Évaluation de la contribution fonctionnelle des espèces lombriciennes anéciques à la décomposition des litières prairiales : variabilité inter-espèces au sein d'une même catégorie écologique

Kevin Hoeffner

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Par
Kevin HOEFFNER

Évaluation de la contribution fonctionnelle des espèces lombriciennes anéciques à la décomposition des litières prairiales

Variabilité inter-espèces au sein d'une même catégorie écologique

Thèse présentée et soutenue à la Station Biologique de Paimpont, le 18/12/2018
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“The show must go on !” – Queen 1991

Liste des articles

I. Hoeffner K., Santonja M., Monard C., Barbe L., Le Moing M., Cluzeau D. (2019). Soil properties, grassland management and landscape diversity drive the assembly of earthworm communities in temperate grassland. En révision dans Pedosphere.

II. Hoeffner K., Butt K., Monard C., van Groenigen J. W., Cluzeau D. (2018). A review of interactions between temperate earthworms and organic matter with a focus on sub-divisions of the anecic earthworm category. En préparation pour Pedobiologia.

III. Hoeffner K., Monard C., Santonja M. Cluzeau D. (2018). Feeding behaviour of epi-anecic earthworm species and their impacts on soil microbial communities. Soil Biology and Biochemistry 125 1–9, doi : 10.1016/j.soilbio.2018.06.017.

IV. Hoeffner K., Santonja M. Cluzeau D, Monard C. (2019). Epi-anecic rather than strict-anecic earthworms enhance soil enzymatic activities. Soil Biology and Biochemistry 132 (2019) 93–100, DOI : 10.1016/j.soilbio.2019.02.001

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INTRODUCTION

A. Enjeux liés au système sol

1. Les sols supports de services écosystémiques

La zone critique comprend la surface terrestre, le couvert végétal et l'ensemble des réservoirs d'eau (rivières, lacs, mers peu profondes ; National Research Council, 2000). Il s'agit donc d'un environnement hétérogène dans lequel des interactions complexes entre la roche, l'eau, l'air et les organismes vivants déterminent la disponibilité des ressources nécessaires à la vie (Brantley et al., 2007; Chorover et al., 2007). A l'interface de la biosphère, de l'hydrosphère et de la lithosphère, le sol a une place centrale au sein de la zone critique (National Research Council, 2000). Le développement des sociétés humaines est en grande partie lié directement ou indirectement aux sols (*e.g.* nourriture, fibres, eau potable, air…). La formalisation des bénéfices que prodiguent les écosystèmes aux sociétés humaines sous le concept de services écosystémiques (Millennium Ecosystem Assessment, 2005), a mis en exergue le rôle multifonctionnel de la zone critique en général et du sol en particulier (Daily, 1997; Blum et al., 2006; Haygarth and Ritz, 2009; Dominati et al., 2010).

A ce jour, la communauté scientifique n'est cependant pas parvenue à un consensus sur une définition précise des services écosystémiques permettant d'envisager toute leur diversité et leur complexité (Costanza et al., 1997; De Groot et al., 2002; Kettunen and Brink, 2006; Wallace, 2007; Maes et al., 2012; Maris, 2014). Au niveau sociétal, les usagers et les bénéficiaires de ces services se sont encore relativement peu appropriés ce concept, qui reste majoritairement réservé à la recherche scientifique (Lamarque et al., 2011; Bispo et al., 2016). Ainsi, en France, la loi Biodiversité de 2016 mentionne les services écosystémiques générés par « Les espaces, ressources et milieux naturels terrestres et marins, les sites, les paysages diurnes et nocturnes, la qualité de l'air, les êtres vivants et la biodiversité » sans pour autant les définir (Article L110-1).

Chaque service écosystémique repose sur des interactions biotiques ