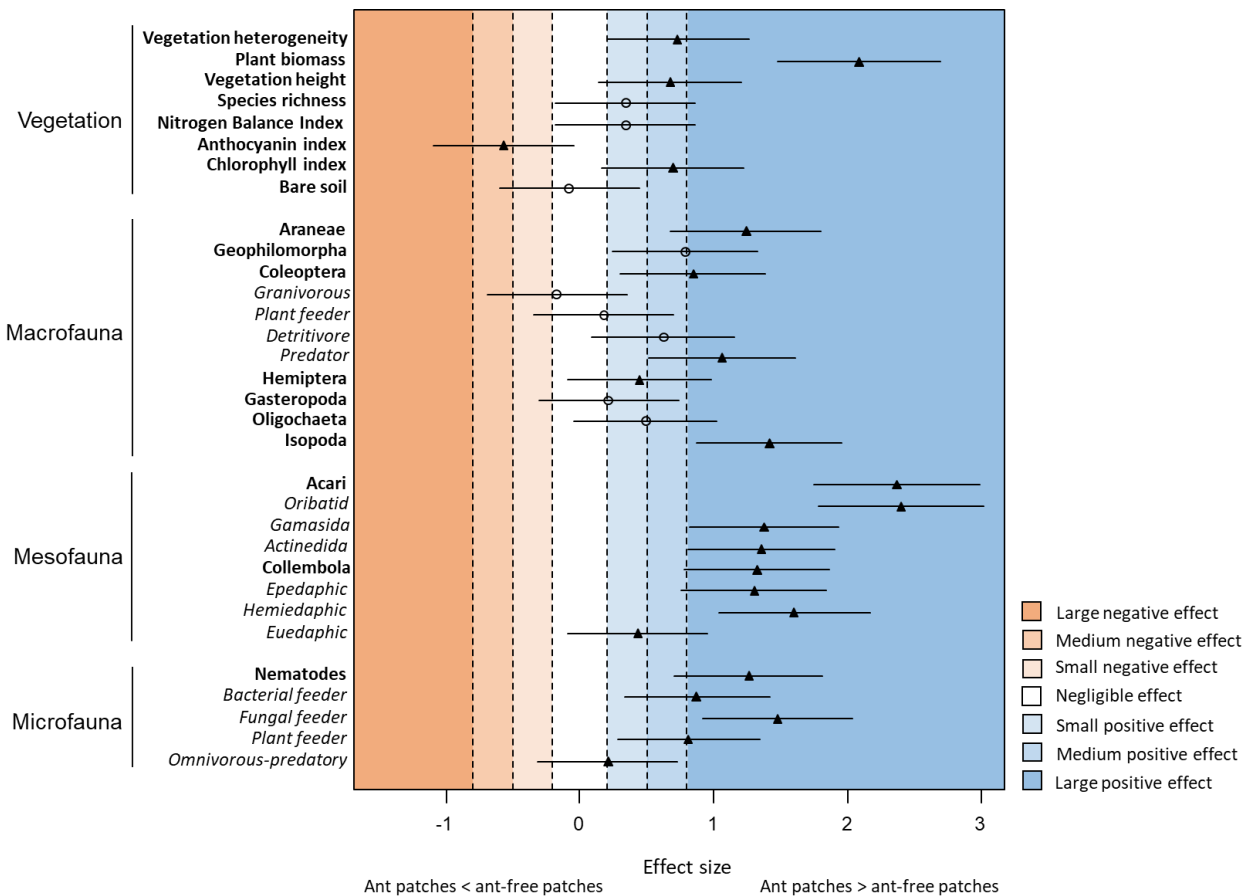


Figure 1.1: Effect size (Cohen’s d) and 95% confidence intervals for the effect of ants on vegetation parameters and above- and below-ground fauna abundances (macrofauna, mesofauna and microfauna). Soil fauna subgroups are in italics. Black triangles represent the mean effect size for variables with a significant difference between ant and ant-free patches. Dots represent the mean effect size for variables with no significant difference between ant and ant-free patches. Effect size > 0 indicates higher values in ant patches than in ant-free patches; effect size < 0 indicates lower values in ant patches than in ant-free patches.

1.3.3 Above- and below-ground fauna

Macrofauna

Abundances of Araneae and coleopteran predators were respectively three and four times higher (large effects) in ant patches than in ant-free patches (Figure 1.1; Table 1.2). Abundances of total Coleoptera, Hemiptera and Isopoda were also higher in ant patches (Table 1.2), but this effect was less marked (Figure 1.1).

Occurrences of Araneae, coleopteran predators, Isopoda, Geophilomorpha, coleopteran detritivores and Oligochaeta were significantly higher in ant patches (Table 1.2).

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Table 1.2: Macrofauna abundance (ind.m⁻²) and occurrence analyses for ant and ant-free patches, spring 2018. Values are means ± standard errors; n= 30. Z value and P value were obtained from zero-inflated GLMM for abundance and from GLMM with binomial distribution for occurrence. Bold values indicate significant differences between ant patches and ant-free patches.

	Abundance				Occurrence	
	Z value	P value	Ant patches	Ant-free patches	Z value	P-value
Araneae	-5.53	< 0.001	80.6 ± 11.3	25.6 ± 5.2	-2.53	0.01
Geophilomorpha	-0.65	0.51	11.6 ± 2.4	3.7 ± 1.5	-2.70	0.007
Coleoptera	-4.00	< 0.001	79.4 ± 13.6	34.1 ± 7.5	-1.30	0.19
<i>Granivorous</i>	0.95	0.34	7.7 ± 5.3	13.3 ± 6.1	0.48	0.63
<i>Plant feeder</i>	-0.97	0.33	10.5 ± 3.7	6.4 ± 2.1	-0.65	0.52
<i>Detritivore</i>	-1.46	0.15	11.0 ± 3.1	2.6 ± 1.3	-2.10	0.04
<i>Predator</i>	-4.72	< 0.001	50.2 ± 10.5	11.7 ± 3.2	-2.28	0.02
Hemiptera	-2.60	0.01	51.9 ± 10.8	27.2 ± 5.5	-1.10	0.28
Gasteropoda	-0.50	0.62	9.4 ± 2.8	7.5 ± 2.8	-0.92	0.36
Oligochaeta	-1.02	0.31	21.5 ± 5.6	16.5 ± 4.2	-2.04	0.04
Isopoda	-3.83	< 0.001	195.3 ± 70.5	2.1 ± 1.3	-3.74	< 0.001

Mesofauna

Total abundances of Acari and Collembola were three times higher (large effect) in the ant patches (Table 1.3; Figure 1.1), where abundances of all Acari and Collembola groups were also significantly higher (Table 1.3), although less so for euedaphic collembolans than for the other mesofauna groups (Figure 1.1).

Microfauna

Total abundance of nematodes was twice as high (large effect) in the ant patches (Table 1.3; Figure 1.1), where abundances of all trophic groups were also significantly higher (Table 1.3), although less so for omnivorous-predatory nematodes than for the other groups (Figure 1.1).

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Table 1.3: Mesofauna and microfauna abundance (ind.m⁻²) analyses for ant and ant-free patches. Values are means \pm standard errors; n= 30. Z value and P values were obtained from GLMM with binomial negative distribution. Bold values indicate significant differences.

	Z value	P value	Ant patches	Ant-free patches
Mesofauna				
Acari	-8.98	< 0.001	29 146.5 \pm 2 564.1	10 030.0 \pm 932.8
<i>Oribatid</i>	-7.24	< 0.001	13 982.5 \pm 1 578.1	2 320.5 \pm 361.5
<i>Gamasida</i>	-5.34	< 0.001	9 911.0 \pm 1 023.0	4 624.0 \pm 548.0
<i>Actinedida</i>	-4.51	< 0.001	5 253.0 \pm 375.2	3 085.5 \pm 423.9
Collembola	-4.83	< 0.001	47 804.0 \pm 7 660.2	16 583.5 \pm 4 718.9
<i>Epedaphic</i>	-4.33	< 0.001	3 578.5 \pm 576.6	1 275.0 \pm 239.9
<i>Hemiedaphic</i>	-4.79	< 0.001	32 147.0 \pm 4945.0	7 310.0 \pm 2 187.8
<i>Euedaphic</i>	-2.00	0.05	12 078.5 \pm 3 696.6	7 998.5 \pm 2 659.7
Microfauna				
Nematodes	-5.43	< 0.001	372 274.5 \pm 40 792.8	186 660.0 \pm 15 830.0
<i>Bacterial feeder</i>	-4.64	< 0.001	249 283.0 \pm 34 142.3	127 300.8 \pm 10 543.8
<i>Fungal feeder</i>	-4.48	< 0.001	103 295.0 \pm 10 316.0	49 839.6 \pm 7 848.5
<i>Plant feeder</i>	-2.57	0.01	18 840.3 \pm 3 223.3	9 205.3 \pm 1 884.4
<i>Omnivorous-predatory</i>	-81.42	< 0.001	855.9 \pm 294.3	314.2 \pm 92.7

1.3.4 Soil respiration

Soil respiration was twice as high in ant patches (2.39 ± 0.27) as in ant-free patches (1.23 ± 0.13) ($t = -4.64$, $P < 0.001$), with a large effect of ants (Cohen's $d = 0.96 \pm 0.55$).

1.3.5 Trophic levels

Total abundance of decomposers (D) was three times higher in ant patches than in ant-free patches ($t = -5.70$, $P < 0.001$), while total abundances of primary consumers (PC) and secondary consumers (SC) were twice as high in ant patches (respectively, $t = -5.70$ and $t = -6.10$, $P < 0.001$). The D:PC and D:SC ratios were respectively 2 times and 1.5 times higher in ant patches than in ant-free patches (respectively, $t = -3.43$ and $t = -86.7$, $P < 0.001$), while the SC:PC ratio was not affected ($t = -0.54$, $P = 0.59$).

1.3.6 Path analysis

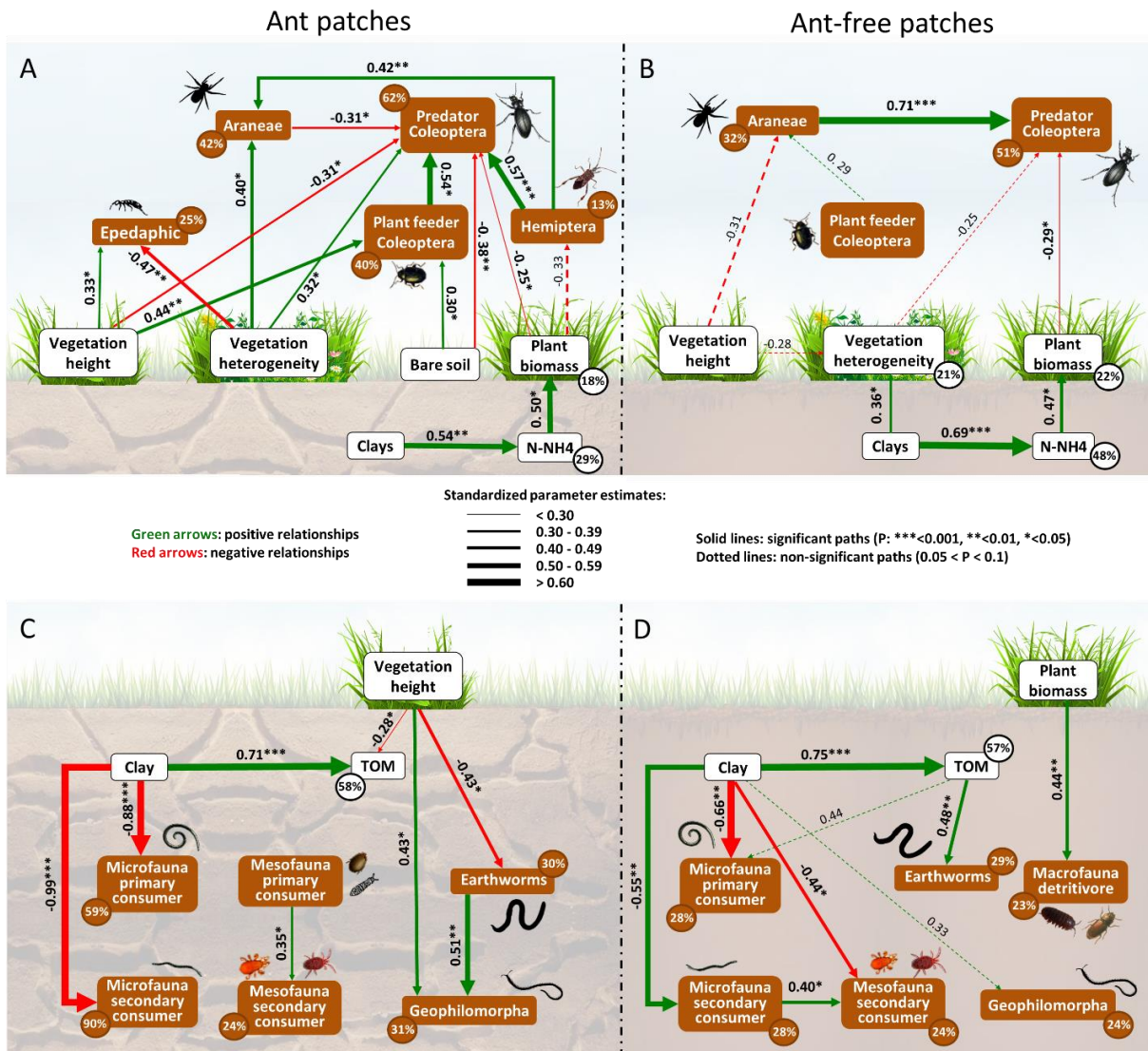


Figure 1.2: Path analysis models. Causal influences of vegetation heterogeneity and height, bare soil percentage, plant biomass, clay and N-NH₄ content on above-ground invertebrate abundance (epedaphic collembolans, Hemiptera, Araneae, plant feeder and predator Coleoptera) in ant patches (df = 18, $\chi^2 = 18.83$, P = 0.40, RMSEA = 0.04, CFI = 0.99) (A) and ant-free patches (df = 18, $\chi^2 = 18.02$, P = 0.46, RMSEA = 0, CFI = 1) (B). Causal influences of vegetation height, plant biomass, TOM and clay content on fauna abundance (microfauna primary and secondary consumers, mesofauna primary and secondary consumers, earthworms, macrofauna detritivores and Geophilomorpha) in ant patches (df = 18, $\chi^2 = 17.78$, P = 0.47, RMSEA = 0, CFI = 1) (C) and ant-free patches (df = 18, $\chi^2 = 23.05$, P = 0.19, RMSEA < 0.1, CFI > 0.9) (D). Numbers next to arrows show standardised parameter estimates (see supplementary material, Table S1.2 and

Table S1.3). Percentages of explained variance are shown with dependent variables.

In the above-ground compartment, we found more significant relationships in ant patches (Figure 1.2A and B). Vegetation heterogeneity and height were important drivers of the ant patches' above-ground food web. Vegetation heterogeneity had a positive direct

effect on predatory Coleoptera and Araneae abundances and, conversely, a negative effect on epedaphic collembolans. Vegetation height had a positive direct effect on epedaphic collembolans and plant feeder Coleoptera and negative effect on predatory Coleoptera. Predatory Coleoptera were positively affected by Hemiptera and plant feeder Coleoptera and negatively by Araneae, while Aranea was only dependent on Hemiptera. In contrast, on ant-free patches, predatory Coleoptera were negatively affected by plant biomass and positively by Araneae. Vegetation heterogeneity and vegetation height had less influence on the above-ground food web in ant-free patches. On both PAs, we observed that clay content had a strong positive effect on N-NH₄ content which in turn positively impacted the plant biomass.

For the soil compartment, ant patches revealed strong shifts in relationships across the food web (Figure 1.2C and D). Both path analyses suggested that clay content could be an important driver of the food web, positively affecting TOM content and negatively affecting microfauna primary consumers. Microfauna secondary consumers were strongly negatively affected by clay content in ant patches but positively affected in ant-free patches.

1.4 Discussion

This study reveals the strong local influence that *M. barbarus* has on both the environmental filter, by modifying soil physico-chemical properties, and the biotic filter, by changing plant communities and altering above- and below-ground fauna abundance, occurrence and community structure. Its engineering activity affects not only these ecosystem components, but also the trophic and non-trophic relationships between them. These new results add to our previous findings on the positive effect of *M. barbarus* on soil and vegetation restoration in a degraded dry grassland (De Almeida, Blight, et al. 2020), and highlight its central role in their ecosystem.

1.4.1 Impacts on the environmental filter

The engineering activity of ants and their impacts on the environmental filter are directly related to specific functional traits called effect traits, such as colony size, nest location or size of workers' mandibles. For example, the capacity of ants to restructure soil depends on their physical capacity to transport material (Dostál et al. 2005). The large proportion of coarse sand particles ranging from 0.2 to 2 mm we recorded in *M. barbarus* nests may therefore be explained by workers' average jaw opening, which ranges from 0.80 to 2.80 mm (Oliveras et al. 2005).

Differences in soil nutrient concentration between ant and ant-free patches may arise from ant foraging as well as nest construction and maintenance (Dostál et al. 2005). The higher concentration of available P in ant patches can be explained by food collected in the nests and the subsequent increase in organic matter mineralisation (Frouz et al. 1997; Dostál et al. 2005; De Almeida, Blight, et al. 2020). Their lower concentration of total N and other cations could be due to higher mineralisation rates of organic matter (Wagner & Jones 2006), assimilation of N by plants and microbes (Brown et al. 2012) and/or replacement of the upper horizon with subsoil usually poor in organic matter (Nkem et al. 2000; Dostál et al. 2005). Ant bioturbation may also lead to reduced Na₂O content (Nkem et al. 2000). Because sodium (Na⁺) can be fixed by clays (Domenico & Schwartz 1998), the measured decrease in clay content could lead to greater leaching of Na₂O in ant patches.

1.4.2 Impacts on the biotic filter

Modifications to soil physico-chemical properties may indirectly affect the biotic filter by creating micro-environments favourable to plant growth. In general, burrowing animals such as ants create patches of disturbed soil that influence vegetation growth and contribute to spatial heterogeneity in plant communities (Davidson et al. 2012). Since we found no changes in plant community composition, the higher plant biomass (De Almeida, Blight, et al. 2020) and height, and Chlorophyll index in *M. barbarus* nest green belts may be explained by the higher proportions of N-NH₄ and available P, known to be responsible for higher plant productivity (Lafleur et al. 2005). Moreover, the decrease in plant Anthocyanin index (i.e. leaf Anthocyanin content), likely due in part to the extremely reduced Na₂O content measured, reflects decreased environmental stress in these xerophytic meadows. It offers better conditions for less salt-tolerant species which could outcompete the more tolerant ones.

The high soil respiration we recorded in ant nests, in line with previous studies (Risch et al. 2005; Ohashi, Finér, et al. 2007), is probably mainly due to ant respiration (Risch et al. 2005; Ohashi, Finér, et al. 2007). However, other possible contributing factors include increases in plant biomass, in root respiration (Ohashi, Kilpeläinen, et al. 2007), in organic matter decomposition (Frouz et al. 1997) and in organism abundances. In ant nest green belts, the abundances of organisms comprising each trophic level increased. Engineered patches with high rates of organic material accumulation serve as microhabitats for soil fauna. Above- and below-ground differences in fauna abundance were strong for the main decomposers,

such as Isopoda, Oribatid Acari or collembolans, which responded positively to the presence of *M. barbarus*. Similar positive effects from a *Messor* species have been documented for some of these organisms (Boulton et al. 2003; Boulton & Amberman 2006). Interestingly, earthworms, also considered as ecosystem engineers, appear to have been unaffected by the presence of ants. The interaction between these two ecosystem engineers might have major local impacts and deserves to be thoroughly investigated.

Throughout the food web, increased abundances were observed in the ant nest green belts and no taxon was less abundant or frequent than in ant-free patches. Decomposer abundances increased more than those of primary and secondary consumers, changing the ratio between the different trophic levels. The higher abundances of decomposers (e.g. Collembola) and primary consumers (e.g. Hemiptera) may indirectly affect their predators (Schuch et al. 2008). In our study, the commonest predators were spiders and ground beetles, generalists feeding on taxa such as Hemiptera, Collembola and other Coleoptera (Lang et al. 1999; Schuch et al. 2008). Although most spider species are averse to ant predation, some have adapted ant-specific capture techniques and favour ants over other prey (Cushing 2012). Such spiders might therefore benefit considerably from the presence of *M. barbarus*; for example, the obligate myrmecophagous spider *Zodarion elegans*, observed mainly in ant patches, which may feed on *Messor* species (Traxler 2016).

1.4.3 Consequences on trophic and non-trophic relationships

Messor barbarus profoundly impacts the above- and below-ground compartments, modifying trophic and non-trophic relationships between organisms and between organisms and their environment. By increasing environmental heterogeneity, ants may affect food web organisation by changing resource distribution patterns (Hastings et al. 2007) or habitat structure. The consequences of these changes for above- and below-ground compartments differ. Here, the number of relationships increased in the above-ground compartment. Above-ground, vegetation heterogeneity and height were the main drivers of relationship complexity in *M. barbarus* patches. The increased heterogeneity and biomass of producers could be responsible for the increased abundance of some primary consumers (e.g. plant feeder Coleoptera) and indirectly of their predators (e.g. Araneae and predator Coleoptera). However, these changes may also be directly related to an increase in potential habitats for some primary or secondary consumer organisms (e.g. plant feeder Coleoptera and Araneae).

Below-ground, clay content was the main driver of interaction complexity. However, the sign and the strength of the relationship between clay content and microfauna secondary consumers differed between ant and ant-free patches. Clays are known to affect soil moisture (Gaur & Mohanty 2016), an increase in clays is associated with an overall increase in soil moisture. In ant patches, the strong negative impact of clay content might suggest that ants provide drier microclimate habitats, filtering the assemblage of microfauna secondary consumers towards specialist species. A taxonomic and functional study should be carried out to confirm this hypothesis.

1.4.4 The necessity of multi-component studies

Since its introduction, the concept of ecosystem engineer has generated considerable interest, but it is also a source of debate within the scientific community. The main concern is that all organisms affect their environments to some degree, which has an impact on other species (Reichman & Seabloom 2002). This requires treating the effects of an organism on its environment and on other organisms as a coherent sequence of interactions and not as a collection of disconnected case studies (Wright & Jones 2006; Berke 2010). A multi-component approach must therefore be favoured when studying the concept of ecosystem engineers. Such studies conducted on both terrestrial (e.g. kangaroo rats (Prugh & Brashares 2012), earthworms (Liu et al. 2019)) and aquatic organisms (e.g. carp (Matsuzaki et al. 2007), crayfish (Usio & Townsend 2004)) have proven their value in studying the ecosystem engineer concept. Our multi-component study adds evidence, to the still limited literature, that some species can strongly affect their entire environment.

1.5 Conclusion

In Mediterranean dry grasslands, the effects of *M. barbarus* observed on above- and below-ground compartments illustrate the significance of the habitat alteration impact both on plant community structure and invertebrates and on their relationships. By altering both environmental and biotic filters at a local scale, *M. barbarus* creates habitat heterogeneity that may in turn increase ecological niches in these highly diverse ecosystems. Their impacts at a larger scale should be investigated for a clearer picture of this ecosystem engineer's ecological role.

SUPPLEMENTARY MATERIAL

Method S1.1: Additional information regarding the biology and ecology of our biological model, *Messor barbarus*.

Messor barbarus (Linnaeus 1767) (Hymenoptera: Formicidae) is a common species that occurs naturally in Southern Europe and Northern Africa Mediterranean grasslands (Lebas et al. 2016). This highly polymorphic granivorous ant lives in monogynous colonies of 500 to 21 000 workers (Cerdan 1989) and reaches a mature size at the age of approximately five years. *Messor barbarus* queens can live as long as 15–20 years under controlled conditions, after which the entire colony dies. It builds nests composed of a dense network of galleries and interconnected chambers that can reach five meters deep and spread out over several square metres – 6 m² in our study site (pers. obs.). *Messor barbarus* makes permanent and temporary trails with length ranges from 1 to 40 m (Detrain et al. 1996).

Supplementary material – Chapitre 1

Method S1.2: Additional information regarding the measure of plant community physiological status.

This fluorometer uses fluorescence technology with multiple excitation (UV, blue, green and red) and detection wavelengths (yellow, red and far-red) to measure constitutive and induced leaf epidermal anthocyanins (Log(Infrared fluorescence excited with red/Infrared fluorescence excited with green)), chlorophylls (Infrared fluorescence excited with green/Red fluorescence excited with green) and nitrogen balance (Infrared fluorescence excited with UV/Red fluorescence excited with green) indices (Cerovic et al. 2008; Agati et al. 2011).

Supplementary material – Chapitre 1

Method S1.3: Additional information on methods used to monitor soil respiration and temperature.

EGM-4 allows automatic calculation of emission rates provided that the volume of the chamber and the surface area of the soil are set. The soil respiration chamber has a surface area of 78 cm and has a system volume of 2 035 ml. CO₂ concentration was monitored every 4.8 s over a period of 124 s. For assimilation data, linear fits were used. Soil temperature at 5 cm depth (STP-1 Soil Temperature Probe, PP systems) was determined in the vicinity of the respiration chamber.

To minimise effects from diurnal variation in temperature, soil C efflux measurements were standardised to 10°C (SR) according to equation 1 (Davidson et al. 2006):

$$SR = SR_i \times Q_{10}^{(T_f - 10)/10},$$

where SR_i and T_i are *in situ* soil respiration and temperature respectively, and assuming a temperature sensitivity factor of Q₁₀=2.

Method S1.4: Initial conceptual models for above-ground (Model_A) and below-ground (Model_B) compartments that were both consistent with our data and made sense biologically. The full models were simplified by stepwise exclusion of non-significant variables until a minimum adequate model was reached. The adequacy of each model was determined by non-significant differences between predicted and observed covariance matrices (chi-squared tests, $P > 0.05$), low root mean squared error of approximation index (RMSEA < 0.1) and high comparative fit index (CFI > 0.90).

Model writing:

model<-'
dependent_variable_1 ~ independent_variable_1 + independent_variable_2
dependent_variable_2 ~ independent_variable_1 + independent_variable_2
etc.'

Model for above-ground compartment

model_A<-'
N-NH4 ~ Clay
Plant_biomass ~ N-NH4 + Vegetation_height + Bare_soil + Clay
Vegetation_heterogeneity ~ Vegetation_height + Clay
Plant_feeder_Coleoptera ~ Vegetation_heterogeneity + Bare_soil + Plant_biomass
+Vegetation_height
Epedaphic ~ Vegetation_heterogeneity + Bare_soil + Plant_biomass +Vegetation_height
Hemiptera ~ Vegetation_heterogeneity + Bare_soil + Plant_biomass +Vegetation_height
Araneae ~ Hemiptera + Plant_feeder_Coleoptera + Vegetation_heterogeneity +
Plant_biomass+ Epedaphic + Macrofauna_detritivore +Vegetation_height + Bare_soil
Predator_Coleoptera ~ Hemiptera + Plant_feeder_Coleoptera + Araneae + Epedaphic +
Bare_soil+ Vegetation_heterogeneity + Plant_biomass + Vegetation_height'

Models for below-ground compartment

Model_B <-'
TOM ~ Clay + Plant_biomass + Vegetation_height
Microfauna_primary_consumer ~ TOM + Clay + Plant_biomass + Vegetation_height
Macrofauna_detritivore ~ TOM + Clay + Plant_biomass + Vegetation_height
Earthworms ~ TOM + Clay + Plant_biomass + Vegetation_height
Mesofauna_primary_consumer ~ Microfauna_primary_consumer + TOM + Clay +
Vegetation_height + Microfauna_secondary_consumer
Microfauna_secondary_consumer ~ Microfauna_primary_consumer + Clay +
Vegetation_height
Mesofauna_secondary_consumer ~ Clay + Vegetation_height +
Mesofauna_primary_consumer
+ Microfauna_secondary_consumer + Microfauna_primary_consumer
Geophilomorpha ~ Clay + Vegetation_height + Mesofauna_primary_consumer +
Mesofauna_secondary_consumer + Macrofauna_detritivore + Earthworms'

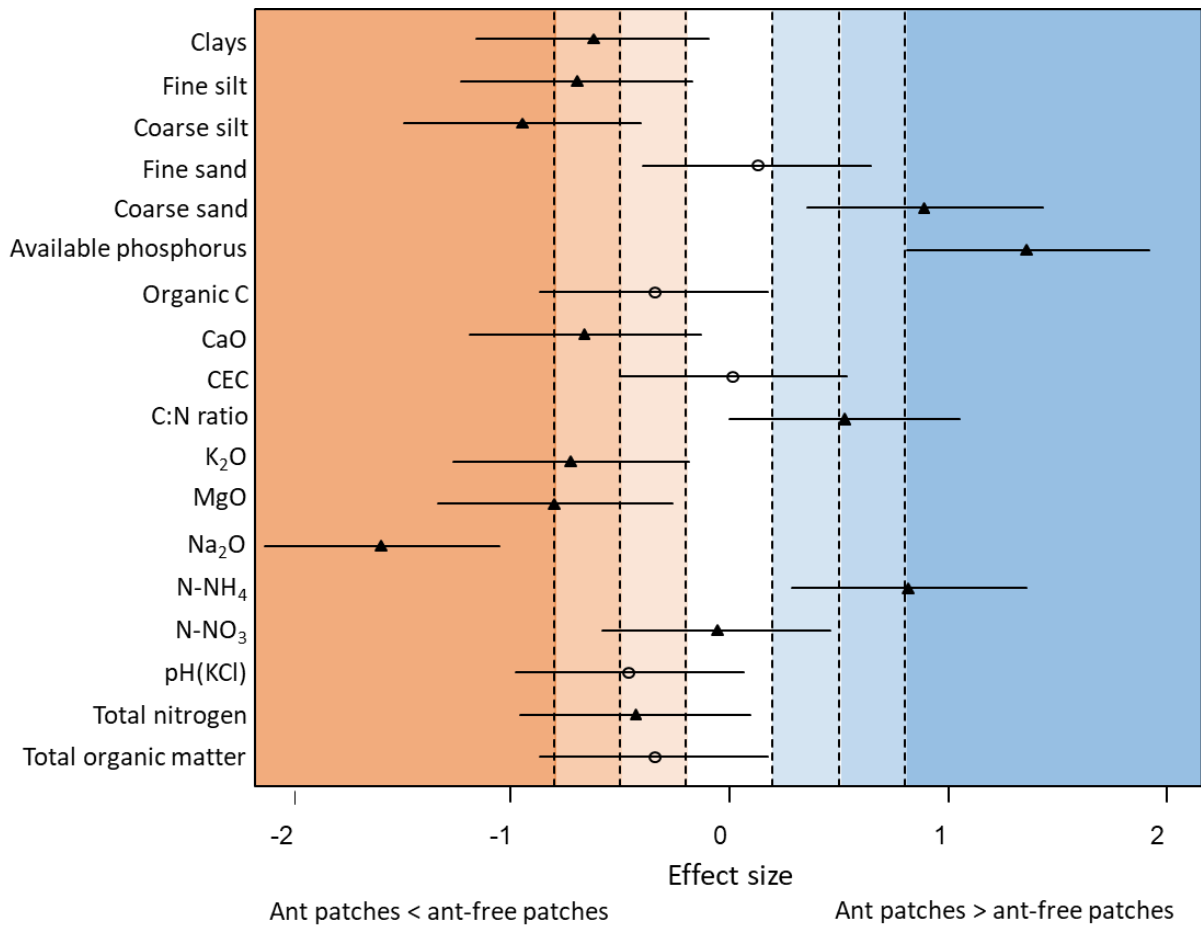
Supplementary material – Chapitre 1

Table S1.1: Soil physico-chemical analyses for ant and ant-free patches. Values are means \pm standard errors; n= 30. T and P value were obtained from linear mixed effect models or GLMM with gamma distribution. Bold values indicate significant differences between ant patches and ant-free patches.

	t value	P-value	Ant patches	Ant-free patches
Soil physical parameters				
Clay (g/Kg of soil)	-2.83	0.01	7.0 \pm 0.4	8.8 \pm 0.6
Fine silt (g/Kg of soil)	-3.16	0.01	13.1 \pm 0.9	17.5 \pm 1.6
Coarse silt (g/Kg of soil)	-4.32	< 0.001	5.6 \pm 0.4	9.2 \pm 0.9
Fine sand (g/Kg of soil)	1.43	0.15	11.9 \pm 0.3	11.2 \pm 0.5
Coarse sand (g/Kg of soil)	2.42	0.02	62.3 \pm 1.5	53.3 \pm 2.7
Soil chemical parameters				
Available P (mg/Kg of soil)	5.89	< 0.001	33.9 \pm 2.5	22.5 \pm 1.2
Organic C (g/Kg of soil)	-1.71	0.09	18.3 \pm 0.8	21.3 \pm 1.8
CaO (mg/Kg of soil)	-3.21	0.001	9 624.8 \pm 180.0	10 589.5 \pm 321.2
CEC (cmol+/kg of soil)	0.09	0.9	8.8 \pm 0.5	8.7 \pm 0.5
C:N ratio	2.32	0.02	11.3 \pm 0.3	10.6 \pm 0.3
K ₂ O (mg/Kg of soil)	-3.68	< 0.001	209.2 \pm 15.1	311.8 \pm 29.5
MgO (mg/Kg of soil)	-4.00	< 0.001	234.3 \pm 21.5	382.6 \pm 44.9
Na ₂ O (mg/Kg of soil)	-9.33	< 0.001	114.2 \pm 44.0	2 194.5 \pm 681.3
N-NH ₄ (mg/Kg of soil)	3.59	< 0.001	4.3 \pm 0.3	3.1 \pm 0.2
N-NO ₃ (mg/Kg of soil)	-2.37	0.02	9.0 \pm 0.7	14.1 \pm 2.8
pH(KCl)	-1.61	0.11	7.49 \pm 0.02	7.57 \pm 0.05
Total nitrogen (mg/Kg of soil)	-2.43	0.02	1 650.9 \pm 95.8	2 108.8 \pm 220.7
Total organic matter (%)	-1.71	0.09	3.2 \pm 0.1	3.7 \pm 0.3

Supplementary material – Chapitre 1

Figure S1.1: Effect size (Cohen’s d) and 95% confidence intervals for the effect of ants on soil physico-chemical parameters. Black triangles represent the mean effect size for variables with a significant difference between ant and ant-free patches. Dots represent the mean effect size for variables with no significant difference between ant and ant-free patches. Effect size > 0 indicates higher values in ant patches than in ant-free patches; effect size < 0 indicates lower values in ant patches than in ant-free patches.



Supplementary material – Chapitre 1

Table S1.2: Estimate, standard error, z-value, P-value and standardised path estimates for models of above-ground compartment in ant patches and ant-free patches depicted in Figure 1.2A and Figure 1.2B.

Response	Predictor	Ant-patches					Ant-free patches				
		Estimate	Standard error	z-value	P-value	Standardized estimate	Estimate	Standard error	z-value	P-value	Standardized estimate
N-NH4	~ Clay	0.24	0.07	3.44	< 0.001	0.54	0.20	0.04	5.22	< 0.001	0.69
Plant biomass	~ N-NH4	0.50	0.20	2.49	0.01	0.50	0.48	0.23	2.11	0.03	0.47
Plant biomass	~ Vegetation height	0.01	0.05	0.21	0.84	0.04	0.10	0.07	1.56	0.12	0.26
Plant biomass	~ Bare soil	0.0007	0.01	0.06	0.95	0.01	-0.02	0.02	-1.37	0.17	-0.23
Plant biomass	~ Clay	-0.11	0.09	-1.26	0.21	-0.25	-0.07	0.07	-1.04	0.30	-0.23
Vegetation heterogeneity	~ Vegetation height	0.08	0.05	1.64	0.10	0.29	-0.11	0.06	-1.70	0.09	-0.27
Vegetation heterogeneity	~ Clay	-0.06	0.08	-0.78	0.44	-0.14	0.10	0.05	2.20	0.03	0.36
Plant feeder Coleoptera	~ Bare soil	0.02	0.01	2.11	0.04	0.30	-0.02	0.02	-1.34	0.18	-0.24
Plant feeder Coleoptera	~ Vegetation heterogeneity	0.22	0.15	1.43	0.15	0.21	0.18	0.18	1.01	0.31	0.18
Plant feeder Coleoptera	~ Plant biomass	0.11	0.14	0.77	0.44	0.11	-0.11	0.18	-0.60	0.55	-0.11
Plant feeder Coleoptera	~ Vegetation height	0.12	0.04	2.94	0.003	0.44	0.10	0.07	1.41	0.16	0.27
Epedaphic	~ Bare soil	0.004	0.01	0.34	0.74	0.05	-0.03	0.02	-1.57	0.12	-0.28
Epedaphic	~ Vegetation heterogeneity	-0.47	0.17	-2.78	0.01	-0.47	0.23	0.18	1.26	0.21	0.22
Epedaphic	~ Plant biomass	-0.02	0.16	-0.12	0.90	-0.02	-0.04	0.18	-0.21	0.84	-0.04
Epedaphic	~ Vegetation height	0.09	0.05	1.97	0.05	0.33	0.004	0.07	0.06	0.96	0.01
Hemiptera	~ Bare soil	-0.002	0.01	-0.14	0.88	-0.03	0.01	0.02	0.66	0.51	0.12

Supplementary material – Chapitre 1

Hemiptera	~ Vegetation heterogeneity	-0.08	0.18	-0.44	0.66	-0.08	0.17	0.18	0.93	0.35	0.17
Hemiptera	~ Plant biomass	-0.33	0.17	-1.88	0.06	-0.33	-0.24	0.18	-1.35	0.18	-0.24
Hemiptera	~ Vegetation height	-0.03	0.05	-0.54	0.59	-0.10	0.07	0.07	0.97	0.33	0.18
Araneae	~ Hemiptera	0.43	0.15	2.77	0.01	0.42	0.16	0.16	0.98	0.33	0.16
Araneae	~ Plant feeder Coleoptera	-0.03	0.19	-0.15	0.88	-0.03	0.29	0.16	1.80	0.07	0.29
Araneae	~ Epedaphic	0.10	0.17	0.60	0.55	0.10	-0.03	0.16	-0.19	0.85	-0.03
Araneae	~ Bare soil	-0.01	0.01	-1.04	0.30	-0.16	-0.03	0.02	-1.62	0.11	-0.28
Araneae	~ Vegetation heterogeneity	0.41	0.18	2.34	0.02	0.40	0.06	0.17	0.36	0.72	0.06
Araneae	~ Plant biomass	-0.22	0.15	-1.44	0.15	-0.22	0.09	0.16	0.57	0.57	0.09
Araneae	~ Vegetation height	-0.03	0.05	-0.67	0.50	-0.12	-0.12	0.07	-1.80	0.07	-0.31
Predator Coleoptera	~ Hemiptera	0.56	0.13	4.15	0.00	0.57	-0.08	0.14	-0.57	0.57	-0.08
Predator Coleoptera	~ Plant feeder Coleoptera	0.53	0.14	3.67	< 0.001	0.54	-0.22	0.14	-1.57	0.12	-0.22
Predator Coleoptera	~ Araneae	-0.29	0.14	-2.04	0.04	-0.31	0.69	0.15	4.57	< 0.001	0.71
Predator Coleoptera	~ Epedaphic	-0.07	0.13	-0.53	0.60	-0.07	-0.01	0.14	-0.05	0.96	-0.01
Predator Coleoptera	~ Bare soil	-0.03	0.01	-3.01	0.003	-0.38	-0.01	0.01	-0.39	0.70	-0.06
Predator Coleoptera	~ Vegetation heterogeneity	0.31	0.15	2.11	0.03	0.32	-0.25	0.14	-1.74	0.08	-0.25
Predator Coleoptera	~ Plant biomass	-0.25	0.12	-2.00	0.05	-0.25	-0.28	0.14	-2.07	0.04	-0.29
Predator Coleoptera	~ Vegetation height	-0.09	0.04	-2.16	0.03	-0.31	0.02	0.06	0.35	0.72	0.05

Supplementary material – Chapitre 1

Table S1.3: Estimate, standard error, z-value, P-value and standardised path estimates for models of below-ground compartment in ant patches and ant-free patches depicted in Figure 1.2C and Figure 1.2D.

Response	Predictor	Ant-patches					Ant-free patches				
		Estimate	Standard error	z-value	P-value	Standardized estimate	Estimate	Standard error	z-value	P-value	Standardized estimate
TOM	~ Clay	0,31	0,05	5,86	< 0.001	0,71	0,21	0,03	6,21	< 0.001	0,75
TOM	~ Plant biomass	0,00	0,00	1,01	0,31	0,12	0,00	0,00	-0,16	0,88	-0,02
TOM	~ Vegetation height	-0,08	0,03	-2,27	0,02	-0,28	-0,04	0,05	-0,84	0,40	-0,10
Microfauna primary consumer	~ Clay	-0,39	0,08	-5,01	< 0.001	-0,88	-0,19	0,07	-2,77	0,01	-0,66
Microfauna primary consumer	~ TOM	0,22	0,18	1,19	0,24	0,22	0,44	0,24	1,86	0,06	0,44
Microfauna primary consumer	~ Plant biomass	0,002	0,002	0,91	0,37	0,11	-0,01	0,01	-1,59	0,11	-0,25
Microfauna primary consumer	~ Vegetation height	-0,01	0,04	-0,22	0,83	-0,03	0,00	0,06	-0,02	0,98	-0,003
Macrofauna detritivore	~ Clay	-0,17	0,11	-1,52	0,13	-0,39	-0,05	0,07	-0,66	0,51	-0,16
Macrofauna detritivore	~ TOM	0,39	0,27	1,46	0,15	0,39	-0,09	0,25	-0,38	0,70	-0,09
Macrofauna detritivore	~ Plant biomass	-0,004	0,003	-1,37	0,17	-0,25	0,02	0,01	2,72	0,01	0,44
Macrofauna detritivore	~ Vegetation height	0,04	0,05	0,83	0,40	0,16	-0,02	0,06	-0,24	0,81	-0,04
Earthworms	~ Clay	-0,05	0,10	-0,49	0,63	-0,11	-0,04	0,07	-0,65	0,51	-0,15

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Earthworms	~ TOM	0,03	0,24	0,13	0,90	0,03	0,48	0,24	2,02	0,04	0,48
Earthworms	~ Plant biomass	0,004	0,002	1,58	0,11	0,25	-0,01	0,01	-1,64	0,10	-0,26
Earthworms	~ Vegetation height	-0,12	0,05	-2,48	0,01	-0,42	-0,08	0,06	-1,35	0,18	-0,21
Mesofauna primary consumer	~ Clay	0,09	0,16	0,57	0,57	0,20	-0,10	0,09	-1,07	0,29	-0,35
Mesofauna primary consumer	~ TOM	-0,05	0,27	-0,20	0,84	-0,05	0,09	0,29	0,31	0,76	0,09
Mesofauna primary consumer	~ Microfauna primary consumer	-0,41	0,61	-0,68	0,50	-0,41	-0,13	0,20	-0,63	0,53	-0,13
Mesofauna primary consumer	~ Microfauna secondary consumer	-0,73	0,55	-1,32	0,19	-0,73	0,11	0,21	0,52	0,60	0,11
Mesofauna primary consumer	~ Vegetation height	0,03	0,05	0,61	0,54	0,12	-0,01	0,07	-0,18	0,86	-0,03
Microfauna secondary consumer	~ Microfauna primary consumer	-0,99	0,09	-11,25	0,00	-0,99	0,17	0,17	1,03	0,30	0,17
Microfauna secondary consumer	~ Clay	-0,02	0,04	-0,54	0,59	-0,05	0,16	0,05	3,30	< 0.001	0,55
Microfauna secondary consumer	~ Vegetation height	-0,01	0,02	-0,45	0,65	-0,03	-0,03	0,06	-0,49	0,62	-0,08
Mesofauna secondary consumer	~ Microfauna primary consumer	0,54	0,57	0,95	0,34	0,54	-0,09	0,18	-0,49	0,63	-0,09

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Mesofauna secondary consumer	~ Microfauna secondary consumer	0,22	0,53	0,41	0,68	0,22	0,40	0,19	2,12	0,03	0,40
Mesofauna secondary consumer	~ Mesofauna primary consumer	0,35	0,17	2,05	0,04	0,35	0,12	0,16	0,74	0,46	0,12
Mesofauna secondary consumer	~ Clay	0,07	0,11	0,62	0,54	0,15	-0,12	0,06	-2,13	0,03	-0,44
Mesofauna secondary consumer	~ Vegetation height	0,05	0,05	1,13	0,26	0,19	-0,07	0,06	-1,17	0,24	-0,19
Geophilomorpha	~ Mesofauna primary consumer	-0,22	0,17	-1,26	0,21	-0,21	-0,15	0,17	-0,88	0,38	-0,14
Geophilomorpha	~ Mesofauna secondary consumer	0,26	0,18	1,48	0,14	0,25	-0,20	0,17	-1,17	0,24	-0,20
Geophilomorpha	~ Macrofauna detritivore	-0,04	0,16	-0,23	0,82	-0,04	-0,0003	0,17	-0,002	1,00	-0,0003
Geophilomorpha	~ Earthworms	0,52	0,18	2,86	0,004	0,51	0,33	0,17	1,91	0,06	0,33
Geophilomorpha	~ Clay	0,01	0,07	0,07	0,94	0,01	0,03	0,05	0,66	0,51	0,11
Geophilomorpha	~ Vegetation height	0,12	0,05	2,39	0,02	0,43	0,09	0,07	1,34	0,18	0,23

TRANSITION VERS LE CHAPITRE 2

Dans le premier chapitre, nous avons démontré que *M. barbarus* joue un rôle au sein de son écosystème par ses interactions directes et indirectes avec différents compartiments (sol, végétation, faune) des pelouses méditerranéennes. Ce résultat soulève plusieurs questions sur son utilisation en restauration écologique. Dans ce deuxième chapitre, nous nous sommes ainsi focalisés sur sa capacité à accélérer la restauration des propriétés physico-chimiques du sol et de la végétation dans un milieu dégradé, et par quelles actions.

La restauration écologique est définie par la Society for Ecological Restoration (SER 2004) comme « *une action intentionnelle qui initie ou accélère l'auto-réparation d'un écosystème qui a été dégradé, endommagé ou détruit, en respectant sa santé, son intégrité et sa gestion durable* ». La restauration écologique est un processus qui permettrait aux écosystèmes dégradés de se rétablir entièrement en récupérant l'intégralité de leurs fonctions, de façon viable sur le long terme. Ceci suppose alors un rétablissement de la richesse spécifique, la composition, la structuration spatiale et temporelle des fonctions de tous les compartiments de l'écosystème mais aussi des interactions entre ces compartiments. La restauration écologique d'un écosystème devrait aussi répondre à trois critères : écologique, socio-économique, mais également culturel (Clewell & Aronson 2013).

La restauration *sensu stricto* doit donc replacer l'écosystème dans sa trajectoire historique, appelée écosystème de référence et défini comme l'état originel antérieur à la perturbation (Hall 2005) avant que le système écologique en question ne franchisse un ou plusieurs seuils écologiques irréversibles (Aronson et al. 1993; Aronson et al. 1995; Hobbs et al. 2009). Parfois, l'écosystème de référence peut être redéfini afin de correspondre aux réelles capacités de rétablissement des espèces et des communautés locales (McDonald et al. 2016). L'écosystème de référence est un outil d'aide permettant de planifier, suivre et évaluer les travaux de restauration écologique même si, *in fine*, il ne peut être atteint en pratique, notamment en raison des changements d'usages intervenus, aux changements climatiques ou encore à d'autres interventions irréversibles (McDonald et al. 2016).

Les interventions utilisées en restauration varient fortement selon les projets et les objectifs assignés en relation avec la nature, l'intensité et la durée de la ou les perturbations passées et des contraintes ou opportunités. Afin de rétablir la structure et la fonctionnalité

des écosystèmes dégradés, deux stratégies générales de restauration sont envisageables. La première, appelée restauration passive (ou régénération naturelle), consiste à supprimer ou modifier le(s) facteur(s) de perturbation à l'origine de la dégradation. La restauration passive est donc basée sur la capacité des écosystèmes à se réparer spontanément, de façon indépendante, dès lors que les conditions le permettent. Lorsque la dégradation est plus importante, qu'un (ou plusieurs) seuil d'irréversibilité est franchi au sein de l'écosystème, la restauration active consiste comme dans le premier cas à éliminer les sources de perturbations ainsi qu'à mettre en œuvre des stratégies afin d'accélérer la récupération de l'écosystème (SER 2004; Rohr et al. 2018).

Certains auteurs (Gann et al. 2019; Atkinson & Bonser 2020) proposent de séparer la restauration active en deux catégories : la régénération assistée et la reconstruction. La régénération assistée consiste à restaurer des sites dégradés (de façon intermédiaire ou plus importante) et nécessite l'élimination des causes de dégradation et des interventions actives pour restaurer les dommages abiotiques et biotiques et déclencher la régénération biotique (e.g. imiter les perturbations naturelles, fournir des ressources clés, réintroduire ou renforcer des populations d'espèces, installer des éléments d'habitat tels que des perchoirs, rochers) (Gann et al. 2019). La reconstruction consiste à restaurer des sites très dégradés. Dans ce cas, non seulement toutes les causes de dégradation doivent être éliminées et tous les dommages abiotiques et biotiques rétablis pour qu'ils correspondent à nouveau à l'écosystème de référence, mais aussi la totalité ou la majorité des organismes doit être réintroduite chaque fois que cela est possible. Les organismes réintroduits (successivement ou non) peuvent, en interagissant avec le compartiment abiotique, favoriser la récupération des propriétés de l'écosystème (Gann et al. 2019). Bien que de nombreux projets de restauration écologique aient permis d'améliorer significativement la biodiversité et/ou des fonctions des écosystèmes, aucune revue indexée ne semble jusqu'à présent témoigner d'une restauration de l'intégralité de l'écosystème dégradé à l'identique de celui de référence (Benayas et al., 2009; Bullock et al., 2011). L'un des verrous majeurs à la réussite des opérations de restauration paraît notamment concerner l'incapacité à mimer des interactions complexes régissant la structure de l'écosystème sur le long et très long terme.

Souvent, les opérations de restauration écologique font appel aux principes de l'ingénierie civile utilisant des engins de travaux publics et consommant des ressources non renouvelables. Alors que ces actions visent à restaurer le vivant, elles contribuent cependant

à des émissions importantes de polluants dont les conséquences environnementales sont notoirement négatives (Dutoit 2014). La mise en place d'opérations de restaurations écologiques plus durables sont néanmoins possibles avec l'ingénierie écologique. Ce concept, parfois décrit comme une discipline parallèle à la restauration écologique, a été défini par Odum (1962) comme « *une manipulation environnementale par l'homme en utilisant une faible quantité d'énergie supplémentaire pour contrôler des systèmes dans lesquels les forces énergétiques principales proviennent encore de sources naturelles* ». Bien que d'autres définitions aient été développées par la suite (Mitsch 1996; voir notamment Bergen et al. 2001; Mitsch 2012), l'idée générale de ce concept reste identique : restaurer des écosystèmes pour protéger, conserver et améliorer l'environnement naturel avec ses biens et ses services, pour qu'ils puissent se maintenir au bénéfice de la société humaine et ses besoins et souvent par le maintien de certaines activités anthropiques. L'ingénierie écologique ajoute donc au concept de restauration écologique la notion d'interventions de restaurations durables et centrées sur le vivant. Le vivant correspond ainsi à un moyen et/ou un objectif d'action (Abbadie 2011).

En agissant à la fois sur les compartiments biotiques et abiotiques, les espèces ingénieuses des écosystèmes semblent ainsi des outils potentiels pertinents en ingénierie écologique (Byers et al. 2006; Wright & Jones 2006; Hastings et al. 2007; Gutiérrez & Jones 2008; Jones et al. 2010). Parmi les ingénieurs des écosystèmes, les fourmis présentes sur tous les continents excepté l'Antarctique et modifiant à la fois les compartiments biotiques et abiotiques de leurs écosystèmes, pourraient alors être des organismes utiles dans le cadre de projets d'ingénierie écologique.

Dans le cas de *M. barbarus*, son milieu "naturel", les prairies méditerranéennes, est en déclin depuis plusieurs décennies tant en terme de superficies que de structure. Un nombre croissant d'études visant leur restauration écologique ont ainsi vu le jour (Buisson & Dutoit 2006; Buisson et al. 2006; Mesléard et al. 2011; Jaunatre et al. 2014a; Mesléard et al. 2017; Buisson et al. 2020) (voir Annexe p. 133-144). Pourtant, plusieurs années après la restauration, la composition des espèces demeure souvent différente de celle de la communauté de référence (Jaunatre 2012; Jaunatre et al. 2014a; De Almeida, Blight, et al. 2020). Dans ce contexte, *Messor barbarus*, en raison de son impact sur les propriétés du sol, la végétation, la faune ainsi que sur les interactions entre et au sein des différents compartiments (Chapitre 1), pourrait être une espèce à considérer lors de projets de

restauration écologiques au sein des pelouses méditerranéennes (Figure T2.1). Cette espèce est d'autant plus intéressante qu'elle a déjà fait l'objet de deux expériences de réintroduction en milieu naturel qui montrent la faisabilité de son introduction. La première, réalisée par Bulot et al. (2014) consiste à transplanter des reines fondatrices de *M. barbarus* sur le milieu à restaurer. Un an après la transplantation les résultats sont encourageants avec un taux de survie des reines compris entre 15 et 35%. Afin d'améliorer ce protocole d'implantation, une deuxième méthode a été mise en place par El Boukhrissi et al. (2019). Au lieu d'une introduction directe dans le milieu, les reines fondatrices sont préalablement placées dans des conteneurs en carton biodégradable avec du sol provenant du site où seront implantées les fourmis. Après une période d'incubation de 10 jours en laboratoire, les conteneurs sont introduits dans le sol du site. Huit mois après la mise en place de l'expérience, la survie des reines est de 71%. Ces résultats montrent la faisabilité technique d'utiliser *M. barbarus* en restauration active via leur introduction mais ne déterminent pas leurs rôles sur les sites à restaurer. Afin de déterminer si cette espèce peut être utilisée en restauration écologique, nous évaluons dans ce chapitre 2 le rôle de *M. barbarus* dans l'accélération de la restauration du sol et de la végétation d'une zone dégradée (Figure T2.1).

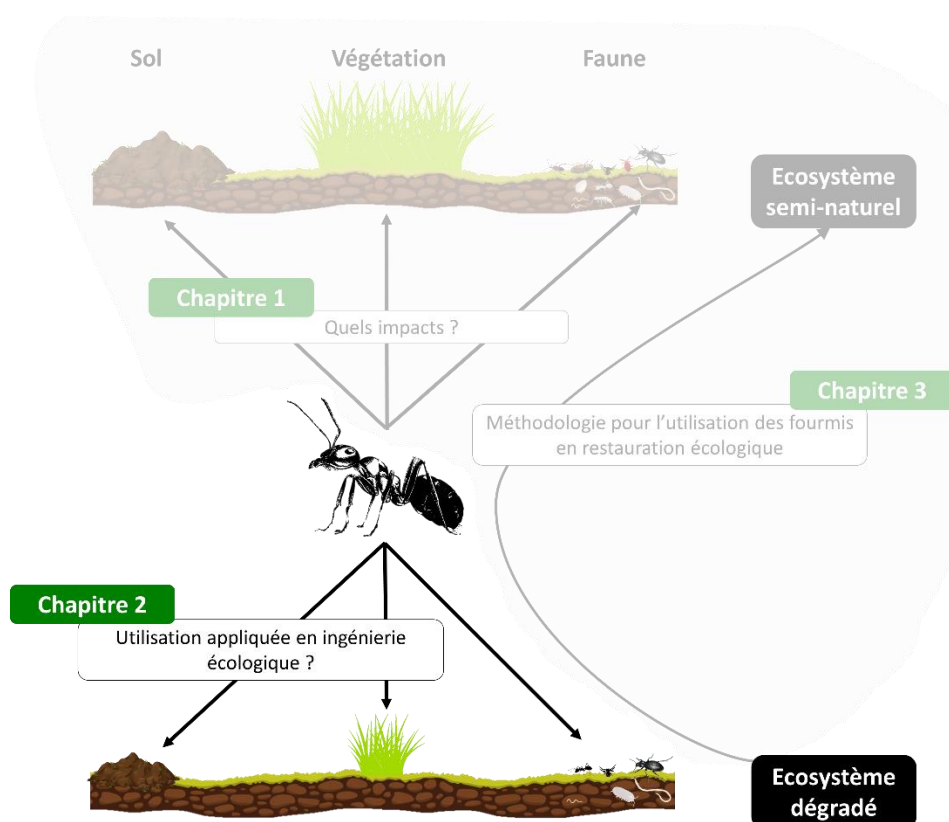


Figure T2.1 : Localisation du chapitre 2 dans l'organisation générale de la thèse.

CHAPITRE 2

Harvester ants as ecological engineers for Mediterranean grassland restoration: impacts on soil and vegetation

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Abstract

Although not widely used, ecosystem engineers represent a promising and sustainable tool in nature-based ecosystem management and restoration. In grassland ecosystems, a few invertebrates that engineer soils have been identified as key species regulating soil nutrients and plant communities' diversity and dynamics. Here, we assessed the role of the harvester ant *Messor barbarus*, an ecological engineer, in a Mediterranean dry grassland under restoration by characterising its nest environment, particularly the soil and vegetation. We found profound differences in soil physical and chemical variables and plant community structure between nests and ant-free patches in the restored grassland. *Messor barbarus* has improved soil fertility, driven the seed bank towards the reference grassland and significantly increased plant biomass, species richness and micro-local-heterogeneity. As biological filters, *M. barbarus* has driven plant communities towards a new trajectory in the restored site. Ant patches are characterised by mesotrophic species, whereas ant-free patches are dominated by species characteristic of compacted soils. They have accelerated the ecological recovery of Mediterranean dry grassland plants by directly and indirectly facilitating their re-establishment. These results illustrate the potential key role of ants as ecological engineers for the conservation and restoration of Mediterranean grasslands.

Keywords: ecosystem engineer, grassland restoration, *Messor barbarus*, plant communities, soil

2.1 Introduction

The well-known effects of ecosystem engineers make them particularly useful in achieving conservation or restoration targets: managing one or a few species can influence community diversity and/or ecosystem functioning. Although still not widely used, ecosystem engineers represent a promising and sustainable tool in nature-based ecosystem management and restoration (Bergen et al. 2001). Terrestrial ecosystem engineers (Jones et al. 1997) play important roles in controlling major ecological functions, such as the dynamics of soil organic matter, mineral nutrients and biological population regulation (Wills & Landis 2018). These organisms directly or indirectly modulate the availability of resources to other species, by causing physical state changes in biotic and abiotic materials (Jones et al., 1997), and can contribute to the resilience of ecosystems after disturbances (Peterson et al. 1998). They are expected to accelerate ecological recovery by reinstating ecosystem functions after species declines (Manning et al. 2015) and by facilitating the re-establishment of other threatened species.

In grassland ecosystems, a few invertebrates that engineer soils, such as ants or earthworms, have been identified as key species regulating soil nutrients and plant community diversity and dynamics (Eisenhauer et al. 2009; Wills & Landis 2018). These organisms build organomineral structures with specific physical, chemical and microbiological properties (Jouquet et al. 2006), engineered patches often therefore considered as “islets” because of their uncommon diversity and quantity of soil organisms and plants (Lavelle et al. 1997).

Ants are “bioturbator agents” influencing soil structure through the production of galleries and the translocation of soil aggregates (Cammaraat & Risch 2008; Bottinelli et al. 2015). They not only change soil physical and chemical properties such as soil water, nutrient content, pH (Folgarait 1998; Frouz & Jilková 2008; Farji-Brener & Werenkraut 2015), but also affect plant communities (Christian 2001; Azcárate & Peco 2006). In terrestrial ecosystems, ants are considered a seed disperser (Wills & Landis 2018). They also transport seeds to good establishment sites (Hanzawa et al., 1988), reduce seed predation (Ness & Morin 2008) and competition with the parent plant. In the Mediterranean region, species from the *Messor* genus are key in many ecological functions such as soil structuration or seed dispersal in dry grasslands (Azcárate & Peco 2007; Bulot et al. 2016). Although they may decrease seed

germination by direct predation (Schöning et al. 2004), they also play a key dispersal role when they lose seeds in suitable conditions for germination on the way to the nest (Wolff & Debussche 1999) or when they deposit them in refuse piles, increasing seedlings recruitment (Azcárate & Peco 2007). This seed dispersal that is not mediated by any particular seed structure is known as diszoochory (Arnan, Retana, et al. 2010). Among *Messor* species, *M. barbarus* is probably the most common and numerically dominant in western Mediterranean grasslands (Lebas et al. 2016). *Messor barbarus* is a polymorphic species living in monogynous colonies of about 8,000 individuals (Cerdan 1989). It builds nests composed of a dense network of galleries and interconnected chambers that can reach five meters deep. Usually, colonies reach a stable size at approximately the age of five (Gordon 1995). *Messor barbarus* makes permanent and temporary trails with length ranges from 1 to 30 m (Cerdan 1989). Previous studies reported their roles on seed redistribution, with in particular an increase in seed density and seed bank species richness in the refuse piles located on the top of the nest (Azcárate & Peco 2007; Bulot et al. 2016). Other studies reported their roles in changes of soil physical and chemical properties (Azcárate & Peco 2007; Martín-Perea et al. 2019), especially with an increase in nutrient content, especially potassium, organic matter and pH. As such, referring to Jones et al. (1994), *M. barbarus* can be considered as an ecosystem engineer in Mediterranean grasslands.

Mediterranean dry grasslands are primary targets of conservation and ecological restoration programs (Buisson & Dutoit 2006; Janišová et al. 2011). Indeed, considered as biodiversity hot spots, they have been not only reduced in area but also in habitat quality and biodiversity since the 20th century changes in land use (Saunders et al. 1991). Considering their key function, harvester ants may be good ecological engineer candidates to restore grassland vegetation and soil, two ecosystem components key to successful ecological restoration (Ruiz-Jaen & Mitchell Aide 2005; Wortley et al. 2013). Ants are used as bio-indicators for the evaluation of restoration success (Andersen et al. 2002; Fagan et al. 2010), or are the target of the restoration (Gibb & Cunningham 2013) but their biological engineering potential to restore degraded grasslands has not been exploited yet.

The objective of our study was to evaluate the role of an ecosystem engineer in the restoration of an ecosystem after its degradation. In 2009, 5.5 ha of the plain of La Crau (south-eastern France; see supplementary material Figure S2.1), a natural reserve, was heavily polluted by an oil leak. Two years later, after the excavation and removal of all the polluted

soil, 72 000 tons of a similar soil were transferred from a nearby active quarry already expanding before the accident (Figure S2.1) (see Bulot et al., 2014b). This highly valuable grassland (Devaux et al. 1983) has been sharply reduced in its area from 50 000 to 11 500 ha since the 1960s (Buisson & Dutoit 2006). This ecosystem is a priority habitat under the EU habitats Directive (EUNIS, 2020).

Seven years after soil replacement, we assessed the role of the harvester ant *M. barbarus* in the restored site, and in the surrounding unaltered dry grassland by characterising soil physico-chemical variables and soil seed banks on the top of the nest, and plant communities in the nest environment compared to grassland off the nest. We specifically hypothesised that *M. barbarus* has (1) modified soil texture by increasing coarse sand content; (2) increased soil chemical parameters (such as available phosphorus, organic C and ammonium); (3) increased soil seed bank density and species richness, and (4) increased plant species richness, cover, dissimilarity index and biomass, towards the reference steppe.

2.2 Materials & methods

2.2.1 Study area

The dry grassland of the plain of la Crau in south-eastern France (43°33'N, 4°52'E) is considered a “steppe” ecosystem of the Mediterranean basin’s rangelands (Devaux et al. 1983; Le Houérou 2001). Climate is Mediterranean with a 15°C mean annual temperature. Mean annual precipitation is 400 mm to 600 mm falling in spring and autumn. A strong north-westerly cold wind called “Mistral”, blowing at more than 50 km.h⁻¹ for 110 days.year⁻¹, induces the dryness of the ecosystem (Devaux et al. 1983). The topography is flat, with siliceous stones covering more than 50% of the soil surface. An impermeable calcareous conglomerate situated 40 cm below the surface makes the alluvial water table inaccessible and contributes, with the strong wind and low precipitations, to soil drought (Molliex et al. 2013). Over the past several thousand years, itinerant sheep-grazing and prescribed fires have been used for the traditional exploitation of the steppe (Devaux et al. 1983; Buisson & Dutoit 2006). The plant community is composed of annuals and forbs and is dominated by *Brachypodium retusum* (Pers.) P.Beauv. and *Thymus vulgaris* L.. Two *Messor* species co-occur on the site (*M. barbarus* and *M. bouvieri*; personal observation), but *M. barbarus* is by far the most abundant.

2.2.2 Experimental design

We selected two study sites, the restored site-a formerly oil degraded site- and a surrounding unaltered dry grassland. At both sites, we randomly selected 10 of the largest *M. barbarus* nests. The selected nests corresponded to class 4 of the Blanco-Moreno et al. (2014) five levels scale. This scale is based on a combination of surface area occupied by the colony, number of entrances, and ant size and behaviour (class 4: nests covering 2–4 m² with 3–4 entrances). No class 5 were observed. Taking ant nest size as a proxy of colony age (Tschinkel 2005), the colonies selected can be assumed to be the first established in the restored site six months after the soil restoration. At both sites, we also defined 10 ant-free patches of 4 m² with no signs of ant activity (nests, refuse piles or tracks). To avoid any border effects from colony activities, ant-free patches were located at least 5 m away from the selected nests.

We could not set up a before-after experimental design because *M. barbarus* nest location is unpredictable. Indeed, after their nuptial flight in autumn, mated queens land randomly. At a fine scale, nest location is strongly influenced by the proximity to long-established colonies (Blanco-Moreno et al. 2014) and not by soil properties (e.g. organic carbon, soil strength or aggregate distribution) (Baraibar et al. 2011), leading to a regular or random distribution (Blanco-Moreno et al. 2014). We assumed that if differences were recorded between ant patches and ant-free patches, they were the results of the engineering activity of *M. barbarus*.

2.2.3 Soil variables

We measured, in the first two centimetres of soil both from ant-free patches and under ant refuse piles in February 2018, a set of variables known to strongly influence vegetation (Cano-Ortiz et al. 2014). Part of the soil was frozen for nitrate and ammonium analysis. The rest of the sample was dried and sieved (2 mm sieve) prior to assessing the following without decarbonisation according to standard international methods (Baize 2018): physico-chemical properties (organic carbon, total nitrogen, P₂O₅ (available phosphorus) (Olsen et al. 1954), CaO, MgO, K₂O, pH(KCl), CEC (Cation Exchange Capacity), C:N ratio, total organic matter) and sizes of fine particles (clay (< 0.002 mm), fine silt (0.002–0.02 mm), coarse silt (0.2–0.05 mm), fine sand (0.05–0.2 mm), coarse sand (0.2–2 mm)).

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2.2.4 Seed Bank

We collected 100 cm³ of the first centimetres of soil under the refuse piles and from the ant-free patches in October 2017. The 40 samples generated (20 from the reference site and 20 from the restored site) were spread on a substrate composed of 1:3 compost-vermiculite mix in germination seed trays (30 cm x 45 cm). All samples were randomly placed in a greenhouse for five months from October 2017 to March 2018, with soil moisture kept constant. Germinant seedlings were removed as soon as they were identified to species using Mamarot (2002). Data collected were number of species and number of seedlings.

2.2.5 Plant community analyses

We sampled plant communities in 2 m x 2 m (4 m²) quadrats placed on each the twenty selected nests (ant patches) and twenty ant-free patches in May 2018. In each quadrat, the abundance of each plant species was defined using a modified Braun-Blanquet scale (Braun-Blanquet et al. 1952) as follows: 0.2 = represented by a single individual in the 4 m² quadrat, 1 = covering less than 5%, 2 = covering between 5% and 25%, 3 = covering between 26% and 50%, 4 = covering between 51% and 75% and finally 5 = covering more than 75%. We visually estimated the percentage of vegetation cover in the same 4 m² quadrat (Jaunatre et al. 2014b).

The micro-local heterogeneity generated by *M. barbarus* activities around the nest (soil excavated by ants and deposited at the surface above ant hills, surrounding refuse piles, ant trails (very clean paths), seed collection by ants, etc.) was assessed by placing three quadrats of 10 cm x 10 cm (0.01 m²) in each 4 m² quadrat. In the ant patches, in order to take into account vegetation micro-patterns created by *M. barbarus*, we placed the three quadrats as follows: one on the “green belt” (dense vegetation belt located on the previous year’s refuse pile where there is a very high density of seedlings), one on the “soil turned over” (top of nest where the soil is heavily disturbed) and one in an area bearing none of these traces of *M. barbarus* activity. In the ant-free patches, we randomly placed three 0.01 m² quadrats in each 4 m² quadrat and counted all plants therein in May 2018, when most of the seedlings could be identified. Then for each of the 40 patches, a micro-local heterogeneity index based on the average of three Bray-Curtis index distances – a dissimilarity index varying between 0 and 1: 0 for similar communities and 1 for distinct communities – was calculated between the three 0.01 m² quadrats.

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Finally, we measured above-ground plant biomass in 50 cm x 50 cm (0.25 m²) quadrats placed on the “green belt” in the ant patches and randomly in the ant-free patches. In each quadrat, we cut the vegetation at ground level during the productivity peak in June 2018. Each sample was then oven-dried at 40°C up to constant weight.

2.2.6 Nest density

We estimated *M. barbarus* nest densities in autumn 2017 by counting the number of nests in six quadrats of 400 m² randomly distributed at both sites.

2.2.7 Statistical analyses

Prior to statistical analysis, all data distributions were examined using the Shapiro–Wilk test of normality. When data were not normally distributed, they were log-transformed. GLMs with Gaussian distribution were computed to study patch type and site effects and their interaction on soil physico-chemical variables, seed bank (seed density and species richness) and plant community characteristics (species richness, vegetation cover, micro-local heterogeneity and above-ground plant biomass). A GLM with gamma distribution was applied to evaluate patch type and site effects and their interaction on soil variables that violated the assumption of a normal distribution. Models were followed by Tukey HSD post-hoc tests using the package “multcomp” (Hothorn et al. 2016). Significance thresholds for post hoc analyses were set at $P < 0.05$. For both site, effect sizes - the Cohen’s *d* index with 95% confidence intervals for each variable (Cohen 1992) - were calculated using the package “effsize” (Torchiano 2019). The magnitude is assessed using the scale provided by Cohen (1992) as follow: $|d| < 0.2$ "negligible", $|d| < 0.5$ "small", $|d| < 0.8$ "medium", $|d| > 0.8$ "large". A positive Cohen’s *d* indicates a higher value of the response variable in ant patches than in ant-free patches.

Changes in seed bank composition were visualised via Non-metric Multidimensional Scaling (NMDS) based on the Bray-Curtis dissimilarity index to ordinate the characteristics of plant communities (composition and abundance). Differences in seed bank composition were tested by permutation multivariate analysis using the Adonis function.

A redundancy analysis (RDA) was performed to evaluate the influence of six physico-chemical soil variables on plant community composition. From a preliminary RDA analysis including the 19 soil variables measured, the six variables contributing most to the RDA axes

were selected. RDA and NMDS analyses and illustrations were performed using the package “vegan” (Oksanen et al. 2016)

A Generalized Linear Model (GLM) with quasi-Poisson distribution was used to compare nest densities between the reference steppe and the restored site. All statistical analyses were performed using the R software version 1.0.44 (R Development Core Team, 2011).

2.3 Results

2.3.1 Soil variables

In the restored site, clay and coarse sand content were respectively lower and higher in the ant patches than in the ant-free patches (respectively: $z = -6.76$, $P < 0.001$; $z = 4.89$, $P < 0.001$; see supplementary material Table S2.1). We found a large negative effect size of ants on clay content (Cohen’s $d = -2.66 \pm 1.29$) and a large positive effect size on coarse sand content (Cohen’s $d = 2.88 \pm 1.34$) (see supplementary material Figure S2.2). In the reference steppe, we found no significant differences in fine particles between ant and ant-free patches (see supplementary material Table S2.1). No significant difference was found in fine silt, coarse silt and fine sand content at either site (see supplementary material Table S2.1).

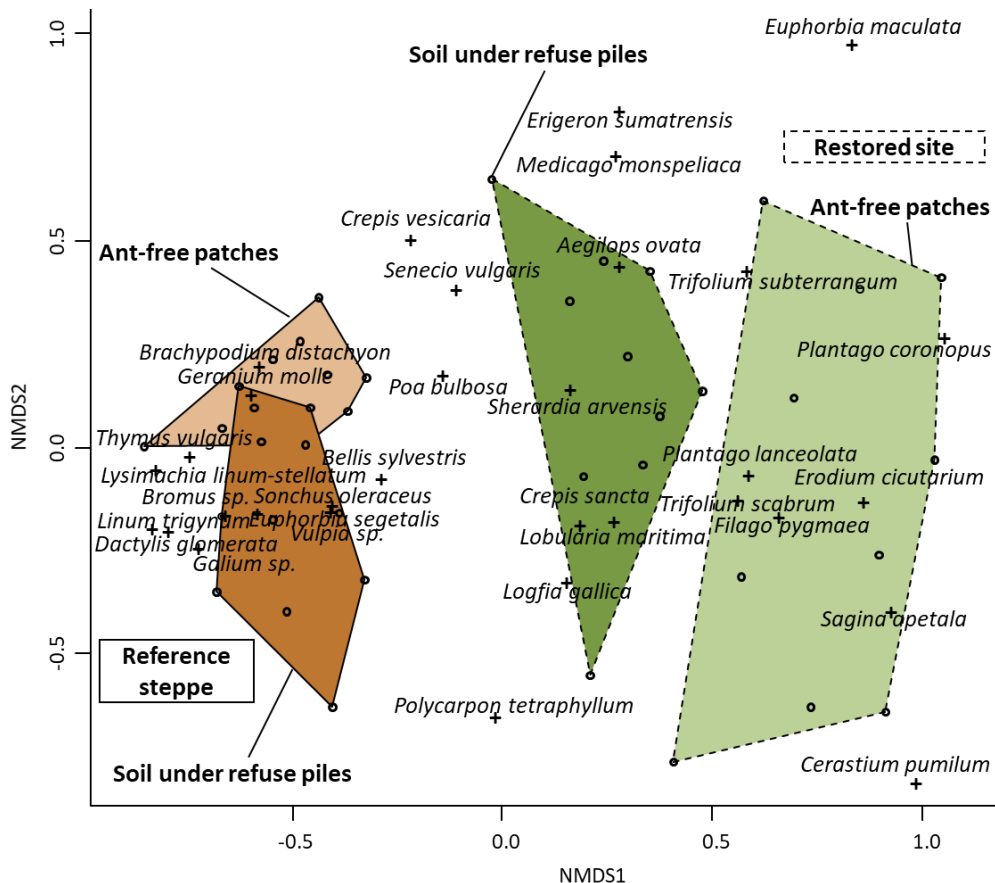
In the restored site, CEC, pH(KCl), K_2O , MgO, CaO and total nitrogen content were significantly higher in ant patches than in ant-free patches (see supplementary material Table S2.1) with a large positive effect size (see supplementary material Figure S2.2). In the reference steppe, there were no significant differences in these variables, except for the C:N ratio, which was significantly higher in ant than in ant-free patches. In both sites, ammonium, nitrate, available phosphorus, organic carbon content and total organic matter (see supplementary material Table S2.1) were significantly higher in ant patches with a large positive effect size (see supplementary material Figure S2.2).

2.3.2 Seed bank

The NMDS ordination (stress = 0.18) discriminated on axis 1 the restored site seed banks from those of the reference steppe (Figure 2.1). In the restored site, seed bank composition and abundance differed between soil under refuse piles and from ant-free patches ($P < 0.01$). Soil samples collected under refuse piles were characterised by *Crepis sancta* (L.) Bornm., *Lobularia maritima* (L.) Desv. and *Logfia gallica* (L.) Cross. & Germ.,

whereas the ant-free patches were characterised by species like *Erodium cicutarium* (L.) L'Hér. and *Filago pygmaea* L. (Figure 2.1). In contrast, the reference steppe seed banks of ant and ant-free patches were not discriminated by NMDS ordination ($P = 0.13$); both were characterized by species such as *Brachypodium distachyon* (L.) P.Beauv., *Lysimachia linum-stellatum* L. or *Linum trigynum* L.. The composition of the seed bank in the restored site ant patches was significantly different from the compositions in the three other modalities ($P < 0.01$; Figure 2.1).

Figure 2.1: Non-metric multidimensional scaling (stress = 0.18) performed on the seed bank of the first centimetres of soil (100 cm³) under the refuse piles and in the ant-free patches. The 10 samples of each treatment are grouped in polygons, with dashed lines for the restored site (ant-free patches in light grey and soil under refuse piles in dark grey) and full lines for the reference steppe (ant-free patches in light grey and soil under refuse piles in dark grey). Dots represent the 40 patches and crosses represent the spatial location of each species. For clarity, only the plant species most correlated to the two first axes are shown.



Both species richness and density were significantly lower in seed banks from ant-free patches in the restored site than from the three other modalities, which did not differ significantly from each other (Table 2.1). In the restored site, we found a large positive effect

size of ants on both species richness (Cohen's $d = 2.59 \pm 1.27$) and density (Cohen's $d = 1.14 \pm 1.01$) (see supplementary material Figure S2.2).

2.3.3 Plant community characteristics

At a scale of 4 m² in the restored site, ant patches had significantly higher plant species richness than ant-free patches ($z = -3.06$, $P = 0.02$; Cohen's $d = 1.21 \pm 1.02$) (Table 2.1; see supplementary material Figure S2.2). In the reference steppe, there were no significant differences between ant and ant-free patches ($z = -1.91$, $P = 0.2$) (Table 2.1). There was no significant inter-site difference in plant species richness for either ant ($z = -2.21$, $P = 0.1$) or ant-free patches ($z = -1.05$, $P = 0.7$) (Table 2.1).

Table 2.1: Effects of ants, site and their interaction on seed bank variables and plant community variables. Values are means \pm standard errors; F value and P value were obtained from GLM with Gaussian distribution. Two values in the same row with a different letter are significantly different according to Tukey post-hoc tests. Significance thresholds were set at a risk of 5%.

			Restored site		Reference steppe	
	F	P-value	Ant-free patches	Ant patches	Ant-free patches	Ant patches
Seed bank variables						
Species richness						
Ants	9.09	0.005	9.4 \pm 0.8	17.6 \pm 1.1	16.5 \pm 1.1	14.7 \pm 1.1
Site	3.92	0.06	a	b	b	b
Ants x Site	22.20	< 0.001				
Density (log(dm³))						
Ants	7.75	0.009	6.1 \pm 0.3	7 \pm 0.1	7.4 \pm 0.1	7.5 \pm 0.2
Site	22.82	< 0.001	a	b	b	b
Ants x Site	4.75	0.04				
Plant community variables						
Specific richness (4m²)						
Ants	12.36	0.001	39 \pm 1.8	45.1 \pm 1.3	36.9 \pm 0.9	40.7 \pm 1.4
Site	5.33	0.03	a	b	a	ab
Ants x Site	0.67	0.42				
Vegetation cover (%) (4m²)						
Ants	4.35	0.04	57 \pm 4.9	65.5 \pm 2.6	69.5 \pm 1.9	74 \pm 2.9
Site	11.36	0.002	a	ab	b	b
Ants x Site	0.41	0.53				
Micro-heterogeneity (0.01m²)						
Ants	15.91	< 0.001	0.63 \pm 0.03	0.80 \pm 0.02	0.68 \pm 0.03	0.71 \pm 0.02
Site	0.51	0.48	a	b	a	ab
Ants x Site	7.23	0.008				

Plant biomass (log(g)) (0.25m²)

Ants	128.85	< 0.001	1.1 ± 0.1	3.0 ± 0.2	2.3 ± 0.1	3.2 ± 0.1
Site	33.08	< 0.001	a	b	c	b
Ants x Site	18.00	< 0.001				

We found no significant difference in vegetation cover between ant and ant-free patches within either site (restored site: $z = 1.93$, $P = 0.2$; reference steppe: $z = 1.02$, $P = 0.7$) (Table 2.1). Similarly, we found no significant difference in vegetation cover between restored site and reference steppe ant patches ($P = 0.2$). However, vegetation cover was significantly lower in restored site ant-free patches than in reference steppe ant-free patches ($z = 2.84$, $P = 0.02$) and ant patches ($z = 3.86$, $P < 0.001$).

At a scale of 0.01 m², the interaction term between patch type and sites was significant for the Bray-Curtis distance (Table 2.1). In the restored site, vegetation heterogeneity was higher in ant patches ($P < 0.001$; Cohen's $d = 1.27 \pm 0.57$) (Table 2.1; see supplementary material Figure S2.2). By contrast, the reference steppe showed no significant differences between ant and ant-free patches ($z = 0.92$, $P = 0.8$). We found no significant difference in micro-local heterogeneity between the two sites ant patches ($z = -2.4$, $P = 0.08$) (Table 2.1).

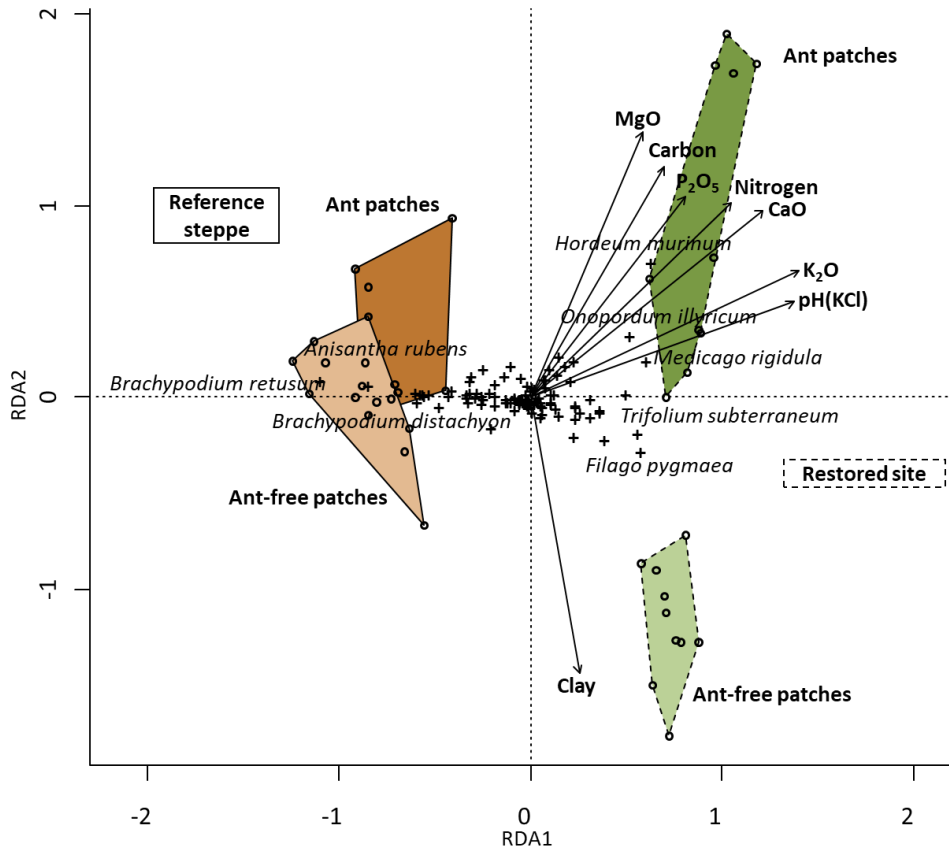
At a scale of 0.25 m², the interaction term between patch type and sites was significant for plant biomass (Table 2.1). In both sites, plant biomass was significantly higher in ant patches (Table 2.1) with a large positive effect size (see supplementary material Figure S2.2). We found no significant difference in plant biomass between the two sites' ant patches ($z = 1.07$, $P = 0.7$).

2.3.4 Soil influence on plant communities

The variation in plant community composition was significantly explained by the first two RDA axes. The first axis (69 % explained, $F = 20.78$, $P < 0.001$) was correlated with soil chemical properties pH(KCl) and K₂O content (Figure 2.2). These variables discriminated the typical plant community of the reference steppe, dominated by grasses (e.g. *B. distachyon*, *B. retusum*, *Anisantha rubens* (L.) Nevski), from the plant community of the compacted soils of the restored site (e.g. *Filago pygmaea* L., *Trifolium subterraneum* L.). The second axis (11 % explained, $F = 3.31$, $P < 0.001$) discriminated clay content from all the other chemical variables. These variables discriminated plant communities of the restored site ant patches, characterised by more nitrophilous and ruderal species (e.g. *Hordeum murinum* L., *Medicago*

rigidula (L.) All., *Medicago truncatula* Gaertn., *Onopordum illyricum* L.), from those of the restored site ant-free patches.

Figure 2.2: Redundancy analysis (RDA) showing the vegetation composition correlations with the following soil variables: proportion of clay, pH(KCl), K₂O, CaO, total nitrogen, P₂O₅, total carbon and MgO. The 10 samples of each treatment were grouped in polygons, with dashed lines for the restored site (ant-free patches in light grey and ant patches in dark grey) and full lines for the reference steppe (ant-free patches in light grey and ant patches in dark grey). Dots represent the 40 patches and crosses represent the spatial location of each species. For clarity, only the plant species most correlated to the two first axes are shown.



2.3.5 Nest density

In autumn 2017, nest density was significantly higher in the restored site ($263 \pm 34 \cdot \text{ha}^{-1}$) than in the surrounding reference steppe ($167 \pm 19 \cdot \text{ha}^{-1}$) ($t = -2.53$, $P = 0.03$).

2.4 Discussion

2.4.1 Ants in the restored site

Seven years after soil restoration, we found strong differences between ant and ant-free patches for both soil physical and chemical, and plant community. We recorded higher soil nutrients, seed bank richness and density, plant community richness, plant biomass, and

heterogeneity in ant patches highlighting the potential role of *M. barbarus* in recovering Mediterranean dry grassland. Nest densities quantified in both sites were consistent with the literature (Azcárate & Peco 2003). In seven years, the *M. barbarus* population has reached high densities and even exceed those of the reference site, which is promising in terms of restoration. Nevertheless, we can expect a decrease in nest density, in the mid-term, due to intraspecific competition (Cushman et al. 1988).

Effects on the soil compartment

The strong impact of *M. barbarus* on the soil compartment supports our first hypothesis that ants, by their engineer's roles, modified soil quality in the restored site. *Messor barbarus* increased the quantity of coarse sand and decreased the amount of clays in the engineered patches, which may have significantly affected the plant community because changes in soil physical properties can facilitate the establishment of plants in a restored site (Li et al. 2010).

Changes we recorded in texture, pH, organic matter and nutrient content (organic C, total nitrogen, N-NH₄, N-NO₃, P₂O₅, CaO, MgO, K₂O, CEC and C:N ratio) suggest a soil fertility enhancement by *Messor barbarus* colonies. Both diet and habitat type determine the magnitude of enrichment in ant-engineered patches, granivorous ants contributing more to soil fertility than omnivorous species (Farji-Brener & Werenkraut 2017). In dry grasslands, harvester ants' nests increase nutrient concentration (MacMahon et al. 2000), probably because of the accumulation of seed debris, ant corpses and other residues of harvester ant activity in refuse piles (MacMahon et al. 2000; Wills & Landis 2018). In both studied sites, ants had a significant positive impact on ammonium and nitrate content, likely through production of metabolic residues or decomposition of the organic matter accumulated in *M. barbarus* refuse piles. This increases nitrogen content, including the different forms available to plants as ammonium and nitrate (Azcárate & Peco 2007).

Effects on vegetation

Messor barbarus colonies drove seed bank and plant communities towards the reference grassland ecosystem. In the restored site, higher species richness and seed density were recorded in ant patches than in ant-free patches. Seed-harvesting ants play an important role in seed dispersal (Wills & Landis 2018), particularly in dry ecosystems, where they are present in high densities (MacMahon et al. 2000; Wills & Landis 2018). Seed-dispersal

mutualisms influence seedling recruitment, population dynamics, species distribution, plant-community composition and gene flow (Nathan & Muller-Landau 2000; Christian 2001). Only seven years after the establishment of colonies, the seed bank composition of restored site ant patches resembled the reference steppe seed bank more than the ant-free seed banks did. Thus, species such as *Galium sp.* and *Vulpia sp.*, naturally present in the reference steppe, were found in ant patches but not in ant-free patches at the restored site.

Two non-exclusive hypotheses may explain this higher similarity between the ant patch seed banks of the restored site and those of the reference site. First, harvester ants retrieve seeds from the environment and lose, abandon or reject some of them in refuse piles (MacMahon et al., 2000; Sánchez et al., 2006; Bulot et al., 2016). Workers of *M. barbarus* are known to prospect over a mean distance of 30 metres (Cerdan 1989), but foraging tracks of more than 50 metres have been observed (personal observation). Several studied nests were located less than 50 metres from the reference steppe, so workers may have prospected in the reference steppe. Second, the nest itself may have acted as a passive trap for seeds due to its coarse texture and/or its height (Brown et al. 2012). Contrary to the reference steppe, with stone cover of more than 50% of the total surface area, the restored site contains very few pebbles. This area being relatively flat, large nests 10 cm high contribute to the micro-relief, which might be sufficient to trap wind-transported seeds.

Messor barbarus colonies also modified plant communities. They changed community composition and increased plant biomass, species richness and micro-local heterogeneity. As discussed above, the higher proportions of ammonium, nitrates and available phosphorus recorded in ant patches may explain our results. This is consistent with recent findings from meta-analysis that plant biomass and fitness can sometimes be higher in ant nest soils (Farji-Brener & Werenkraut 2017), probably because of increased nutrients, including ammonium (Lafleur et al. 2005). In grasslands, small-scale disturbances created by ecosystem engineers, such as ant nests and badger mounds, can play a significant role in maintaining species richness and spatial heterogeneity (Platt 1975), and may contribute to species diversity in restored grasslands that lack the diversity of the native ecosystem (Lane & BassiriRad 2005).

2.4.2 Ants in the reference ecosystem

Interestingly, *M. barbarus* impacts were more significant in the restored site than in the reference steppe. Two explanations appear tenable. First, the role of ecological engineers

like *M. barbarus* is context-dependent and less pronounced in natural or semi-natural ecosystems. Second, because *M. barbarus* has inhabited the reference steppe for thousands of years, a large part of, or even the entire, reference steppe may already have been engineered by *M. barbarus*, including our ant-free patches. In some cases, changes in the quality of the organic matter within anthills have been detected 20 years after colony disappearance (Kristiansen & Amelung 2001). For nests built by *Formica montana* and *Acanthamyops claviger*, nutritional differences between mound and surrounding soils were high eight years after site restoration and disappeared after 26 years (Lane & BassiriRad 2005). Here, the reference steppe soil may have retained “memory” of the ants’ engineering after nest abandonment, making their impacts at the patch scale less detectable. This suggests that where engineers have occupied the entire habitat in natural and semi-natural ecosystems, their impacts are expressed at a large scale (Jones et al. 1997).

2.4.3 Implications in grassland restoration

The soil parameters condition the presence of plant populations and communities (Ehrenfeld et al. 2005). In the restored site, the main drivers of the plant community change are the MgO, organic C and P₂O₅ content; essential elements for plant survival and growth (Gurevitch et al. 2002). In western Mediterranean grasslands, changes in their amount may benefit or disadvantage some species (Cano-Ortiz et al. 2014). Although *M. barbarus* has changed the trajectory of plant communities, differences were still significant between the restored site and the reference steppe. The reference plant community was mainly composed of oligotrophic species such as *B. distachyon*, whereas ant patches in the restored site were characterised by more mesotrophic species such as *H. murinum* or *O. illyricum*. Through nest soil modification, ants can act as a biological filter promoting or impeding plant species according to their ecological traits. For example, *Lasius flavus* provides suitable habitat conditions for the establishment of plant species adapted to poor organic nutrient availability (Ehrle et al. 2019).

The mesotrophic species established in the restored site ant patches only represented part of the species pool present in the seed bank. After nest abandonment (around 10 years), nutrients may well decrease in the soil, allowing steppe plant species such as *Galium sp.* and *Vulpia sp.*, present in the ant patches’ seed bank, to establish populations. This may in turn reduce differences in plant community composition between restored site and reference

ecosystem. Monitoring these ant patches after nest abandonment would help to assess the dynamics of their long-term impact and its final implications for the restoration of Mediterranean grasslands.

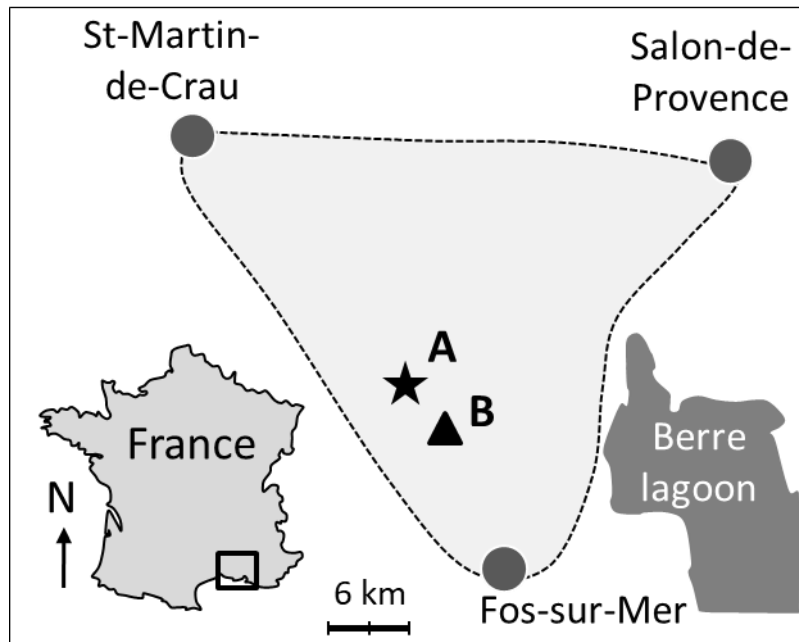
Interactions between individuals or populations are the basis of the rules for assembling species (Lortie et al. 2004) and play one of the major roles in the functioning and evolution of ecosystems. Given their involvement in many ecological processes such as seed dispersal or soil functioning (nutrient recycling), ant disappearance is a key issue for ecosystem conservation and restoration. Although no *Messor* species are endangered in South-Western Europe, they can locally disappear because of a pollution such as in La Crau or following the invasion of invasive species (Blight et al. 2014), with major ecological consequences (e.g. Gómez & Oliveras 2003). Disappearance of key ant species can lead to severe consequences for plants both at a population (Rodríguez-Cabal et al. 2012) and community level (Christian 2001). In South African shrublands, the disruption of native ant communities by the invasive Argentine ant lead to a disproportionate reduction in the densities of large-seeded plants (Christian 2001). The preservation of mutualistic interactions involving ants is therefore essential for conserving and restoring natural communities.

2.5 Conclusion

Since the soil restoration in 2011, *M. barbarus* has improved the soil fertility, driving the seed bank towards the reference grassland and significantly increasing plant biomass, species richness and micro-local-heterogeneity. They have accelerated the ecological recovery of Mediterranean dry grasslands plants by directly and indirectly facilitating their re-establishment. *These results highlight the potential key role of harvester ants as ecological engineers for the conservation and management of these ecosystems. Thus, the recolonization of these habitats by M. barbarus seems to be necessary for their restoration.* In the case, where it could not naturally colonize (e.g. a site distant from a source population), its reintroduction already tested (Bulot et al., 2014a) can be a valuable tool in ecosystem restoration.

SUPPLEMENTARY MATERIAL

Figure S2.1: Location of the plain of La Crau in south-eastern France. Light grey area between St-Martin-de-Crau, Salon-de-Provence and Fos-sur-Mer is characterised by the same steppe vegetation and the same geological substratum. The star (A) represents the location of the studied site and the triangle (B) represents the quarry from which the soil for the restoration comes from.



Supplementary material – Chapitre 2

Table S2.1: Soil analyses for ant and ant-free patches at restored and reference sites, February 2018. Values are means \pm standard errors. F value and p value were obtained from GLMs with Gaussian distribution or gamma distribution (for MgO and N-NO₃) on ants' effects. Two values in the same row with a different letter are significantly different according to Tukey post-hoc tests. Significance thresholds were set at a risk of 5%.

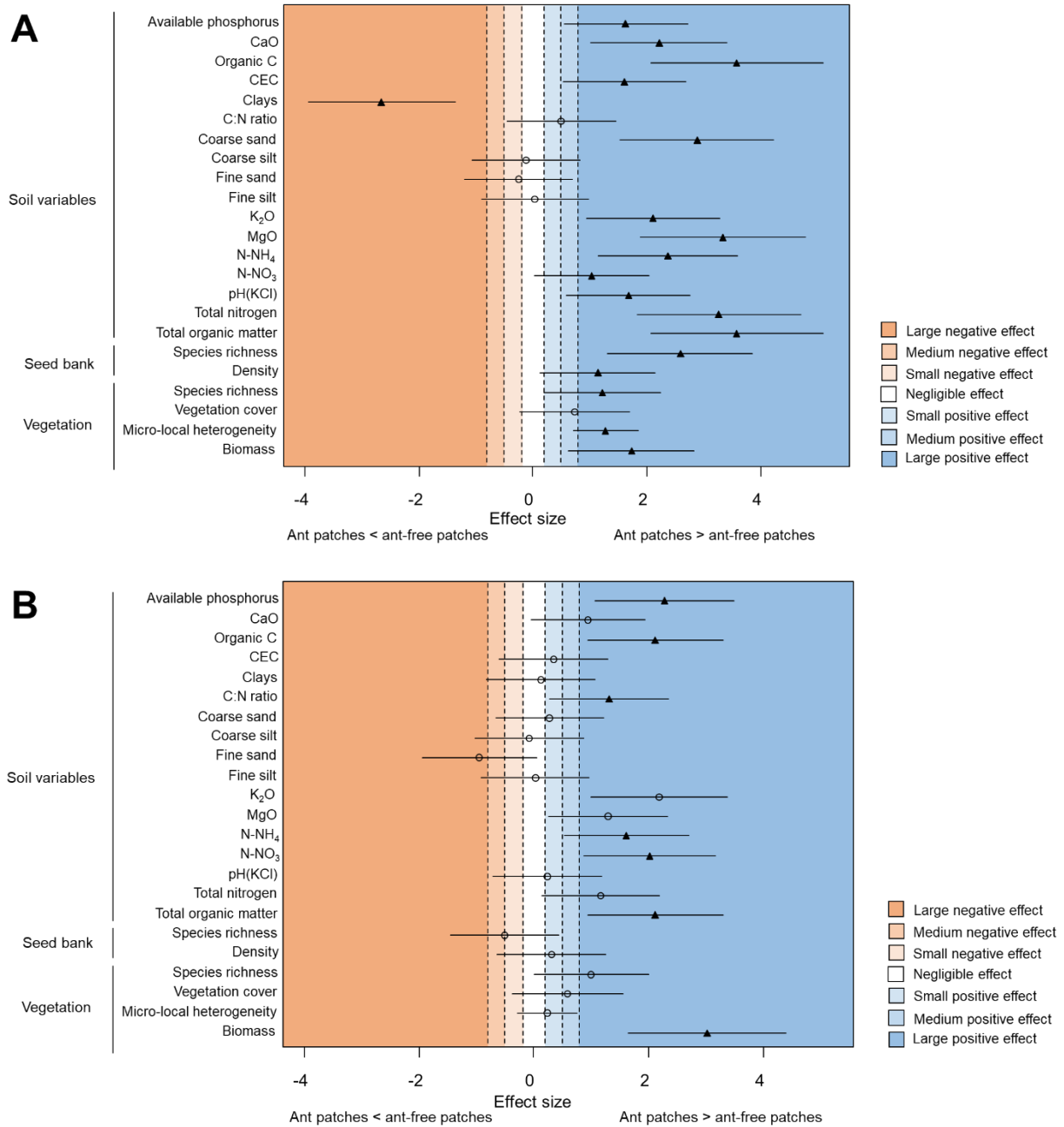
	F	P	Restored site		Reference steppe	
			Ant-free patches	Ant patches	Ant-free patches	Ant patches
Available phosphorus log(mg/Kg of soil)						
Patch type	122.10	< 0.001	1.9 \pm 0.1	4.5 \pm 0.3	0.9 \pm 0.2	2.5 \pm 0.1
Site	66.20	< 0.001	a	b	c	a
Patch type x Site	7.73	0.009				
CaO (mg/Kg of soil)						
Patch type	28.91	< 0.001	2418.2 \pm 82.1	3673.7 \pm 240	1972.3 \pm 58.4	2177.3 \pm 77.6
Site	51.13	< 0.001	a	b	a	a
Patch type x Site	14.96	< 0.001				
Organic C (g/Kg of soil)						
Patch type	86.66	< 0.001	15.1 \pm 0.7	25.4 \pm 1.1	15.5 \pm 0.4	19.1 \pm 0.7
Site	15.83	< 0.001	a	b	a	c
Patch type x Site	20.25	< 0.001				
CEC (meq/100g of soil)						
Patch type	10.40	0.003	10.6 \pm 0.2	11.7 \pm 0.3	10.3 \pm 0.2	10.5 \pm 0.1
Site	15.59	< 0.001	a	b	a	a
Patch type x Site	5.04	0.03				
Clays (g/Kg of soil)						
Patch type	21.32	< 0.001	224.2 \pm 5	177.2 \pm 6.1	189.2 \pm 4.2	190.8 \pm 4.1
Site	4.74	0.04	a	b	b	b
Patch type x Site	24.43	< 0.001				
C:N ratio						
Patch type	9.00	0.005	8.7 \pm 0.2	9 \pm 0.2	9.7 \pm 0.2	10.8 \pm 0.3
Site	35.33	< 0.001	a	ab	b	c
Patch type x Site	2.68	0.1				
Coarse sand (g/Kg of soil)						
Patch type	15.87	< 0.001	203.4 \pm 4.4	255.4 \pm 6.8	222.6 \pm 7.2	230.5 \pm 10.5
Site	0.14	0.7	a	b	a	ab
Patch type x Site	8.60	0.006				
Coarse silt (g/Kg of soil)						
Patch type	0.004	0.95	132 \pm 4.8	130.5 \pm 2.9	154.7 \pm 4.1	155.7 \pm 3.4
Site	38.08	< 0.001	a	a	b	b
Patch type x Site	0.10	0.75				
Fine sand (log(g/Kg of soil))						
Patch type	2.51	0.1	5.4 \pm 0.03	5.4 \pm 0.02	5.5 \pm 0.01	5.5 \pm 0.02
Site	13.62	< 0.001	a	a	b	ab
Patch type x Site	0.55	0.5				

Supplementary material – Chapitre 2

Fine silt (g/Kg of soil)						
Patch type	0.008	0.9	212.5 ± 9	213.2 ± 3.5	185.6 ± 3.1	185.9 ± 5
Site	22.97	< 0.001	a	a	b	b
Patch type x Site	0.001	0.97				
K₂O (mg/Kg of soil)						
Patch type	33.89	< 0.001	291 ± 27.8	489 ± 31.6	126.4 ± 8.3	182.3 ± 7.8
Site	116.77	< 0.001	a	b	c	c
Patch type x Site	10.62	0.002				
MgO (mg/Kg of soil)						
Patch type	62.23	< 0.001	170.4 ± 6.7	312.5 ± 17.9	191 ± 9.1	222.9 ± 6.3
Site	9.79	0.002	a	b	a	a
Patch type x Site	24.96	< 0.001				
N-NH₄ (log(mg/Kg of soil))						
Patch type	66.34	< 0.001	1.8 ± 0.1	3.0 ± 0.1	1.8 ± 0.1	2.4 ± 0.1
Site	11.37	0.002	a	b	a	c
Patch type x Site	8.25	0.007				
N-NO₃ (mg/Kg of soil)						
Patch type	6.29	0.02	11.1 ± 1.4	44.9 ± 14.7	6.5 ± 0.5	9.6 ± 0.5
Site	7.29	0.01	a	b	c	a
Patch type x Site	4.34	0.04				
pH(KCl)						
Patch type	9.48	0.004	6.4 ± 0.1	6.9 ± 0.1	6 ± 0.1	6.1 ± 0.1
Site	58.29	< 0.001	a	b	c	c
Patch type x Site	5.47	0.02				
Total nitrogen (mg/Kg of soil)						
Patch type	59.96	< 0.001	1738.4 ± 63.9	2829.1 ± 134.9	1600.6 ± 42.3	1765.9 ± 47.2
Site	54.82	< 0.001	a	b	a	a
Patch type x Site	32.55	< 0.001				
Total organic matter (%)						
Patch type	86.66	< 0.001	2.6 ± 0.1	4.4 ± 0.2	2.7 ± 0.1	3.3 ± 0.1
Site	15.83	< 0.001	a	b	a	c
Patch type x Site	20.25	< 0.001				

Supplementary material – Chapitre 2

Figure S2.2: Effect size (Cohen's d) and 95% confidence intervals for the effect of ants on soil, seed bank and vegetation in (A) the restored site and (B) the reference steppe. Black triangles represent the mean effect size for variables with a significant difference between ant and ant-free patches. Dots represent the mean effect size for variables with no significant difference between ant and ant-free patches. Effect size > 0 indicate greater values in ant patches than in ant-free patches; effect size < 0 indicate lower values in ant patches than in ant-free patches.



TRANSITION VERS LE CHAPITRE 3

Bien que les fourmis aient des impacts multi-compartiments dans leur écosystème (Chapitre 1) permettant d'accélérer le processus de restauration des propriétés physico-chimiques du sol et des communautés végétales (Chapitre 2), elles n'ont, jusqu'alors, que très peu été utilisées en ingénierie écologique. Cette sous-utilisation pourrait pour partie être liée à l'absence de méthodes concrètes d'application mais aussi à un manque de connaissances fondamentales des gestionnaires d'espaces sur les fourmis. Une méthodologie d'application basée sur les traits pourrait faciliter leur utilisation.

Un trait est défini comme étant « *toute caractéristique morphologique, physiologique ou phénologique, mesurable à l'échelle de l'individu, de la cellule à l'organisme, sans référence à l'environnement ou à tout autre niveau d'organisation* » (Violle et al. 2007). Les traits peuvent ainsi être utilisés pour quantifier, décrire, comparer la manière dont les espèces répondent et agissent sur leur environnement (Lavorel & Garnier 2002). On distingue les traits d'effets qui représentent la contribution des espèces à des processus écosystémiques des traits de réponses qui correspondent à la réponse ou l'adaptation des espèces à des contraintes environnementales (e.g. pâturage, conditions climatiques) et dont la valeur varie en fonction du changement des conditions environnementales (Lavorel et al. 1997; Lavorel & Garnier 2002). Ces deux catégories ne sont pas exclusives : un même trait peut à la fois être considéré comme trait de réponse et trait d'effet.

Les traits sont largement utilisés en écologie et notamment pour prédire quantitativement la réponse des espèces aux changements environnementaux, l'assemblage des communautés qui en résultent et l'impact sur les propriétés de l'écosystème. La plupart des traits pouvant être mesurés sur une grande majorité des espèces d'un même groupe, les analyses utilisant les traits plutôt que l'identité taxonomique sont plus facilement comparables entre communautés et à travers différentes échelles spatiales (Ackerly 2003). Cette démarche peut être plus facilement appliquée lors de projets de restauration écologique (Reich et al. 1997; Carlucci et al. 2020).

Lors de projets de restauration passive, les traits peuvent être des outils pour mesurer la réponse des communautés à la suppression des perturbations, et observer la trajectoire de la restauration (Pywell et al. 2003; Fournier et al. 2012; Díaz-García et al. 2017; Giannini et al.

2017). Lors de projets de restauration active, la réintroduction ou le renforcement de populations, notamment d'espèces ingénieuses, peut être un moyen d'action pour que l'écosystème retrouve toute ses fonctionnalités (Byers et al. 2006; Gann et al. 2019). Afin de choisir l'espèce (ou les espèces) à réintroduire ou renforcer, capable d'effectuer les fonctions cibles déterminées par les objectifs de restauration, une approche taxonomique ainsi qu'une connaissance très large et approfondie du rôle de chaque espèce au sein de l'écosystème sont nécessaires. Les traits d'effet peuvent alors être utilisés pour faciliter le choix des espèces cibles. Ils seront sélectionnés en fonction des objectifs de restauration et donc des fonctions à restaurer. Le choix d'une ou plusieurs espèces est alors restreint par le nombre de fonctions sur lesquelles elles peuvent agir via leurs traits d'effet.

Afin de faciliter l'emploi de fourmis lors de projets de restauration écologiques (passive ou active) par les gestionnaires (myrmécologues ou non), nous proposons dans le troisième chapitre, une méthode de sélection des fourmis basée sur les traits permettant d'identifier les espèces les plus appropriées en fonction des objectifs de restauration (Figure T3.1).

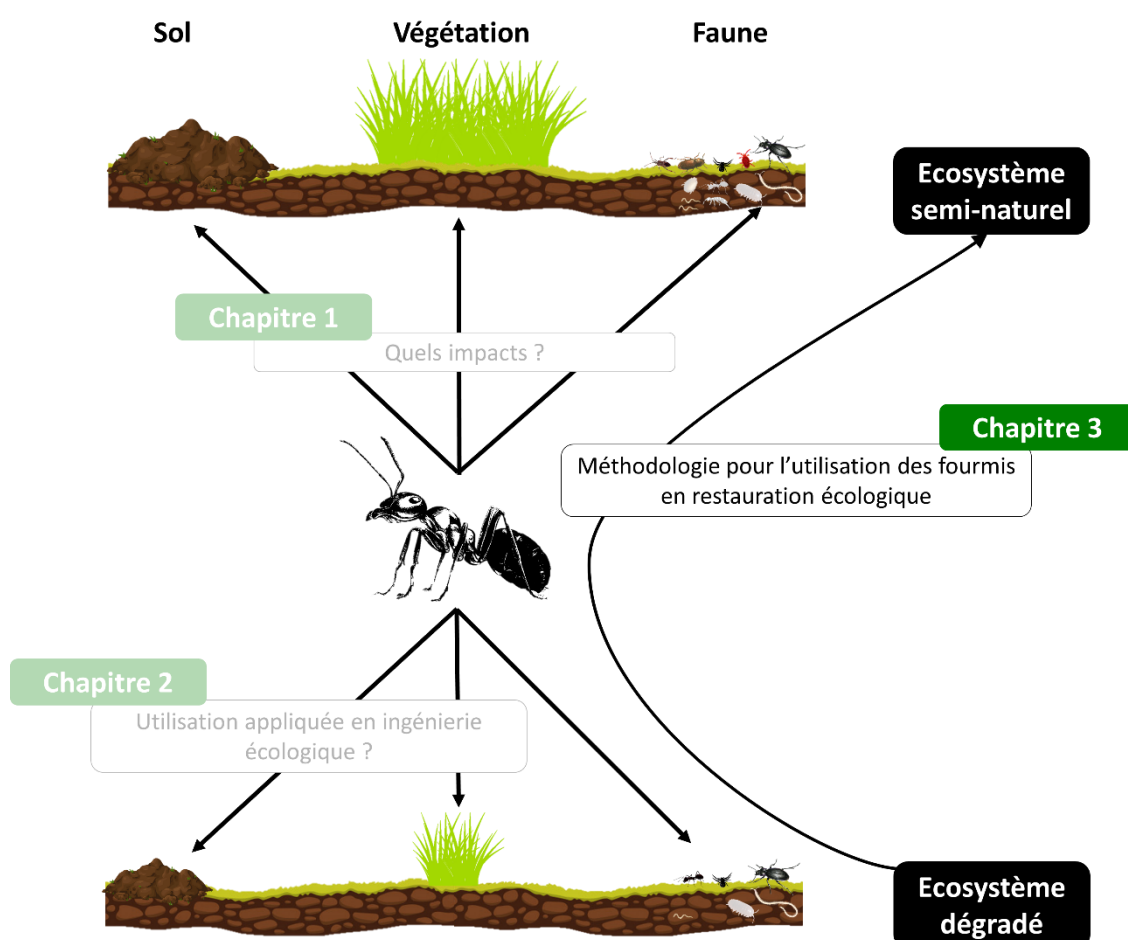


Figure T3.1 : Localisation du chapitre 3 dans l'organisation générale de la thèse.

CHAPITRE 3

A trait-based approach to promote ants in restoration ecology

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Abstract

The current global changes require change our practices for more environment friendly methods. This is also needed in nature conservation including in ecological restoration. Indeed, current methods essentially use civil engineering principles, which are strongly consuming in non-renewable energy. The sustainability of these techniques then raises questions. This integration of nature conversation aspect in ecological restoration operations can thus be done through the direct use of species to restore degraded ecosystems. In such projects, ants, because of their central role on ecosystem functioning and their presence on almost all continents, have promising potential. Nevertheless, most stakeholders are not myrmecologists and may therefore face difficulties in identifying good candidate species. In order to overcome this issue and facilitate the ant selection phase, we propose a trait based methodology. We first sum up the potential of ants in restoration ecology, then we listed ant effect traits or life-history traits known to affect abiotic (physical and chemical soil properties) and biotic compartments (plant and fauna communities) and/or relevant to assess restoration success. Then, we propose a trait-based methodology based on the previously identified traits. This methodology could be adapted for other organisms, particularly biota, whose functions on the environment are sometimes well known but which are not, however, the target of applications in ecological restoration.

Keywords: ants, ecological engineering, effect traits, life-history traits, active restoration, monitoring.

3.1 Introduction

Among the various methods for assessing biodiversity, approaches based on traits developed since 30 years (Vandewalle et al. 2010) reshape how ecologists measure diversity, assess coexistences between species assess functional roles and for example restore habitats (Fukami et al. 2005; Cadotte et al. 2011). Functional traits are defined as any measurable morphological, physiological, behavioural or phenological feature from the cell to the whole-organism level of an individual (Violle et al. 2007; Pey et al. 2014). They can reflect environmental tolerance, i.e. response traits (Statzner et al. 2001; Violle et al. 2007), and/or affect ecosystem processes, i.e. effect traits (Lavorel & Garnier 2002; Violle et al. 2007). From an ecological point of view, a species gathers individuals with a set of phenotypic, physiologic and behavioural traits that determine when and where they can exist and how they interact with individuals from their own species, from other species (McGill et al. 2006), and with their habitat. By measuring and understanding the diversity of species traits, conservation and restoration decisions could be largely improved (Cadotte et al. 2011).

Ecosystem restoration plays a vital role in conserving biodiversity worldwide (Aronson & Alexander 2013). Restoration aims to increase biodiversity and ecosystem services by returning structure and function of human degraded ecosystems (Benayas et al. 2009). The removal of the disturbance – event leading to an alteration or loss of biomass (Grime 1977) and to the total or partial destruction of populations, communities or ecosystems (Pickett & White 2013) – that degraded the ecosystem is the first step of a restoration project. Each active restoration project – when ecosystem is managed by a range of human interventions to accelerate/influence the successional trajectory of recovery – is divided into three phases: the pre-operational phase during which it is essential to accurately define the restoration objectives and the ecological functions to restore; the operational phase which consists in setting up the project; and finally the post-operational phase consists in monitoring the success of the restoration (Choi 2004; Clewell & Aronson 2013). The operational phase often involves the use of civil engineering principles and techniques based on heavy interventions (public works machinery), with high consumption of non-renewable resources and significant emissions of pollutants (Dutoit 2014). In order to avoid the environmental consequences that civil engineering has, “ecological engineering”, one component or complementary approach of restoration ecology (Aronson et al. 2016), relies in part on the direct use of species to

restore degraded ecosystems (Mitsch & Jørgensen 2003). This nature-based solution – inspired by, supported by or copied from nature using internal mechanisms for changing the ecosystem in the expected direction, making them stable and useful for ecosystem services (Nesshöver et al. 2017) – promotes species that directly or indirectly modify the physics and chemistry of their habitat and influencing other organisms (Jones et al. 1997).

A key issue in ecological engineering is to identify the appropriate species to introduce and/or promote, preferably a native species (Sotka & Byers 2019), that will deliver the ecological functions targeted. In that respect, a methodology based on species traits may help stakeholders upstream of the operational phase. It will guide managers to the right set of species based on the desired functions to restore. It allows a first screening of species, simplifying the selection process for stakeholders that do not always have a sufficient knowledge on the ecology and biology of the species regional pool. Phytoremediation – the use of green or higher terrestrial plants for treating chemically or radioactively polluted soils – is a good example of such an approach in which the plants are selected for some of their traits such as growth rate, the level of tolerance, accumulation and translocation of metals (Sarma 2011). A trait-based approach can be relevant in the post-operational phase. A monitoring has to be set up to evaluate the success of the operation. In that regards, the use of traits can also help stakeholders by facilitating the identification of relevant species. This approach allows to indirectly estimate the recovery of ecosystem multifunctionality even if the species composition has not been entirely restored (Engst et al. 2016).

Whereas plant species are commonly used to restore degraded ecosystems (e.g. Sarma 2011), such approach remains underexplored for invertebrates (Snyder & Hendrix 2008; Jouquet et al. 2014). Because of their well-known key role in soil, earthworms are the soil organisms most frequently used in ecological engineering (Jouquet et al. 2014), and have been successfully used to restore soils (e.g. Butt 2008), as were termites too sometimes (Jouquet et al. 2014). However, despite promising potential (De Almeida, Blight, et al. 2020), ants are still underexploited in ecological engineering. Indeed, their social life and related traits, most of ants' form long-live sedentary colonies, as well as their well-known ecological roles make them relevant in ecological engineering projects. They occur throughout all continents except Antarctica and are among the most diverse and abundant organisms on earth (Hölldobler & Wilson 1990). Ants have a strong impact on the different ecosystems they occupy, such as temperate grasslands (Wills & Landis 2018), tropical forests (Lu et al. 2019; Swanson et al.

2019) and semi-arid pine forests (Çakır 2019). In all these environments, ants play key ecological roles as soil engineers, predators, nutrient cyclers and/or regulators of plant growth and reproduction (Zelikova et al. 2011; Del Toro et al. 2012; De Almeida, Mesléard, et al. 2020). Regarding these effects ants have great potential for applications in active restoration projects, so far rarely explored.

We then aim to highlight ants' potential for restoration ecology and to propose a solid methodology based on ants' traits to facilitate their application in restoration projects. Thus, we first evaluate the potential of ants in restoration ecology; we then identified a list of effect traits known to affect the following three ecosystem compartments: the soil (physics and chemistry), the plant community, and the soil fauna, that can be relevant in the operational phase of restoration project as well as life-history traits that can be relevant in the post-operational phase, i.e. monitoring for restoration success. Finally, we proposed an applicable methodology for stakeholders based on the previously identified traits to facilitate the use of ants in restoration projects.

3.2 Ants in restoration ecology

Social insects can largely impact other species and modify the structure and ecosystem functioning. Its capacity may be explained by a combination of unique characteristics of these organisms (Elizalde et al. 2020) that may be relevant in restoration ecology. They contribute, relative to vertebrates and other arthropods, to a high proportion in terms of numbers of individuals and biomass within terrestrial ecosystems (Wilson 1990). Because of their colonial lifestyle, these insects can influence their ecosystem at both individual and colony level (Keller 1995; Elizalde et al. 2020). Among them, ants have a widespread distribution, high biomass and numerical abundance in many ecosystems and long lifespan up to 28 years old (Shilovsky et al. 2018). These colonial organisms are relatively stationary and little subject to seasonality in their occurrence (Andersen & Majer 2004). Besides their construction and food storage capacity, they can also be involved in mutualistic relationships (Elizalde et al. 2020). These diverse characteristics, useful for the success of restoration programs, make ants particularly interesting organisms in this context.

3.2.1 Ants' ecological functions

Ants have a central role on ecosystem functioning (Del Toro et al. 2012; Elizalde et al. 2020). Their engineering activity affects not only each ecosystem component separately, but

also their relationships (De Almeida, Mesléard, et al. 2020). They have the ability to monopolize space and food resources, and therefore influence other species in the areas they occupy (Andersen, 1992). By their structural engineering activities, particularly during nest construction and maintenance, ants can move large amount of underground soil to superficial layers (Cammeraat & Risch 2008). They can also concentrate organic matter and produce large quantities of organic waste that are deposited inside the nest or on the soil surface (Frouz & Jilková 2008). By these activities, ants can change soil physico-chemical properties and water infiltration that indirectly affect the surrounding vegetation (e.g. plant biomass, diversity, abundance) (Dostál et al. 2005).

The magnitude and direction (positive, negative or neutral) of the effects on the soil vary between ant species (Frouz & Jilková 2008) and environments (Farji-Brener & Werenkraut 2017). Most studies showed an increase in organic matter in ant nests (Cammeraat et al. 2002; Azcárate & Peco 2007). Similarly, ant activity generally increases soil nitrogen, phosphorus and potassium contents. However, no effect (Leal et al. 2007) or even a decrease in nitrogen or potassium contents were also recorded (Eldridge & Myers 1998; Dostál et al. 2005). As a consequence, ants have contrasting effects on plants. They can positively or negatively modify plant growth (Saha et al. 2012; De Almeida, Mesléard, et al. 2020) or diversity (Schütz et al. 2008; Saha et al. 2012), depending on their nutrient requirements.

Beside these indirect effects on plants, ants through seed dispersal can profoundly affect plant population and community structure. Myrmecochory – seed dispersal mediated by the elaiosome, a lipid-rich seed appendage that mainly attracts non-granivorous ants and provides rewards for seed dispersal – involves more than ten thousand plant species across many terrestrial ecosystems (Lengyel et al. 2009). Christian (2001) showed that the preservation of seed-dispersal mutualistic interactions is essential for maintaining plant natural communities. It may shape the initial spatial distribution of plants within populations and define the context for future ecological, demographic and genetic interactions among emerging seedlings (Kalisz et al. 1999). Granivorous ants can also disperse seeds, but the positive effects on vegetation (plant species-richness and diversity) are balanced by the predation costs on plant population (Arnan et al. 2012). Although several studies have found a higher amount of seed (Schütz et al. 2008; Bulot et al. 2016; De Almeida, Blight, et al. 2020) and seed germination in ant nests than in surrounding areas, the relative effect of seed

dispersal provided by ants depends on ant community composition (Prior et al. 2015), but also on seed availability (Servigne & Detrain, 2008; Arnan et al. 2012). Ants could also act as biotic defences, protecting plants against herbivores and parasites, thus modifying plant population and community dynamics. In return, plants offer benefits such as shelter and food rewards (Rosumek et al. 2009).

Ants have also an impact on other invertebrates. They can negatively affect populations through predation and competition, whereas some species take advantage of their presence by feeding on them. Sanders & Platner (2007) thus recorded three times the density of linyphiid spiders in ant-free patches, while *Formica* ant abundance was higher in spider-free patches. Ants can also indirectly affect invertebrates by modifying the abiotic compartment and plant communities. This impact is mostly positive for macro-, meso- and microfauna (Boulton & Amberman, 2006; Çakır, 2019; Sanders & Van Veen, 2011), as well as bacterial and fungal communities (Boulton et al. 2003; Boulton & Amberman 2006). The higher abundance of bacteria and fungi may indirectly increase bacterial and fungal predators such as nematodes and collembolans (Boulton et al. 2003; Boulton & Amberman 2006). An increase in plant heterogeneity and biomass in ant patches can thus be responsible for increased abundance of detritivores such as Collembola and some plant feeder such as Coleoptera, and indirectly of their predators (e.g. Araneae and predator Coleoptera) (De Almeida, Mesléard, et al. 2020).

3.2.2 Ants in ecological engineering projects

Because of their multi-compartments impacts, ants have a great potential in restoration projects (Lu et al. 2019; Nicolai 2019; De Almeida, Mesléard, et al. 2020). By their bioturbation and organic matter concentration activities, ants can help restore degraded soils at the early stage of the succession whereas as seed disperser they may be key when it comes to help the recolonization and dispersion of plants. In passive restoration - when disturbances are eliminated and recolonisation occurs naturally – only few studies showed that ants can accelerate the restoration (e.g. Nicolai et al. 2008). Nicolai (2019) has demonstrated that two years after a wildfire, *Pogonomyrmex rugosus* (Emery, 1895) nest edges have improved resources that yielded faster recovery of grass inflorescences than on burned controls. As areas of high grass reproduction during recovery, ant patches may act as source for seed dispersal into surrounding habitat (Nicolai 2019). Besides, it was also found in a tropical forest

that the enrichment of microbial carbon in nest soils increased with restoration age (until 53 years old) (Lu et al. 2019). The higher soil enrichment by ants, including in organic matter, available nitrogen, ammonium, nitrate, at the early stage might promote the spontaneous forest restorations while at the older restoration stage, by strongly stimulating microbial growth and opening up space, ants improve plant development (Lu et al. 2019).

Although ants impacts are mostly small-scale and limited to the nest, they can play a significant role in maintaining plant species richness and spatial heterogeneity at site scale (Platt 1975). For example, they contribute to restore the level of native ecosystem plant diversity in degraded grasslands (Lane & BassiriRad 2005). Their nests may act as source for the recolonisation of other organisms throughout the degraded ecosystem. Moreover, their impacts are not only detected during nest occupation but can be recorded up to more than 20 years after colony disappearance (Kristiansen & Amelung 2001; Lane & BassiriRad 2005), thus increasing the spatial and temporal impact of ants in the environment.

Despite the advantages of their presence already demonstrated in passive restoration, the only example in the literature of their use in active restoration was conducted in the Plain of la Crau (South of France) with *Messor barbarus* (Linnaeus, 1767) (De Almeida, Blight, et al. 2020). In a restored grassland, seven years after mated-queens' introduction, *M. barbarus* has improved soil fertility, driven the soil seed bank towards the reference ecosystem and significantly increased plant biomass, species richness and micro-local heterogeneity. By directly and indirectly facilitating plant establishment, *M. barbarus* accelerated the grassland restoration compared to controls without *M. barbarus* nests, trails or refuse piles.

3.2.3 Environmental monitoring

Beside the actual operation phase, environmental monitoring is also crucial in restoration ecology. In a recent literature survey, Casimiro, Sansevero, & Queiroz (2019) found 470 articles in which ants were associated to the study of environmental changes. The use of ants as bioindicators has emerged in the mid-1970s in Australia to assess the restoration of abandoned quarries (Andersen & Majer 2004). Since then it has spread to other countries, mainly in Brazil and Europe (Casimiro et al. 2019). Whereas abundance and species richness are the most commonly used ecological metrics for assessing success of ants' recovery, it seems that the recovery of ant functional groups takes place more quickly than the species richness.

A global model of ant community dynamics based on nine functional groups has been developed for Australian communities in relation to environmental stressors (factors limiting ant productivity) and disturbance (factors eliminating ant biomass) (Andersen 1995; Andersen & Majer 2004). Ant community functional composition provide information on the state of ecosystem degradation. For example, generalized Myrmicinae have wider environmental tolerance than dominant Dolichoderinae or highly specialized functional groups such as cryptic species and specialist predators. Disturbances have also important indirect effects on competitive hierarchies through changes in dominant species abundance.

However, the nature of these functional groups developed for Australian communities represent some constraints to their use in restoration. Their qualitative character makes them imprecise to apply them to other ant communities. Andersen's functional groups mix life history traits, taxonomy and competitive abilities. An approach based on quantitative traits that avoids taxonomy would allow a more precise evaluation of ant community response to environmental change and would facilitate its application irrespective of the biogeographic region.

3.3 Identification of ants' traits relevant for restoration

The type of restoration and its objectives depend on the state of the degraded ecosystem (Figure 3.1). When the target ecosystem has been profoundly impacted, the priority is to restore the soil physical and chemical properties by identifying the ecological functions of interest (e.g. soil structure, water infiltration, soil fertility). When the abiotic compartment has been less degraded, the priority is to restore the biotic component (vegetation, fauna, bacteria and fungi), by rehabilitating biotic interactions (e.g. seed dispersal, predation, competition, facilitation, symbiosis). When the removal of disturbances is sufficient or after the restoration of the abiotic and/or biotic components monitoring of community and population changes is necessary to ensure that all target functions have been restored. In each case, ants with particular traits appear relevant to fulfil the restoration objectives (Figure 3.1).

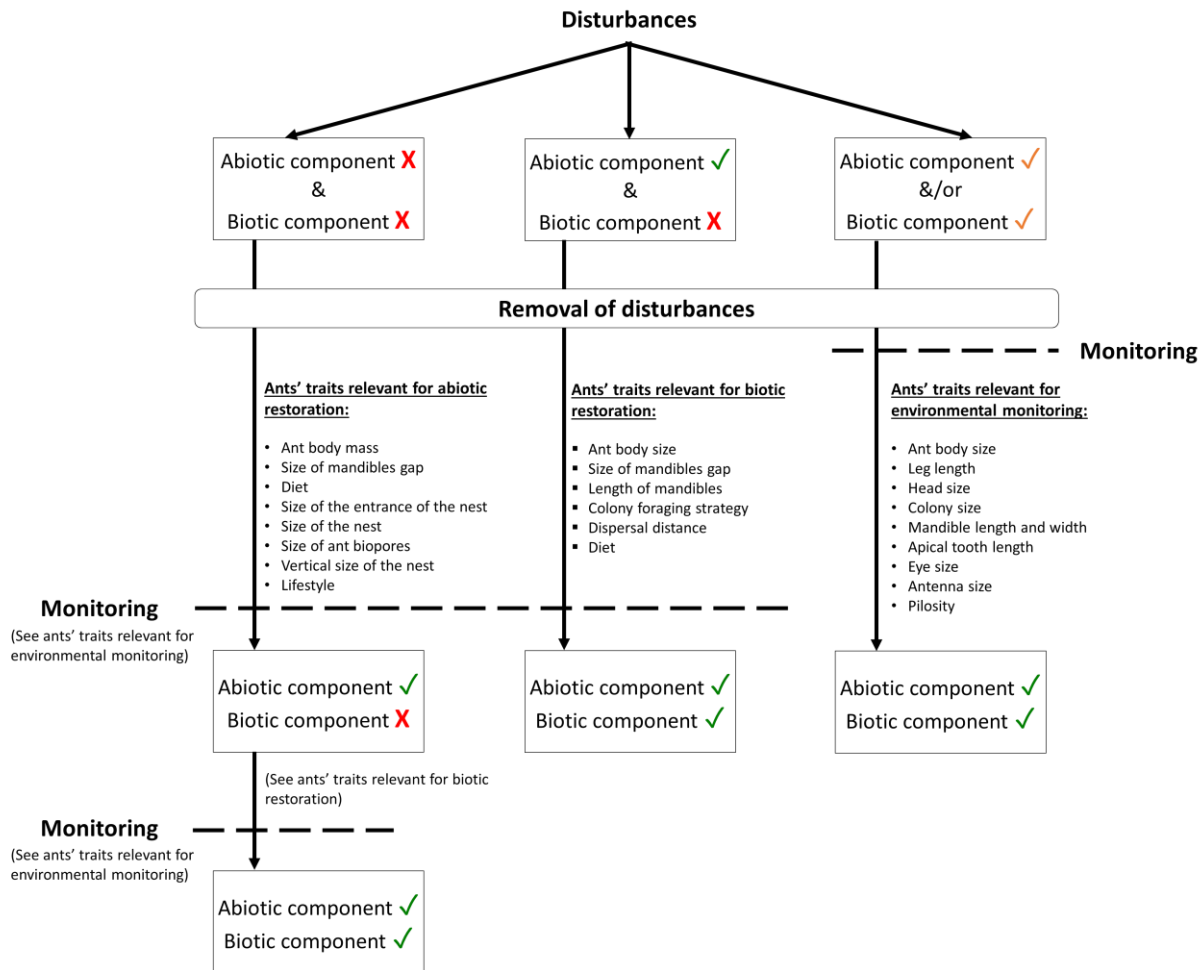


Figure 3.1: Ant individual and colony traits relevant in restoration projects. When the target ecosystem is heavily degraded (abiotic and biotic component), after the removal of disturbances the soil functions must be restored first, followed by the restoration of the biotic component functions. When the ecosystem is less degraded, only the biotic component functions has to be restored after disturbances removal. After the restoration of each component or when the ecosystem is slightly degraded, monitoring of community and population changes is necessary to ensure that all target functions have been restored or are in the process of restoration. Deeply degraded components are represented by “X”, slightly degraded components by “✓”, and suitable components by “✓”.

3.3.1 Relevant ant traits for ecological engineering

Abiotic component

Ants' capacity to restructure soil depends on their physical capacity to transport material (Dostál et al. 2005). During nest construction and maintenance, worker ants use their mandibles to transport mineral particles and soil pellets outside the nest. The size of their mandible gap, which is positively correlated with the ant body size (Oliveras et al. 2005), influences soil texture-contrast (Oliveras et al. 2005) (Figure 3.1; Table 3.1). Indeed it determines the size of soil particles excavated, the larger they are, the more they are able to transport large particles (Dostál et al. 2005; Martín-Perea et al. 2019). The nest vertical size

may also influence the size of soil particles excavated. The excavation of soil material by ants increases soil (macro)porosity, which in turn alters the ratio between soil solid and air phase and leads to lower bulk densities (Cammeraat & Risch 2008). Moreover, the size of ant nest biopores has important consequences on water infiltration and on soil erosion caused by water flowing overland (Lobry de Bruyn & Conacher 1994). A greater soil pore volume in the nest mound increases water infiltration to greater depths (MacMahon et al. 2000). Water infiltration and retention are also related to nest architecture particularly the size of the nest and the size of the nest entrance (MacMahon et al. 2000) (Figure 3.1; Table 3.1), factors that varies among ant species (Cammeraat & Risch 2008).

Ants also contribute to soil's chemical changes. Their diet determines the magnitude of soil enrichment (Figure 3.1). The accumulation of food and waste generated from plant sources, for example in the ant refuse piles, may improve soil fertility more than that coming from animal sources (Farji-Brener & Werenkraut 2017). Thus, leaf-cutting and granivorous ants contribute more to soil fertility than omnivorous species (Farji-Brener & Werenkraut 2017).

Biotic component

Ants involved in seed dispersal directly influence plants community composition and structure. The spatial effects of seed dispersal may depend on the colony foraging strategy (MacMahon et al. 2000) with prospection distance increases from individual to group foraging species (Lanan 2014). The ant body size also predict the seeds dispersal distance (Christian 2001; Ness et al. 2004), which influences plant establishment, seed germination and seedling growth (Christian 2001; Fernandes et al. 2020). Larger species disperse seeds further than do smaller ants (Horvitz & Schemske 1986; Gómez & Espadaler 1998; Ness et al. 2004). The ant physical capacity to transport material has also consequences on the vegetation component. Beyond the attractiveness of seeds, the success of their transport may be linked to the ant mandible gap and thus to the ants' physical ability to carry seeds of a specific morphology or size (Oliveras et al. 2005) (Figure 3.1; Table 3.1). The larger the ants' mandible gap and body, the larger seeds can be transported. Finally, in the particular case of leaf-cutting ants, the ant body size and leg size was found to determine the leaf-transport rate, with relative long leg being advantageous (Burd 2000).

Ants' diet clearly determines their impact on the biotic compartment, with more noticeable effects on vegetation, such as granivorous species, whereas predatory species will

have a stronger impact on fauna (Vandermeer et al. 2010; Offenberg 2015). As a consequence, granivorous ants are expected to have a greater effect at the earlier stages of the restoration (colonization), while predators will have greater impacts at the more advanced stages (population control). For predatory or generalists species, the worker body and mandibles gap size determine the size of prey taken and hunted (Oliveras et al. 2005). Also, length of mandibles is related to diet and longer mandibles leads to more predation events (Parr et al. 2017) (Figure 3.1; Table 3.1).

Changes in soil physico-chemical properties and vegetation influence positively and indirectly above- and below-ground fauna abundance and occurrence and change their community structure (Sanders et al. 2008; Schuch et al. 2008; Sanders & Van Veen 2011). In ant nests, the high rates of organic material accumulation increase food resources for detritivores and indirectly increase microhabitats for soil fauna through vegetation modification. Nests are also a refuge for myrmecophilous species such as lycaenid butterflies (e.g. *Maculinea telius* and *M. nausithosus*) (Wynhoff et al. 2008) or some beetles (e.g. *Atheta talpa* and *Monotoma coniciocollis*) (Päivinen et al. 2004). Other species are predators of the brood or adults such as spiders from the genus *Zodariinae* (e.g. *Zodarion elegans*) (Traxler 2016; De Almeida, Mesléard, et al. 2020), some beetles (e.g. *Zyras humeralis*) (Päivinen et al. 2004) or hoverflies of the subfamily *Microdontinae* (Reemer 2013).

3.3.2 Relevant ant traits for environmental monitoring

A bioindicator species must have several characteristics, the most notable being ease of measurement, sensitivity to environmental disturbances, and predictable responses to environmental disturbances (Niemi & McDonald 2004; Ribas et al. 2012). Ants fulfil these three criteria; their traits are relatively easy to measure, they are also sensitive to environmental disturbances and some traits can be used to monitor a response to disturbances.

Ant traits are closely related to environmental complexity (Nooten et al. 2019). Their body size and ant leg length tends to be correlated to habitat complexity. They are larger with greater leg length and larger heads in open spaces than in complex habitats (Kaspari & Weiser 1999; Sarty et al. 2006; Weiser & Kaspari 2006; Gibb & Parr 2010; Yates et al. 2014).

Mandibular and eye traits also vary in relation to habitat-complexity; longer apical teeth and smaller mandible sizes in relation to their overall length were recorded in open

habitats (Yates et al. 2014; Gibb et al. 2015). Salas-Lopez et al. (2018) found that mandible length and width, apical tooth length and eye size increased with land-use intensification. Besides, in areas disturbed by fires, average colony size, worker size, worker polymorphism and the ratio between queen and worker size were higher than in unburned areas (Arnan et al. 2013). Ants with longer antenna scapes, which are important for perceiving the environment and locating resources or prey items (Weiser & Kaspari 2006; Silva & Brandão 2010), prevailed in habitats with dense herb/grass layer (Nooten et al. 2019). Besides, ants tend to be smaller in warm and wet habitats (Gibb et al. 2018).

Some traits can provide information on the availability of resources. The body size of an organism determines the quantity of resources consumed. Therefore, monitoring ant body size in a community might provide information on the quantity of resources available in a degraded or restored habitat. Moreover, dark colours, large body sizes and physiological tolerance to low-temperatures are indicative of low-temperature traits. Similarly, hair can increase tolerance to dehydration (Wittlinger et al. 2007). Monitoring these traits in a degraded ecosystem provide information on the evolution of microclimate conditions in degraded and restored habitats. Ant body size also decrease with an increase in soil compaction (Schmidt et al. 2017).

3.3.3 Traits related to population introduction, establishment and dispersion

In active restoration, in order to facilitate the introduction and/or promotion of a species, its establishment and spread within an environment, some life history traits must be considered. First of all, it is essential to facilitate the operational phase by choosing a species that is easy to collect and introduce. To do so, the species must be common near the introduction site. The number of queens per colony and the dispersal method are also two important factors. Queens from colonies dispersing by budding may be more difficult to collect (digging out the pre-existing colony) and the excavation may degrade the donor site. For species that reproduce during nuptial flight, collecting mated queens should be easier and less impact on the donor site. As in any introduction process, a special attention must be paid to species involved in hybridogenesis. In this reproductive system, known as "social hybridisation" and varying among species (Eyer et al. 2013; Romiguier et al. 2017; Lavanchy & Schwandertanja 2019), workers are the result of the hybridisation of parents of different genetic origin. It is thus important that both lineages are still present on the site (e.g. collection

and introduction of both lineages) to ensure that the population can grow successfully in the future.

The operational phase will be a success only if the population does not collapse in the following years. Firstly, it is necessary that the environmental conditions of the degraded site meet the species requirements such as thermal and moisture habitat preferences. Territoriality must be also considered in order to ensure that the candidate species will persist in coexistence with species already present. The location of the nest (hypogaeic, under stones, dead wood, litter, arboreal) has also to be considered in order to determine the introduction zones and the type of shelters that could be set up to facilitate their establishment and dispersal. Finally, species reproducing by mating flight, although they take more time to build a stable and mature colony than species reproducing by budding, have a higher dispersal distance capacity.

3.4 Applicable methodology for stakeholders

For restoration projects requiring the use of ants, stakeholders must have a very good knowledge of the biology and ecology of the local ant community to be able to select the most appropriate species. A screening of species based on their effect and life history traits allows stakeholders to overcome this limit. We propose a six steps methodology to facilitate the selection and the use of ants in restoration programs (Figure 3.2).

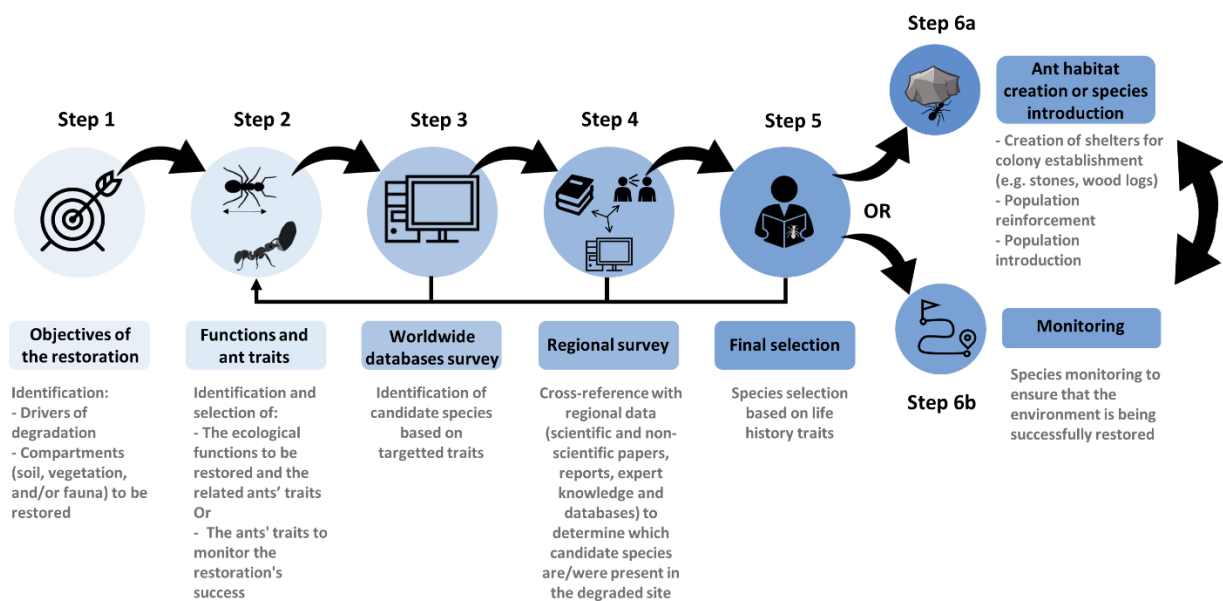


Figure 3.2: Methodology applicable to stakeholders in the use of ants in restoration projects

When an environment has been degraded, stakeholders should first identify the causes of the degradation, and the consequences on the ecosystem components functioning (soil, vegetation and/or fauna) (step 1; Figure 3.2). Once this assessment has been realised, stakeholders select a number of ecological functions to be restored (e.g. soil structure or seed dispersal) and the associated ants' traits (e.g. size of mandibles gap, diet, ant body and colony size) or ants' traits relevant to monitor the restoration's success (step 2; Figure 3.2). Based on these traits, a first screening in ant worldwide databases provides a list of candidate species having these traits and able to restore the targeted functions (step 3; Figure 3.2). Several ant databases have been developed over the past few years including GABI project which focuses on distribution records (Guénard et al. 2017) and GlobalAnts which focuses on ant traits with associated georeferenced assemblage-level data (Parr et al. 2017).

Because priority must be given to local species, the results of the ant worldwide databases interrogation are then cross-referenced with regional data (including scientific papers, grey literature, expert or empirical knowledge and databases) to identify which candidate species are/were naturally occurring in the geographic region concerned (step 4; Figure 3.2). The final ant species selection is based on traits related to population establishment and dispersion and/or relevant for environmental monitoring (step 5; Figure 3.2). This step 5 ensures the success of the operational phase (step 6; Figure 3.2), which consists on : improving the habitat of the selected species and/or introducing it (step 6a); or monitoring species (step 6b). In some cases, providing shelters (e.g. stones, wood logs) may be sufficient to reinforce populations by decreasing nesting site competition.

If no candidate species have been identified in steps 3, 4 or 5 based on the list of effect traits, one should return to step 2. This can happen when: the database presents gap in available traits (step 3); no species in the regional pool fills the specifications (e.g. too many traits have been implemented in the survey) (step 4); the candidate species introduction appears to be impossible at step 5 (e.g. not enough source populations). When a query with less traits implemented in the survey is not sufficient to obtain a candidate species in step 3, 4 or 5, it is necessary to split the functions to be restored in several queries to obtain a set of complementary species. The best species would be those that provide many of the needed services. However, the chances to identify a single ant species able to restore all the expected functions is very low. The functional complementarity of species between present and

reintroduced, or between reintroduced species as to be considered to maximise the success of the restoration.

3.5 Application and limits of the methodology

3.5.1 Application

The methodology we proposed is addressed to stakeholders with no expertise on the regional pool of ants. When stakeholders have expertise in the local myrmecofauna, our methodology has to be used proceeding directly from step 2 to step 4. This was the approach chosen in 2010 to restore a grassland destroyed by an oil leak (5.5ha) in the Plain of la Crau (France) (Bulot, Dutoit, et al. 2014). The polluted soil was excavated and “identical” soil was transferred (see Bulot et al. 2017 for more information), but a fine crust at the soil surface limited plant’s establishment. To restore soil physico-chemical properties (decompaction and increase of soil nutrients) and vegetation compartment (seeds redistribution), the granivorous ant *M. barbarus* was reintroduced. This species gathered all the required traits to restore the functions of interest. First, among the most abundant native species those that disperse seeds and nest in the soil were identified from regional literature (Cerdan 1989). At this step, two species were retained: *M. barbarus* and *Pheidole pallidula* (Nylander, 1849). They both disperse through a nuptial flight and have high densities of workers. Finally, *M. barbarus* was chosen based on its higher mandible gap and larger body size (Bulot, Dutoit, et al. 2014; De Almeida, Blight, et al. 2020). As previously mentioned, granivorous ants are known to increase soil nutrients content, workers’ mandible gap size is directly related to the capacity of the species to displace large soil particles and seeds, effect on soil is more pronounced in permanent nest, and dispersion through nuptial flights allows a more rapid colonization of the 5.5 ha of degraded grassland.

3.5.2 Limits

The proposed methodology implies database searches based on traits to identify species, whereas only the reverse is currently possible on ant databases. To this purpose, developing a new database does not seem necessary, but change in the search requests in the already existing databases is requisite. GlobalAnts appears the most appropriate database in our context, but it needs to be improved to fit the proposed methodology. This database, although more complete than the others, is not yet exhaustive. In order to choose the most

appropriate ant species for restoration, a most comprehensive database is needed. This lack in data availability remains the main limitation of our methodology.

The positive effects of ants on the ecosystem have been detailed here, however some ants can also induce disservices. For example, leaf-cutting ants can improve soil conditions (Farji-Brener & Silva 1995), assist in seed dispersal (Farji-Brener & Ghermandi 2004), but can also together drastically cut the new growing vegetation (Corrêa et al. 2010; Meyer et al. 2011). The costs and benefits of introducing a species to a site must always be precisely weighted and strictly depends of the local context.

3.5.3 Perspectives

Interaction between ecological engineers has to be considered in restoration ecology because complementarity in traits and functions may accelerate the restoration process. The association of ants with other engineers may allow a temporal continuity and/or a complementarity in the realized functions. The asynchrony of earthworms and ants' activity peak (falls/winter and spring/summer respectively) which may allow a continuity in the soil structuration function, soil chemical fertility and seed burrowing is a clear example of functional complementarity. In the same way, Nicolai (2019) showed that due to different granivory rates and seed selections (Samson et al. 1992), two years after a fire in a semi-arid grassland kangaroo rats created nests enhancing density of recovering dicots while ants on the edges of nests facilitated reproduction of recovering grasses.

3.6 Conclusion

In conclusion, some taxonomic groups perform key ecological functions that ensure the ecosystem functioning. A trait-based approach that is focused on both on their effect and response traits, facilitate their use in restoration projects. Traits are selected according to the predefined objectives and the ecosystem considered.

Although our methodology focuses on the effect and life history traits of ants, it could be easily applied to other biota. So far, these organisms have not been widely used in ecological restoration projects. This methodology intends to facilitate their use and potentially decrease the costs related to restoration by promoting nature-based solutions.

Chapitre 3

Table 3.1: Ants traits and the associated impacted processes of soil, vegetation and fauna components, and unit of measurement.

Components	Impacted processes	Traits	Measures	Sources
Soil	Soil structure	•Size of mandibles gap	•mm	Dostál et al. 2005; Oliveras et al. 2005; Cammeraat & Risch 2008; Martín-Perea et al. 2019
		•Mandibles length	•mm	
		•Ant body size	•mg	
		•Production of biogenic structure	•Burrowing or not burrowing	
	Soil (macro)porosity	Production of biogenic structure	Burrowing or not burrowing	Cammeraat & Risch 2008
	Bulk density	Production of biogenic structure	Burrowing or not burrowing	Cammeraat & Risch 2008
Infiltration rate and water retention capacity	•Size of ant biopores •Nest vertical size (deep) •Size of the nest •Nest entrance size	•mm	Lobry de Bruyn & Conacher 1994; MacMahon et al. 2000; Cammeraat & Risch 2008	
		•mm		
		•m		
		•mm		
Soil erosion	Size of ant biopores	mm	Lobry de Bruyn & Conacher 1994	
Magnitude of enrichment	Diet	Generalist predator, specialist predator, generalist, seed harvester, seed harvester and generalist, sugar feeder and generalist	Farji-Brener & Werenkraut 2017; Elizalde et al. 2020	
Vegetation	Seed predation	•Colony foraging strategy •Diet	•Individual or group •Generalist predator, specialist predator, generalist, seed harvester, seed harvester and generalist, sugar feeder and generalist	MacMahon et al. 2000

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	Seed dispersal	<ul style="list-style-type: none"> •Ant body size •Size of mandibles gap •Diet 	<ul style="list-style-type: none"> •mm •mm •Generalist predator, specialist predator, generalist, seed harvester, seed harvester and generalist, sugar feeder and generalist 	Ness et al. 2004; Oliveras et al. 2005
	Seed germination	<ul style="list-style-type: none"> •Ant body size •Dispersal distance 	<ul style="list-style-type: none"> •mm •m 	Ness et al. 2004; Fernandes et al. 2020
	Seedling growth	<ul style="list-style-type: none"> •Ant body size •Dispersal distance 	<ul style="list-style-type: none"> •mm •m 	Ness et al. 2004; Fernandes et al. 2020
	Plant establishment	<ul style="list-style-type: none"> •Ant body size •Dispersal distance 	<ul style="list-style-type: none"> •mm •m 	Ness et al. 2004; Fernandes et al. 2020
Faune	Population regulation	<ul style="list-style-type: none"> •Size of mandibles gap •Prey-predator size 	mm	Oliveras et al. 2005; Elizalde et al. 2020
	Predation	<ul style="list-style-type: none"> •Diet •Length of mandibles 	<ul style="list-style-type: none"> •Generalist predator, specialist predator, generalist, seed harvester, seed harvester and generalist, sugar feeder and generalist •mm 	Parr et al. 2017

DISCUSSION – CONCLUSIONS
GENERALES

Les fourmis sont souvent présentées comme des espèces ingénieures des écosystèmes. Cependant cette généralisation ne paraît pas justifiée. En effet, bien que la plupart des fourmis ait un impact sur la structure des écosystèmes via la modification physique, le maintien ou la création d'habitats, certaines espèces ne sont pourtant pas des espèces ingénieures au sens propre de la définition (e.g. fourmis nomades). Les six facteurs proposés par Jones et al. (1997, 1994) peuvent alors être pertinents pour déterminer le rôle de chaque espèce au sein de son écosystème. Cependant, jusqu'à présent, aucune étude exhaustive n'a été effectuée en utilisant le sixième facteur afin de caractériser l'impact des fourmis dans leur milieu et d'offrir vision réellement globale de leur rôle d'ingénieur. Le premier objectif de cette thèse a donc été d'étudier les effets multi-compartiments directs et indirects d'une espèce dite ingénieure, *M. barbarus*, sur son écosystème : les pelouses méditerranéennes. Au regard de nos résultats, son utilisation comme outil en écologie de la restauration peut être envisagée. Nous avons donc évalué son rôle dans l'accélération de la restauration écologique d'un milieu dégradé puis proposé une méthodologie appliquée de l'utilisation des fourmis basée sur leurs traits.

D.1 Apports de la thèse au concept d'ingénieur des écosystèmes

D.1.1. Messor barbarus : espèce ingénieure des écosystèmes dans les pelouses méditerranéennes en milieu semi-naturel et dégradé

Ce travail de thèse nous a permis de déterminer la nature et l'amplitude de l'impact de *M. barbarus* sur le sol, la végétation et une partie des invertébrés. Un des résultats majeurs porte sur le rôle primordial joué par *M. barbarus* dans la structuration des communautés au sein des pelouses méditerranéennes (Chapitres 1 et 2). *Messor barbarus* agit, en effet, à la fois sur le filtre abiotique, en modifiant les propriétés physico-chimiques du sol, et le filtre biotique en modifiant les communautés de plantes (e.g. biomasse, hétérogénéité, hauteur de la végétation, états physiologiques) des invertébrés de surface et souterrains (abondance et occurrence). *Messor barbarus* change aussi directement et indirectement les relations trophiques et non-trophiques au sein de l'écosystème. Ainsi, parmi les six facteurs proposés par Jones et al. (1997, 1994) pour caractériser le rôle d'une espèce ingénieure, nous nous sommes attachés au dernier à savoir le nombre et le type de ressources directement et indirectement modifiés par les constructions, la manière dont ces ressources sont contrôlées et le nombre d'espèces dépendantes de ces ressources.

Bien que dans les deux milieux semi-naturels étudiés (Plaine de la Crau et Réserve Naturelle de Camargue) *M. barbarus* agisse globalement positivement sur la fertilité du sol et les communautés végétales (Chapitres 1 et 2), des différences sont à noter pour certaines variables étudiées. Alors que nous avons mesuré une augmentation de la quantité de matière organique et du carbone organique dans les nids de fourmis dans la Plaine de la Crau (Chapitre 2), ces variables ne diffèrent pas entre les deux modalités testées sur la réserve naturelle de Camargue (Chapitre 1). Les différences les plus marquées ont cependant été enregistrées entre le milieu de référence et la zone à restaurer dans la Plaine de la Crau (Chapitre 1). En effet, la quasi-totalité des variables diffèrent entre nids et zones contrôles dans la zone à restaurer, ce qui n'est pas le cas dans la steppe de référence.

Pourquoi cet effet de l'ingénieur est-il plus marqué sur une zone dégradée ?

Nos résultats suggèrent un impact contexte-dépendant de l'espèce ingénieure dont l'effet serait moindre dans un habitat préservé et mature (pelouses et prairies multiséculaires de la Crau et de Camargue). Cependant, il a été montré que l'impact des fourmis ne s'arrête pas à la mort de la colonie mais peut être visible jusqu'à plus de 20 ans après l'abandon du nid chez certaines espèces (Kristiansen & Amelung 2001; Kristiansen et al. 2001). Dans les milieux semi-naturels, où les fourmis sont présentes depuis des centaines voire des milliers d'années, les zones dites « contrôles » car sans traces visibles d'activité des fourmis ont pu être remaniées par le passé et « garder en mémoire » le travail des fourmis. Cela pourrait expliquer les faibles différences mesurées entre les nids et les zones contrôles dans ces milieux et remettant alors en question la validité des contrôles. A l'inverse, les zones contrôles considérées sur les sites dégradés et dépourvues de toutes « traces » d'activité de fourmis correspondraient à de « vraies » zones contrôles. Une approche à l'échelle locale de l'impact des fourmis ne semble donc pas pertinente dans les écosystèmes naturels car celui-ci est déjà discernable à l'échelle du site.

Comment peut-on mesurer l'impact d'un ingénieur sur un milieu non perturbé ?

Afin de mesurer l'impact d'un ingénieur de l'écosystème, il est alors nécessaire d'effectuer une approche corrélative en comparant plusieurs sites de même nature avec et sans fourmis. Cependant, il semble compliqué voire impossible d'identifier plusieurs sites semi-naturels de même nature non colonisés par les fourmis. Une alternative expérimentale serait alors de retirer les fourmis sur des placettes de plusieurs dizaines de mètres carrés et

de suivre ensuite la dynamique temporelle des variables ciblées. Cette expérimentation était envisagée au début du projet de thèse, mais n'a pu être mise en place pour des raisons techniques (impossibilité d'utiliser des appâts chimiques dans la réserve). Cette méthode a cependant été choisie dans une étude récente afin d'étudier l'impact des communautés de fourmis sur l'abondance d'autres invertébrés, le processus de décomposition de la matière organique et l'herbivorie (Parr et al. 2016). L'ensemble de la communauté de fourmis présente sur les sites ciblés, constitué notamment de fourmis prédatrices, a été supprimé. Après seulement cinq mois d'exclusion, une augmentation significative de l'abondance de certains arthropodes ainsi qu'une modification du processus de décomposition de la matière organique et de l'herbivorie ont été enregistrées. Cette étude confirme donc qu'il est possible de supprimer l'ensemble de la myrmécofaune sur de grandes superficies (1 ha) bien que la rémanence de leurs actions antérieures à court terme n'ait pu être complètement discriminée de la dynamique mesurée après leur suppression.

D.1.2. Intérêt d'une approche multi-compartiments pour l'étude des ingénieurs des écosystèmes ?

Notre étude réalisée dans une réserve naturelle de Camargue (Chapitre 1) constitue une des rares portant sur l'effet d'une fourmi sur trois compartiments. Bien que nous ayons pris en compte l'effet de *M. barbarus* sur une cinquantaine de variables, nous n'avons pas pu caractériser de façon exhaustive l'effet de cette espèce. Pour obtenir une vision globale de son rôle sur son écosystème, il serait nécessaire d'effectuer des mesures complémentaires comme la quantification de la biomasse microbienne et fongique, éléments jouant des rôles importants dans les écosystèmes notamment en tant que décomposeurs de la matière organique (Berg & McClaugherty 2008; Nielsen et al. 2011; Paul 2014).

D'un point de vue technique, de telles études exhaustives se révèlent complexes à mettre en place, ce qui peut expliquer leur faible nombre dans la littérature. Trois contraintes majeures à la mise en place d'études multi-compartiments peuvent être soulevées. Tout d'abord, ces études demandent des connaissances solides sur l'ensemble des compartiments. Elles nécessitent donc la mise en commun des connaissances de nombreux experts, ce qui peut être vu comme une limite à leurs mises en place. La deuxième contrainte identifiée est le temps de récolte, de mesures en laboratoire, d'identifications et d'analyses de résultats nécessaires. La troisième contrainte fait référence à l'identification des relations qui lient deux

organismes. Dans certains cas, il est difficile d'évaluer si une relation est purement trophique entre l'ingénieur des écosystèmes et un autre organisme ou si la relation dépend d'une troisième variable indirectement liée à l'activité de l'ingénieur. Par exemple, nous avons observé la destruction de nombreux nids par des sangliers sur la réserve naturelle de Camargue. Il est compliqué de caractériser cette relation. Si le nid retourné l'a été uniquement pour la prédation des fourmis la relation ne doit pas être prise en compte pour caractériser l'impact de *M. barbarus*. En revanche, si le sanglier est également attiré par l'augmentation de la biomasse végétale et de l'abondance des autres invertébrés indirectement induite par l'activité des fourmis, la relation doit être prise en compte dans la description du sixième facteur de Jones et al. (1997, 1994).

Le constat fait sur la difficulté à mettre en place des protocoles robustes pour quantifier l'ensemble des impacts d'un ingénieur pose la question de la faisabilité de telles études. Un compromis doit donc être établi entre la précision de la donnée et la quantité de données accumulées. Nous avons fait le choix dans le premier chapitre de ne pas conduire une étude au niveau de l'espèce mais à celui des groupes trophiques ou formes de vie. Une approche à ce niveau nécessite moins de connaissances taxonomiques, moins de temps à investir (acquisition et traitement des données) mais également moins de matériel (souvent spécifique à chaque groupe). Par exemple, la charge de travail pour identifier l'ensemble des collemboles jusqu'à l'espèce avait été estimée au minimum à deux mois à temps plein dans le cadre de notre étude, en comptant le temps d'apprentissage de cette technique. L'identification des formes de vie a permis de réduire ce temps par un facteur trois. De plus, l'identification à l'espèce nécessite un matériel spécifique (un microscope ayant un grossissement d'environ 600 avec un objectif à contraste de phase) qui n'est pas toujours disponible dans les laboratoires non spécialistes alors que l'identification des formes de vie a pu être réalisée avec une loupe binoculaire.

Afin de simplifier l'étude des ingénieurs, une approche multi-compartiments peut être maintenue en sélectionnant uniquement des variables clés. Pour les fourmis, la texture du sol ainsi que quelques éléments chimiques principaux, tels que le carbone et l'azote total, le pH, le phosphore assimilable ou encore la capacité d'échange cationique peuvent suffire pour caractériser le compartiment abiotique. Pour les communautés végétales, des mesures rapides telles que la biomasse ou encore l'hétérogénéité, devraient suffire à quantifier

l'impact de l'espèce ingénieure sur la végétation sans pour autant avoir des connaissances précises de la composition floristique locale.

Ce rôle joué par les espèces ingénieures, identifié grâce à des études multi-compartiments en complément de connaissances fondamentales sur le fonctionnement des écosystèmes, pourrait être un outil précieux en restauration écologique.

D.2 Apports de la thèse en restauration écologique

D.2.1 Nids de fourmis : zones refuges et/ou sources pour la recolonisation ?

Les nids de fourmis sont parfois qualifiés de « zones sources » (Sánchez-Piñero & Gómez 1995; Laakso et al. 1998; Farji-Brener 2010; Kachamakova et al. 2019; Nicolai 2019), zones à partir desquelles les organismes peuvent se disperser et coloniser le reste de l'écosystème. Nos résultats montrent que les nids de *M. barbarus* sont plus colonisés par certaines espèces de la steppe, sept ans après leur installation, pouvant alors constituer des zones refuges. Quant à leur rôle de source pour le reste de la zone dégradée, nous ne pouvons qu'émettre l'hypothèse que les nids agiront de la même manière que les *nuclei* utilisés dans les projets de restauration. Des disques de bois ont ainsi été recommandés comme refuges pour l'entomofaune lors de programmes de restauration dans des forêts présentant peu ou pas de troncs d'arbres morts (Bowie & Frampton 2004; Woldendorp & Keenan 2005) car ils fournissent un habitat avec une humidité et un nombre de niches écologiques plus élevés (Bowie & Frampton 2004). Les *nuclei* servent initialement de refuges, puis de sources de population conduisant à une restauration plus rapide des communautés végétales et animales (Reis et al. 2003; Blight et al. 2011).

Les espèces végétales de la steppe peuvent se disperser par le vent et/ou par les fourmis. Les nids pourraient ainsi jouer de rôle de zone source pour ces organismes. A l'inverse, les capacités de dispersion de la faune et notamment de la faune endogée peuvent être très limitées (pas de vecteur de dispersion) réduisant les chances de colonisation de nouveaux nids. Alors qu'un transfert d'espèces entre nids aux conditions micro-locales favorables est probable, il est moins évident entre nids et zones dégradées non travaillées par les fourmis. En effet, nos résultats montrent clairement l'existence d'un filtre abiotique limitant toujours l'installation des plantes de la steppe dans les zones non colonisées par les fourmis à restaurer. Ce rôle de zones refuges ou sources des nids de *M. barbarus* pourrait

alors être un processus clé dans la vitesse de restauration du site, afin de passer d'une restauration locale à une restauration de l'ensemble du site.

D.2.2 Effet des ingénieurs en restauration écologique : de l'échelle locale à l'échelle du site

Nos résultats ont montré que *M. barbarus* accélère, au niveau de son nid, la restauration du compartiment abiotique et de la banque de graines sept ans après son installation sur une pelouse méditerranéenne en cours de restauration de la Plaine de la Crau (Chapitre 2). Son action est pour l'instant locale, au niveau du nid. Comme attendu, la zone dégradée sans nids de fourmis ne semble pas évoluer vers des communautés végétales de type coussouls (Dutoit obs. pers.) mais vers des encroutements biologiques appelés localement « tonsures » dans la plaine de Crau (Rieux et al. 1977) – portion de la steppe présentant une végétation rase constituée en majorité de bryophytes et lichens ainsi qu'un fort recouvrement de sol nu. *Messor barbarus* semble être un « levier d'action » conduisant les communautés végétales au niveau de son nid plutôt vers des communautés de type « friche mésophiles méditerranéenne » composées d'espèces plus mésophiles que celles de la steppe, plutôt oligotrophes. Bien que ces communautés végétales ne soient pas les communautés de coussouls attendues, une partie des espèces de la steppe de référence sont cependant présentes dans la banque de graines des nids. Après l'abandon du nid, les taux de nutriments dans le sol devraient diminuer par lessivage suite à l'absence d'apports et de remontées d'éléments par les fourmis permettant potentiellement à ces plantes de s'exprimer au bout de quelques dizaines d'années. Cette hypothèse est notamment confortée par l'observation de dizaines de zones de tonsures dans la plaine de Crau dont l'évolution vers la végétation de la steppe de référence semble être dépendante de l'installation puis de l'abandon de nids de fourmis de *M. barbarus* (Martin et Dutoit obs. pers.).

Bien que nous ayons validé le rôle de *M. barbarus* pour la restauration du sol et de la végétation à l'échelle du nid, son rôle dans la restauration du site n'est pas encore avéré. En effet, la superficie travaillée par les fourmis depuis les premières colonisations (aidées et naturelles) ne couvre aujourd'hui qu'une faible surface estimée à 2,6 % de la surface totale de la zone dégradée (environ 5 ha). Il sera donc nécessaire dans les prochaines années d'estimer à la fois la dynamique de cette population (établissement de nouvelles colonies et disparition des plus anciennes) mais également la rémanence des impacts après l'abandon du nid afin de déterminer quand la restauration passera de l'échelle locale du nid (quelques m²) à l'échelle

du site (quelques hectares). Les reines de *M. barbarus* peuvent vivre près de 25 ans en conditions contrôlées de laboratoire. Dans ses travaux de thèse, Cerdan (1989) estime de 8 à 10 ans la vie d'une colonie en milieu naturelle. Une étude sur ce sujet pourrait ainsi non seulement fournir des données intéressantes sur la dynamique d'une population de fourmis mais elle permettrait également d'estimer le temps nécessaire pour restaurer un site dégradé en se basant sur le travail d'ingénierie des fourmis.

D.2.3 Toutes les fourmis natives peuvent-elles être utiles en restauration écologique ?

Toutes les fourmis, qu'elles puissent être qualifiées d'espèces ingénieures ou non, doivent être considérées dans le cadre de projets de restauration écologique. Les fourmis ingénieures des écosystèmes seront les seules à pouvoir agir directement sur les propriétés physico-chimiques du sol et devront donc être utilisées lorsque l'écosystème est particulièrement dégradé et que le compartiment abiotique doit être restauré. D'autres espèces pourront alors être utilisées en complément afin de restaurer les relations biotiques importantes pour le fonctionnement de l'écosystème. Bien que les espèces ingénieures montrent des impacts directs et indirects importants sur leur environnement, les autres espèces sont capables d'effets plus ciblés qui rentrent dans le cadre d'objectifs de restauration. Par exemple, les espèces nomades, qui ne sont donc pas qualifiées d'espèces ingénieures des écosystèmes au sens strict de la définition, ont pourtant un rôle important dans leur écosystème. En tant que prédatrices redoutables, elles contribuent ainsi à la régulation de nombreuses populations d'invertébrés (e.g. fourmis, coléoptères, orthoptères) et de petits vertébrés. En restauration écologique, les espèces à réintroduire ne doivent pas être choisies parce qu'elles sont reconnues à dire d'experts comme ingénieures des écosystèmes mais pour les fonctions qu'elles assurent. L'ensemble des espèces natives d'un milieu à restaurer est donc à considérer dans le cadre d'une utilisation potentielle pour la restauration écologique.

D.3 Perspectives de recherche

D.3.1 Effet du nid sur le piégeage des graines

Messor barbarus est une espèce généraliste qui collecte en priorité les graines présentes en abondance et préférentiellement les graines longues et denses (Azcárate et al. 2005). L'accumulation de graines dans le dépotoir est donc souvent considérée comme une

conséquence de cette action de prédation. Or les fourmis, en remaniant le sol, créent des zones de micro-relief susceptibles d'atteindre 10 cm de haut pour *M. barbarus* (obs. pers.). Ce micro-relief pourrait suffire à piéger des graines, d'autant qu'en l'absence de galets dans la zone à restaurer de la Plaine de la Crau, ces structures constituent quasiment les seuls reliefs. Une étude sur le sujet complémentaire aux observations déjà faites sur l'impact direct de *M. barbarus* sur la distribution de graines via la prédation pourrait être intéressante. Les graines transportées par *M. barbarus* sont susceptibles d'être différentes des graines potentiellement retenues par leurs constructions. Une expérience de piégeage de graines, par exemple avec des films « collants » apposés sur les nids de fourmis avec exclusion de celles-ci ou avec des structures mimant la taille et la hauteur d'un nid viserait à déterminer si *M. barbarus* agit sur la redistribution des graines et donc sur les communautés végétales, à la fois via le transport des graines et via la construction de son nid. Une étude taxonomique des graines pourrait, dans ce cas, être effectuée mais une étude basée sur les traits des graines retenues semble particulièrement intéressante à mener dans la mesure où les résultats seraient généralisables à d'autres milieux.

D.3.2 Effet des fourmis sur la dégradation de la matière organique

Nous avons mesuré une augmentation de la quantité de nitrates et d'ammonium dans les nids de *M. barbarus* en comparaison des zones contrôles (Chapitres 1 et 2). Cela suggère une dégradation de la matière organique plus rapide au niveau des nids qu'au niveau des zones contrôles. La plus forte abondance d'invertébrés, notamment décomposeurs, au niveau des nids pourrait faciliter une accélération de la dégradation de la matière organique et ainsi mettre à disposition plus rapidement les nutriments pour les plantes. Cela induirait alors une augmentation de la productivité et au final de la biomasse des plantes (comme mesuré dans nos études) mais également une diminution de la compétition pour les ressources. Si tel est le cas, cette capacité pourrait être utilisée en restauration écologique lorsque le sol doit être enrichi. L'utilisation de fourmis moissonneuses accélérerait alors le processus.

Afin de tester l'hypothèse selon laquelle la matière organique se décompose plus rapidement au niveau des nids de fourmis, des sacs à litière devraient être posés respectivement sur des nids de fourmis et dans les zones contrôles. Des mesures répétées dans le temps fourniraient alors des informations sur les dynamiques de dégradation de la matière organique au niveau des deux modalités. Ce dispositif pourrait être couplé avec une

étude de la dynamique du cycle de l'azote afin de mesurer si cet élément est plus rapidement mis à disposition des plantes au niveau des nids.

D.3.3 Effet synergique possible entre deux espèces ingénieuses des écosystèmes ?

Dans l'étude réalisée en Camargue (Chapitre 1), nous n'avons pas mesuré de différences d'abondance des vers de terre entre les nids et les zones contrôles ; leur occurrence apparaît cependant significativement supérieure au niveau des nids. Bien que la présence de vers de terre dans les nids de fourmis ait déjà été observée dans des nids de *Formica rufa* (Laakso & Setälä 1997; Laakso et al. 1998), nous nous attendions à recenser moins de vers dans les nids notamment à cause des potentielles interactions directes entre vers et fourmis (ex. morsures).

Comme mentionné précédemment, les vers de terre sont également considérés comme des espèces ingénieuses des écosystèmes. Bien que ces deux organismes n'aient pas des rôles strictement identiques sur les compartiments biotiques et abiotiques, certains sont susceptibles d'être redondants pour parti. Les fourmis mélangent le sol verticalement ce qui conduit à des modifications importantes dans la composition chimique, la structure du sol, l'infiltration d'eau au niveau des nids ainsi qu'une modification de la faune et des communautés végétales (Dostál et al. 2005; Boulton & Amberman 2006; Cammeraat & Risch 2008; Frouz & Jilková 2008; De Almeida, Mesléard, et al. 2020). Les vers de terres, via leurs mouvements à la fois verticaux et horizontaux, ont davantage d'impacts sur la densité apparente du sol ainsi que sur l'épaisseur des couches organiques. Ils incorporent des matériaux de litière et d'humus dans les horizons du sol plus profonds (Blanchart et al. 1999; Blouin et al. 2013; Le Bayon et al. 2017). En se nourrissant de graines et en les dispersant, les vers peuvent également avoir un impact sur la banque de graines (Eisenhauer et al. 2010; Forey et al. 2011; Clause et al. 2016). Ces deux organismes ont tendance à accroître l'hétérogénéité de l'écosystème, comme c'est le cas pour une grande majorité des ingénieurs des écosystèmes.

Une action combinée de deux ingénieurs des écosystèmes pourrait accentuer leurs propres effets sur l'écosystème. Cet effet combiné ou synergique de deux ingénieurs dans un même écosystème ne facilite pas la détermination de l'impact de chacun d'entre eux, d'autant que certaines fonctions sont assurées par les deux espèces. La quantification de l'effet des ingénieurs par les six facteurs proposés par Jones et al. (1997, 1994) est alors d'autant plus

complexe. Ce potentiel effet synergique entre deux ingénieurs, prometteur, semble à considérer en restauration écologique. Dans le cas de réintroduction d'espèces, l'utilisation de plusieurs espèces complémentaires pourrait ainsi être un choix judicieux pour accroître l'effet levier, ou lorsque plusieurs fonctions doivent être restaurées et qu'aucune espèce n'est capable de le faire seule.

Ce supposé effet synergique sur la végétation et le sol pourrait être mesuré en mésocosmes. Afin de tester l'effet combiné d'une espèce de fourmis avec une espèce de vers de terre, des mesures physico-chimiques du sol, de l'activité microbienne et le suivi d'une espèce végétale pourraient notamment être réalisées à travers quatre modalités : un contrôle (sans faune présente), un traitement avec fourmis et sans vers, un traitement avec fourmis et vers, un traitement avec vers et sans fourmis. Un suivi de la germination des graines et de la croissance des plantules serait alors conduit et les propriétés physico-chimiques du sol ainsi que de l'activité microbienne analysées à la fin de la période de suivi. Cette expérimentation, jamais menée jusqu'à présent, permettrait de déterminer s'il existe ou non un effet synergique de ces deux ingénieurs. Une étude similaire réalisée en mésocosmes a ainsi permis de mettre en évidence un effet synergique sur la diminution de la biomasse de la litière de surface entre deux espèces ingénieuses de vers de terre de deux classes différentes (un épi-anécique et un endogé) (Xia et al. 2011).

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ANNEXE

Key issues in Northwestern Mediterranean dry grassland restoration

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REVIEW ARTICLE

Key issues in Northwestern Mediterranean dry grassland restoration

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Dry grasslands of the Northwestern Mediterranean Basin are seminatural species-rich ecosystems, composed of many annual species and some structuring perennial species. As these grasslands have been used as rangelands for centuries, human management (grazing; fire regimes) is one of their main ecological and evolutionary drivers, along with the Mediterranean climate. Despite EU conservation policy, damage to such grasslands still occurs and efficient ecological restoration is needed. This article reviews restoration constraints, effective techniques, and research perspectives for restoring them in the light of their ecological characteristics. Major restoration constraints specifically include (1) a poor contribution of the internal (seed bank) and external (seed rain) species pools, and (2) low establishment of some perennial species. The latter may be the result of the low seed quantity or quality of perennial species, stochastic or extended droughts, high soil nutrient content that may lead to competition (e.g. between weed and target species), inappropriate grazing management or fire regimes leading to shrub encroachment, or the death of target species seedlings in early stages of restoration. Successful restoration techniques include seeding, transplanting, hay transfer, and timely soil transfer, all of which have to be adapted to regional plant phenology and to local Mediterranean climate. They must be combined with appropriate grazing or fire management. Their success may depend on years with particular rainfall amounts or patterns or on particular target plant species. We discuss research perspectives contributing to a better understanding of seed dispersal, seedling recruitment, and soil processes and suggest the benefits of using ecosystem engineer species.

Key words: drought, grazing and fire management, local adaptation, Natura 2000 habitat #6220, rangeland, seed bank, seed-ling recruitment, species pool

Implications for Practice

- Constraints for the restoration of dry grasslands of the Northwestern Mediterranean Basin include poor dispersal, low establishment of perennial species, poor quality of the long-term seed bank, and competition between weeds and target species.
- Successful restoration techniques include soil and hay transfer, seeding, and transplanting before autumn rains, applied along with appropriate management (winter-spring grazing and summer fires).
- Restoration success is highly dependent on interannual climatic variability which creates contingency in restoration outcomes, which are generally better in wetter years.
- More research is needed to improve the restoration of dry grasslands of the Northwestern Mediterranean Basin, particularly in the areas of seed dispersal, population biology of key species, soils, ecosystem engineers, fire regimes, and plant-arthropod relationships.

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Introduction

Mediterranean grasslands are characterized by warm, dry summers and cool, wet winters. Grasslands of the Mediterranean Basin are seminatural ecosystems and one of many “Mediterranean cultural landscapes” (Grove & Rackham 2001). They are largely maintained by human land management, such as grazing and pastoral fires (San Miguel 2008). Their long historical use as rangelands has played an important role in determining their recent ecological and evolutionary dynamics, and has resulted in their relatively high tolerance to grazing (Perevolotsky & Seligman 1998). Because they are often species-rich and support wildlife as well as extensive grazing, and therefore sustainable human livelihoods, seminatural Mediterranean grasslands are protected under the EU habitat directive (San Miguel 2008). For ecological restoration purposes, these ecosystems are considered as appropriate references (Gann et al. 2019).

Because conservation and restoration practices in the Mediterranean Basin vary with latitude and longitude (Nunes et al. 2016), we focus here on Northwestern (European Union) Mediterranean-type ecosystems. Despite EU conservation policy, ecological restoration is still needed for some of these grasslands. They are still sometimes subjected to degradation and even well-managed rangelands may support fewer species than they once did, or could if restored. This article reviews and discusses the restoration constraints and techniques of dry grasslands of the Northwestern Mediterranean Basin (NwMedB hereafter), in the light of their ecological characteristics. Among these grasslands, this article emphasizes species-rich dry grasslands, “Pseudo-steppe with grasses and annuals (Thero-Brachypodieta),” most of which are in Spain, Italy, Portugal, and France and cover over 26,000 km² in these countries (Table S1, Supporting Information; EUNIS 2020). These Mediterranean dry grasslands are found in plains, hills, and plateaus, and this article thus excludes watered meadows and alpine grasslands. We here give a general summary of multiple aspects of the restoration of these communities, which may also apply to the restoration of other ecosystems (e.g. similar grasslands or the same grassland type in Eastern Europe; Fig. S1, Table S1). This article is not a comprehensive literature survey or meta-analysis, but instead draws upon experts in various aspects of Mediterranean grassland restoration. Mediterranean grasslands share many restoration challenges with other ecosystems, but also show specific constraints, in particular the strong summer drought and the unique land-use issues of this region. This article aims to highlight some of these differences, while still being useful for ecosystems with similar challenges.

Major Constraints for the Restoration of Northwestern Mediterranean Grassland Plant Communities

Limited Spatial Seed Dispersal

Plant recruitment via seed rain in degraded grasslands is generally described as low, including in NwMedB grasslands (Gomez & Espadaler 1994; Buisson & Dutoit 2004; Buisson et al. 2006; although see the section below on Using Animals to Restore Plant Communities). This may be because small-

seeded species without specialized dispersal structures are over-represented in these grasslands (Azcarate et al. 2002). Small seeds without specialized dispersal appendices are mainly barochorous (gravity-dispersed) and do not disperse very far. They penetrate the soil more easily where they escape secondary dispersal (Peco et al. 2003). They therefore do not easily reach degraded areas. Longer seeds or dispersal units with specific structures are preferentially preyed upon and potentially dispersed by ants (Azcarate et al. 2005).

Although seeds of NwMedB herbaceous species can be dispersed by ants, rabbits, deer, and livestock, dispersal distances have rarely been evaluated. Assessing the contribution of local versus distant diaspore sources is difficult, which may explain why estimating seed dispersal distance has generally been overlooked in seed rain studies in grasslands (Arruda et al. 2018). Moreover, dispersal by livestock is reduced compared to temperate grasslands because grazing rarely occurs at the time of seed dispersal (summer, when forage quantity and quality are low) (Joffre et al. 1988; Buisson et al. 2006).

Life History Traits and Regeneration

Plants of NwMedB grasslands are adapted to summer drought, unpredictable rainfall events, grazing, and fire (Blondel et al. 2010). Some species, such as *Dactylis glomerata* and *Brachypodium retusum*, undergo vegetative summer dormancy, resulting in a reduction of growth sometimes combined with the senescence of aboveground parts (e.g. Volaire & Norton 2006). Many perennial plants can survive and resprout from a belowground bud bank (rhizomes; roots) after such stresses and disturbances (De Luis et al. 2004; Grigulis et al. 2005; Nedjimi 2016). Such plants are vulnerable to intense soil disturbance that destroys the bud bank and underground organs (Fernández Ales et al. 1993). Additionally, decades of cultivation also can greatly reduce native seed banks (Römermann et al. 2005; Fig. 1).

Annual plants are common in the Mediterranean Basin (Blondel et al. 2010), especially in areas with strong summer droughts (Clary 2008). They survive the unfavorable dry season (summer) as seeds. They can produce a small amount of large seeds, which may better survive ruminant ingestion (Peco et al. 2006a). However, they are predominantly represented by species that produce large number of small seeds that may live longer and have a better chance to be found in the persistent seed bank (Peco et al. 2003; Blondel et al. 2010). Delayed germination (via hardseededness or other mechanisms) is a characteristic of some Mediterranean annual forbs, particularly Fabaceae (Azcarate et al. 2002; Peco et al. 2003). However, many species and seeds germinate with autumn rain to benefit from bare ground left after summer drought. As an adaptation to their variable environment, some of these species form a small persistent seed bank allowing them to germinate in later years to cope with the unpredictability of rainfall (Ortega et al. 1997). All together, few seeds join the long-term seed bank (Ortega et al. 1997; Römermann et al. 2005; Buisson et al. 2006, 2018; Fig. 1), which therefore stays relatively poor in species and seeds.

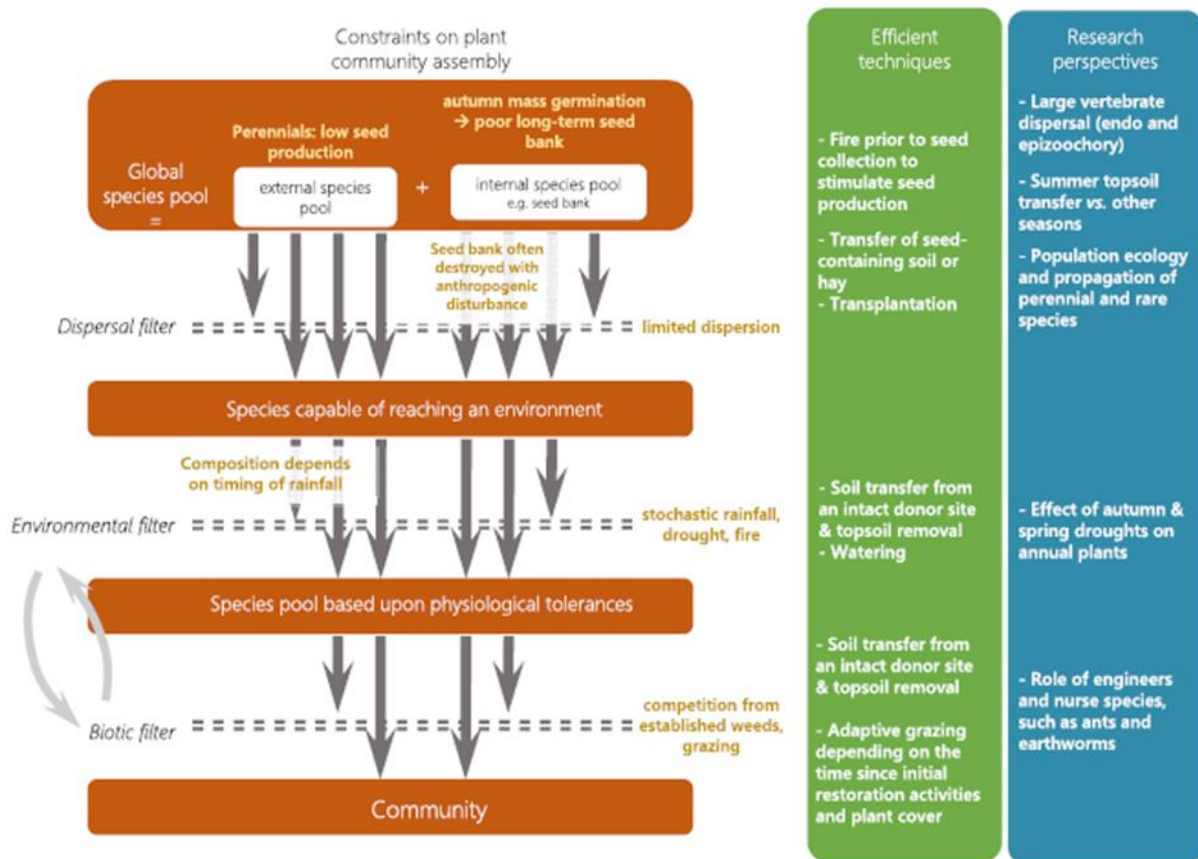


Figure 1. The main processes or filters structuring a plant community are represented on the left side of the figure (in orange), modified from Lortie et al. (2004). Each process/filter is represented by a double horizontal line and the corresponding description is in italics on their left. Community assembly constraints in Northwestern Mediterranean Basin dry grasslands are written in pale orange on the right of the double horizontal lines. Dark gray arrows depict the movement of species through the filters. Light gray arrows represent the feedback loop between the abiotic environment and the biota. To the right of the figure, in a green column the successful techniques for the restoration of Northwestern Mediterranean Basin dry grasslands, and in a blue column the research perspectives.

Seedling Recruitment and Establishment

Mediterranean grassland species mainly germinate with autumn or winter rains. The optimal conditions for germination (temperatures and rainfall timing) strongly vary across plant species (Espigares & Peco 1993). For example, some species of the genera *Lotus*, *Melilotus*, *Medicago*, and *Trifolium* acquire increased germination efficiency with cold stratification or fluctuating temperatures (Assche et al. 2003). The timing of the rainy season, and the associated temperatures, and rainfall patterns may thus change plant species composition from one year to another (Fig. 1). Such annual climatic variability may produce strong contingency in restoration outcomes (Stuble et al. 2017).

Typical Mediterranean perennial grassland species often show a relatively low seedling recruitment (e.g. *B. retusum*, *Asphodelus* sp.), which may be due to (1) low seed production or quality (Coiffait-Gombault et al. 2012; Vidaller et al. 2019a), (2) on-site competition with established plants (Buisson et al. 2015), or (3) drought stress (Bochet et al. 2007; Vidaller et al. 2019a). Sporadic spring droughts, such as in 2003, may also lead to high mortality of well-established adult perennial plants (Buisson et al. 2015). Other perennial species, particularly found in Spain and Italy, show high allocation to sexual reproduction (*Stipa tenacissima*, *Ampelodesmos mauritanicus*),

ability to form soil seed banks (*S. tenacissima*), and high resistance to drought stress (*Lygeum spartum*, *S. tenacissima*) (Grigulis et al. 2005; Cortina et al. 2009; Nedjimi 2016). They are therefore particularly promising for the restoration of degraded areas where they would naturally occur (Mariotti & Zotti 2010).

Most NwMedB grasslands grow on soils with low nutrient content, which, depending on species characteristics, may reduce competitive interactions allowing higher species richness (Maestre et al. 2009). If the degradation is due to intensive agriculture, persisting fertilizer residues (Standish et al. 2006) may favor weeds in competition with target species (Zefferman et al. 2015) and jeopardize the recovery of species-rich plant communities (Fig. 1), even if target propagule dispersal is artificially increased (Jaunatre et al. 2014a).

Inadequate Grazing Management

Grazing is one of the main drivers of plant diversity in Mediterranean grasslands (Peco et al. 1983; Carmona et al. 2015), and domestic grazing is recognized as a major factor in the maintenance of many Mediterranean open habitats (Noy-Meir et al. 1989; Perevolotsky & Seligman 1998; Fig. 1). Because

of a history of livestock grazing, Mediterranean herbaceous communities are considered highly resilient to stocking rate variability (Sternberg et al. 2000) although overgrazing can lead to soil degradation and desertification (Sales-Baptista et al. 2016). Grazing abandonment may cause major shifts in plant and invertebrate communities and to shrub encroachment (Saatkamp et al. 2010).

Techniques to Overcome Constraints for the Restoration of Northwestern Mediterranean Grassland Plant Communities

Opportunities and Limits of Soil Transfer

After major soil disturbance (e.g. road construction, quarries, pipeline laying), Mediterranean grassland recovery may be very slow (Römermann et al. 2005; Pueyo et al. 2009; Coiffait-Gombault et al. 2011; Helm et al. 2019). While finding commercial seed sources of local Mediterranean species is still a challenge, soil transfer, as loose soil or as turf, has already produced encouraging results, at least over shorttime periods, as compared to other ecological restoration techniques (e.g. hay transfer; Jaunatre et al. 2014a). Soil transfer is defined as the translocation of soil material and its native biota. It involves the transfer of the soil substrate and its assemblage of species from an intact donor site to an area requiring restoration (Pywell et al. 1995). This may not only overcome dispersal limitation, but also some other biotic and abiotic filters that constrain the establishment and development of plant communities (Lortie et al. 2004; Fig. 1). The transfer of a soil from a donor to a restoration site provides physico-chemical elements, soil organic matter, and a part of the pedofauna, flora, and microbial biomass (bacterial, algal, and fungal; Wubs et al. 2016). Soil transfer has proven useful for the recovery of species-rich Mediterranean grasslands on typical Mediterranean soils (e.g. Haplic Cambisol), in various situations: (1) restoration of abandoned intensive orchards (Jaunatre et al. 2014a), (2) after a pipeline leak (Bulot et al. 2014b; Bulot et al. 2017), or even (3) long-term restoration of abandoned dry quarries (Chenot et al. 2017).

Stockpiling of soil may lead to the colonization by weeds (i.e. undesirable native ruderals or invasives) often promoted (1) directly, by piles collecting seed rain, and (2) indirectly, by the release of nitrate due to accelerated decomposition of organic matter (Bruehlheide & Flintrop 2000) during stockpiling. Translocated soil, even without stockpiling, may also carry a higher nutrient content due to the disturbance of soil structure (Bruehlheide & Flintrop 2000), and compaction that may occur during transfer by construction machineries (Trueman et al. 2007). Direct or timely transfers may minimize the loss of propagule viability, soil structure, or microbial biomass (Anderson et al. 2008).

Despite these advantages, soil transfer should be applied with caution, because soil formation is a long-term process and soils are not a renewable resource. Mediterranean soils often develop over thousands of years (Molliex et al. 2013), and destruction may be effectively irreversible. Soil transfer should be limited

to cases in which the destruction of the soil of the donor site is preplanned or has already occurred independently of the restoration project (Bulot et al. 2014b). Moreover, all transfers of large quantities of soil require the use of unsustainable civil engineering methods (i.e. hydrocarbon consumption, pollutant emissions, soil alteration, and compaction).

To limit the environmental impact, soil should be transferred without the delays associated with stocking (Bulot et al. 2014b; Bulot et al. 2017; Buisson et al. 2018). Dilution of loose soil reduces the volume of soil removed or restores larger areas (Jaunatre et al. 2014a; Dutoit et al. 2019a). Transferring scattered turfs that would allow perennial plants to survive transfers and act as nuclei for plant recolonization has provided inconsistent results (Dutoit et al. 2019b).

Establishment of Key Species

Low seedling recruitment in Mediterranean grasslands may result from a low number of available seeds and/or drought-mediated low germination and establishment. To optimize restoration strategies, knowledge of these underlying mechanisms is required. For example, if recruitment is seed-limited, seed addition is the best strategy, but if the density of viable seeds is sufficient, priority should be given to an improvement of environmental conditions, or timing of seeding. In the following sections, we explore the use of population biology and population genetic methods in ecological restoration and explain their particular importance in Mediterranean systems using *Brachypodium retusum* as a model species. In NwMedB grasslands, *B. retusum* is the dominant species showing numerous interactions with other typical species (Saiz & Alados 2011). Its establishment and performance are important indicators of restoration success.

Population Genetics and Seed Provenance

Hay transfer using local species-rich source communities may be a cost-efficient restoration technique. Its success depends on hay seed density (number of seeds/m²) and competition at the restoration site (whether sown on bare ground or within settled vegetation) (Coiffait-Gombault et al. 2011; Jaunatre et al. 2014b; Fig. 1). The effectiveness of hay transfer in different seasons has not been studied, but most likely the transfer or sowing of seeds just before the first autumn rains is adequate to maximize germination while minimizing seed loss by ants or wind. Appropriate source communities are not always available or may not contain enough viable seeds of the desired species (Kiehl et al. 2010; Coiffait-Gombault et al. 2011). Active restoration by sowing or planting often requires the purchase of plant commercial material, usually as seed (Vander Mijnsbrugge et al. 2010). This plant material is often not strictly local since seed production is usually based on relatively few populations, raising the question of how local the seed material used in ecological restoration needs to be (McKay et al. 2005).

Vidaller et al. (2018) demonstrated that adaptive population differentiation in germination and early growth of *B. retusum* contributes to different colonization patterns, and that the

provenance needs to be carefully considered in restoration planning. Summer drought, stone cover, and grazing intensity are major drivers of adaptation in this species (Vidaller et al. 2018). Thus, populations originating from sites with higher summer rainfall or lower grazing pressure may not establish as well on restoration sites with strong summer drought or high grazing pressure. However, locally adapted does not necessarily mean geographically close. Distant populations growing in similar environments may be more appropriate for sowing at a given restoration site (Bischoff 2014). Additionally, even local seed sources may be inappropriate if inbred and genetically impoverished (Broadhurst et al. 2008), or if changing climate has changed (or will change) the local climatic envelope (Durka et al. 2017).

Conditions of Establishment and Their Manipulation to Improve Restoration Success

In NwMedB grasslands, soil stability, water availability, and grazing regimes are known to be limiting factors for plant species establishment (Cortina et al. 2009; Pueyo et al. 2009; Vidaller et al. 2019a, 2019b; Fig. 1). An increase in soil water availability generally favors seedling recruitment (Bochet et al. 2007; Pueyo et al. 2009), but germination is not necessarily moisture-limited if seeds are sown in autumn (Vidaller et al. 2019a). A higher soil moisture may also increase competition by ruderal species outcompeting desirable dry grassland species (Masson et al. 2015). Traditional grazing in winter and spring is essential to maintain NwMedB grasslands but can cause considerable damage by increasing plant mortality and decreasing plant performance in the early stages of restoration (Buisson et al. 2015; Vidaller et al. 2019a). Initial watering has a positive effect on *B. retusum* performance in grazed but not in ungrazed plots, suggesting that watering may compensate for grazing stress. Grazing can reduce seedling survival and performance over at least 2 years (Vidaller et al. 2019a). Bunchgrasses, such as *Lygeum spartum*, can be successfully reintroduced to degraded grasslands if environmental conditions are improved (e.g. decreasing water runoff and increasing water availability; Pueyo et al. 2009). *Lygeum spartum* and *Stipa tenacissima* have the potential to reduce soil erosion and improve their nearby environment and thus facilitate the establishment of other species (Cortina et al. 2009; Nedjimi 2016).

The role of fire is less clear than that of grazing but several studies have shown that typical Mediterranean grassland species benefit from occasional fire (Naveh 1975; Grigulis et al. 2005; Vidaller et al. 2019a), especially from summer fire mimicking natural fires (Vidaller et al. 2019b), suggesting that a trade-off similar to grazing may be assumed for fire. High fire frequency may reduce performance and survival but occasional fires may help to preserve grasslands, in particular if sites are under-grazed or if grazing has been abandoned (Vidaller et al. 2019b). Fire strongly increases seed production of *B. retusum* and *Ampelodesmos mauritanica* (Grigulis et al. 2005; Vidaller et al. 2019b) and has a positive effect on community diversity (Incerti et al. 2013; Vidaller et al. 2019b). This suggests that an adequate fire regime, in particular summer fire, is a key factor in

preserving and restoring steppe ecosystems (Fig. 1). Moreover, fire may replace grazing as a conservation measure since the species composition of abandoned grasslands showed a post-fire community shift toward typical steppe communities (Vidaller et al. 2019b). Managing strategies, including type, intensity, and timing of grazing, prescribed burning or watering, need to be based on reliable information on the specific influence of these factors on plant populations.

On soils with high nutrient content, competition by nontarget species but also competition with over-abundant target species (e.g. with *Anisantha* spp.) may decrease perennial species establishment and growth (Buisson et al. 2015) as well as annual species establishment (Jaunatre et al. 2014a). Topsoil removal (or other soil impoverishment techniques) decreases soil nutrient content and arable weed seed bank, and may improve target species establishment (Jaunatre et al. 2014b).

Using Animals to Restore Plant Communities

Constraints in the restoration of Mediterranean grasslands may be overcome by the promotion or reintroduction of animals considered ecological engineers. These organisms directly or indirectly modulate the availability of resources to other species by causing physical state changes in biotic and abiotic materials (Jones et al. 1994), and they can contribute to the resilience of ecosystems after disturbances (Peterson et al. 1998). Although few studies have experimentally tested the use of ecological engineers to restore Mediterranean grasslands (e.g. Bulot et al. 2014a; De Almeida et al. 2020), we suggest several promising lines of research in the following sections.

Seed Dispersal

Seed dispersal is a central plant–animal mutualism influencing seedling recruitment, population dynamics, species distributions, plant-community composition, and gene flow (Nathan & Muller-Landau 2000). Seed dispersal can also reduce competition with the parent plant, and losses to seed predators and pathogens (Howe & Smallwood 1982).

Arthropods are ubiquitous, and one of the most important animal groups involved in seed dispersal and establishment (Lengyel et al. 2009). Among arthropods, ants are the primary seed dispersers in terrestrial ecosystems (Wills & Landis 2018) including Mediterranean grasslands. In these grasslands, species from the genus *Messor* are the most abundant granivorous ants (Cerdá & Retana 1994; Gomez & Espadaler 1994; Azcárate & Peco 2007). Depending on the plant species, 46–100% of the newly produced seeds can be removed by these harvester ants (Westerman et al. 2012). Only a small percentage of these is dropped on the way and dispersed, but an ant colony can transport up to 50,000 seeds a day and the average number of nests per hectare can reach 200 (Detrain & Tasse 2000; Arnan et al. 2010). In the Mediterranean region, foraging tracks of more than 80 m have been observed for the ant species *Messor barbarus* (Detrain et al. 1996), which may increase the potential of plant recovery from the nearby reference site. Within this context, where *M. barbarus* cannot naturally colonize, its

reintroduction using mated queens already tested by Bulot et al. (2014a) may be a valuable tool to restore its populations (De Almeida et al. 2020).

Seedling Recruitment

Seed dispersal, especially by ants, benefits plants not only by reducing competition and predation, or minimizing the effects of fire through burial, but also by depositing seeds in more productive sites (Ness & Bronstein 2004). Granivorous ants are ecologically important because of their strong effects on plant communities, including nutrient cycling and microclimate modification. Although they do directly prey upon seeds (Schöning et al. 2004), they play a key dispersal role when they accidentally drop seeds, during the transport, in microsites with suitable conditions for germination (Detrain & Tasse 2000) or in refuse piles, increasing seedling recruitment (Retana et al. 2004; Azcárate & Peco 2007; Bulot et al. 2016).

Bulot et al. (2014a) experimentally reintroduced *M. barbarus* to later test their potential as ecosystem engineers. This ant species was chosen based on its key functions (see section above) in Mediterranean grasslands (Azcárate & Peco 2007). Seven years after the establishment of the first colonies, ants increased soil nutrients. They accelerated the ecological recovery of Mediterranean dry grassland plants by directly and indirectly facilitating their reestablishment (De Almeida et al. 2020). This innovative experiment provides a promising option in using invertebrates to overcome seedling limitation in Mediterranean grassland restoration.

Grazing Management

In cases of long-term high and destructive livestock pressure, grassland restoration may require the temporary exclusion of grazing. However, while grazing exclusion has initial positive effects including the recovery of the herbaceous cover and an increase in species richness, it can also lead to a decrease in plant species richness and to shrub encroachment (Golodets et al. 2010; Saatkamp et al. 2010). Grazing abandonment may lead to a reduction in the number and cover of small, early flowering and annual species and to their replacement by perennials, in particular graminoids (Noy-Meir et al. 1989; Peco et al. 2006b; Peco et al. 2012; Mesléard et al. 2017; Saatkamp et al. 2018). It may further decrease soil multifunctionality (Peco et al. 2017). Grazing exclusion also affects arthropod communities by (1) increasing ant and beetle species richness (Blight et al. 2011; Azcárate & Peco 2012), (2) shifting beetle composition toward assemblages less typical of NwMedB grasslands (Fadda et al. 2008; García-Tejero et al. 2013), or (3) decreasing Orthoptera abundance (Fonderflick et al. 2014). The decrease in livestock is now considered a major cause of changes in Mediterranean landscapes (Perevolotsky & Seligman 1998) and livestock has been reintroduced to preserve or restore open habitats in protected areas and in many rangelands where it had disappeared or declined. Traditional grazing is used in NwMedB grasslands to control colonization by woody species and to enhance species richness, in particular that of annual or

subordinate species (Bernués et al. 2005; Mesléard et al. 2017; Koerner et al. 2018) as well as for the conservation of threatened species (Bröder et al. 2019).

Grazing can therefore be a major restoration tool and must be carefully considered in restoration planning, as its outcome may depend on productivity (de Bello et al. 2007). It should be adapted to fluctuating environmental conditions linked to the Mediterranean climate, particularly stochastic rainfall events, and to climate change which increases the frequency of such events (Carmona et al. 2012). Drought may reduce the number of species by limiting germination and survival or, in contrast, favor the coexistence of species by the reduction of dominant plant cover (Espigares & Peco 1995; Pérez-Camacho et al. 2012). To avoid soil degradation, desertification or shrub encroachment, grazing intensity should be reduced in dry years and, at the opposite, increased when a lot of fodder is available.

Conclusion and Research Perspectives

Much research is still needed to maximize NwMedB grassland restoration success (Fig. 1):

Dispersal

Both wild and domestic vertebrates are seed dispersers in dry grasslands (Benthien et al. 2016) because they facilitate seed dispersal in two ways: ingestion of seeds or transport of seeds attached to their fur. Studies on dispersal by mammals remain scarce in NwMedB grasslands (Malo & Suárez 1995, 1996), and dispersal distances are rarely evaluated although most grasslands are grazed. Further research should focus on evaluating the fate of seeds transported by both vertebrate and invertebrate animals, on its contribution to restoration, and on the spatial implementation of grazing in order to optimize seed dispersal.

Plant Material

Ecological restoration projects in the Mediterranean Basin still lack wider use of native species of local provenance (Nunes et al. 2016). Research is needed on the population biology and propagation of keystone species. Incentives for the use of local populations in restoration projects (e.g. the French label “*végétal local*”; Malaval et al. 2015) should continue to be supported. Combining studies of neutral markers (not selected by environmental conditions: amplified fragment length polymorphism, microsatellites) and adaptive quantitative traits (under selection by environmental conditions) for NwMedB grassland plants will help improve our understanding of the relative importance of drift and adaptive processes to the overall genetic differentiation among populations (Vidaller et al. 2020).

Soil Transfer and the Soil Black Box

Because many annual species germinate with autumn rains, it is likely that topsoil transfer carried out at the end of summer, right before the first rains, would maximize recovery from the seed bank. While spring and autumn soil transfers have been

implemented and compared in the same area (Buisson et al. 2018), late summer transfer remains to be tested using an experimental design that allows a comparison of transfers in different seasons.

Soil inversion (burial of the topsoil under a layer of subsoil) has been used in temperate grassland restoration to decrease soil nutrients and reduce the seed bank of undesirable species (Glen et al. 2017). Various versions of this (compaction, soil inversion, topsoil removal) should be tested on the same Mediterranean ecosystem-type using similar protocols.

Establishment

Other less-studied organisms can play a role in seedling recruitment, such as nurse plant species (Cortina et al. 2009; Incerti et al. 2013) or earthworms. The latter have been used in mesic grassland restoration (Butt 2008) to improve soil structure and physico-chemistry and to ingest seeds that are later deposited in their casts (Forey et al. 2011). These seeds show higher establishment in this nutrient-rich biostructure than those of the surrounding soil seed bank (Lavelle et al. 2006). Although no similar studies have been carried out in Mediterranean grasslands, we might expect a similar influence of earthworms on their plant communities.

Also, further research of safe sites to improve germination and seedling establishment should be conducted. Their positive role (e.g. modifying microclimate) in the revegetation process, in interaction with grazing, has been shown in stressful environments (Buisson et al. 2015).

Occasional spring droughts may lead to high mortality of perennial species (Buisson et al. 2015) but the effects of such droughts on reproductive success of annual species and on the community composition of the following year still has to be evaluated, taking into account the phenological stage at which such events occur.

Fire is already used as a grassland management or restoration tool under various climates (Bond & Keeley 2005; Pyke et al. 2010). These studies have shown that fire-prone ecosystem restoration requires appropriate fire regimes for species establishment and survival. Such knowledge is not available for Mediterranean grasslands because the use of fire has been abandoned for more than a century and is now controversial. Detailed studies on the effect of various fire regimes are needed to best inform restoration and conservation (Vidaller et al. 2019b).

Grassland Invertebrates

In the Mediterranean Basin, arthropods, such as spiders (García et al. 2009), beetles (Alignan et al. 2018b), and Orthoptera (Alignan et al. 2014, 2018a) are used as bioindicators of restoration success because of their high sensitivity to changes in vegetation structure (height and density) or composition. Continuing to explore plant-arthropod relationships and ecosystem engineers, such as ants and earthworms, will contribute to a more integrative response for overcoming key constraints in NWMedB grassland restoration.

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Supporting Information

The following information may be found in the online version of this article:

Figure S1. Natura 2000 sites containing habitat #6220 are represented as green dots on a map of all Natura 2000 areas.

Table S1. Type of grasslands covered by the paper and for which the paper may be relevant.

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Abstract

The main objective of this thesis was double: (i) to assess the impact of an ant species on its ecosystem, in order to (ii) deduce potential applications in the field of ecological restoration.

Ants are among the most abundant organisms in terrestrial ecosystems and occupy a wide range of geographical areas. They play key ecological roles in many ecosystems as soil engineers, predators or regulators of plant growth and reproduction. However, the information collected locally is often fragmented and does not provide a complete overview of the impact of a species on its environment.

Messor barbarus (L.), known to redistribute seeds and to modify the soil physico-chemical properties, is widespread in South-Western Europe, particularly in Mediterranean grasslands. Therefore, it may play a major role in the composition and structuring of these ecosystems, which are characterised by high biodiversity but whose abundance and surface area have decreased drastically in recent decades.

Through a multi-compartment study, we confirmed the hypothesis that *M. barbarus* is an ecological engineer in Mediterranean grasslands. This species changes this habitat by modifying, as expected, soil physico-chemical properties. These modifications are associated with an increase in both biomass and heterogeneity of plant communities, as well as changes in above- and belowground fauna (abundance, occurrence and structure of communities). *Messor barbarus* profoundly changes trophic and non-trophic relationships within and between species and their habitat. The heterogeneity created locally by the activity of *M. barbarus* leads to a diversification of ecological niches within these grasslands.

Despite their major role in the functioning of ecosystems, ants are rarely considered in restoration ecology. In our study site, corresponding to a dry grassland rehabilitated after an oil leak and a soil transfer, *M. barbarus* contributed to accelerate the restoration of the soil physico-chemical properties but also of the seed bank in the medium term - seven years after the rehabilitation. These results make this species a good candidate for ecological engineering.

In order to generalise the use of ants in restoration ecology, we propose a trait-based methodology for stakeholders. We evaluated the potential of ants in restoration ecology, then listed all the traits known to affect abiotic and biotic compartments and/or relevant to monitor the success of the restoration phase. The proposed methodology provides a first selection of potentially relevant species according to the restoration objectives.

Keywords: Ecosystem engineer, ants, Mediterranean grasslands, ecological restoration, interactions, soil physico-chemical properties, plant communities, fauna, functional and life history traits

Résumé

L'objectif principal de cette thèse était double : (i) mesurer l'impact d'une espèce de fourmi sur son écosystème, afin (ii) d'en déduire des applications potentielles dans le domaine de la restauration écologique.

Les fourmis sont parmi les organismes les plus abondants des écosystèmes terrestres et occupent des zones géographiques très variées. Elles jouent des rôles écologiques clés dans de nombreux écosystèmes comme ingénieurs du sol, prédateurs ou régulateurs de la croissance et de la reproduction des plantes. Cependant les données collectées localement sont souvent parcellaires et ne permettent pas d'avoir une vision complète de l'impact d'une espèce sur son milieu.

Messor barbarus (L.), connue pour redistribuer les graines et pour modifier les propriétés physico-chimiques du sol, est largement répandue dans le Sud-Ouest de l'Europe notamment au sein des pelouses méditerranéennes. Elle pourrait donc jouer un rôle majeur dans la composition et structuration de ces pelouses caractérisées par une forte biodiversité mais dont le nombre et la superficie ont drastiquement diminué ces dernières décennies.

Dans un premier temps, par une étude multi-compartiments, nous avons confirmé l'hypothèse selon laquelle *M. barbarus* est une ingénieure de l'écosystème au sein des pelouses méditerranéennes. Elle transforme cet habitat en modifiant, comme attendu, les propriétés physico-chimiques du sol. Ces modifications sont associées à une augmentation de la biomasse et de l'hétérogénéité des communautés végétales ainsi qu'à des changements dans les faunes épigée et endogée (abondance, occurrence et structure des communautés). De plus, *M. barbarus* modifie profondément les relations trophiques et non trophiques interspécifiques et entre les espèces et leur habitat. L'hétérogénéité créée à l'échelle locale par l'activité de cette fourmi, entraîne une diversification des niches écologiques au sein de ces pelouses.

Malgré leur rôle souvent majeur sur le fonctionnement des écosystèmes, les fourmis ne sont que très rarement considérées en restauration écologique. Sur notre site d'étude, un chantier de réhabilitation d'une pelouse sèche après une fuite d'hydrocarbures et un transfert de sol, *M. barbarus* a permis d'accélérer la restauration des propriétés physico-chimiques du sol mais aussi de la banque de graines à moyen terme - sept ans après la réhabilitation du site. Ces résultats font donc de cette espèce une bonne candidate en ingénierie écologique.

Afin de généraliser l'utilisation des fourmis en restauration écologique, nous proposons une méthodologie à destination des gestionnaires basée sur l'utilisation de traits fonctionnels et d'histoire de vie. Pour cela nous avons évalué le potentiel des fourmis en écologie de la restauration, puis nous avons listé l'ensemble des traits connus pour affecter les compartiments abiotiques et biotiques et/ou pertinent pour effectuer un suivi du succès de la phase de restauration. La méthodologie proposée permet une première sélection des espèces potentiellement utilisables en fonction des objectifs de restauration.

Mots-clés : Ingénieur de l'écosystème, fourmis, pelouses méditerranéennes, restauration écologique, interactions, propriétés physico-chimiques du sol, communautés végétales, faune, traits fonctionnels et d'histoire de vie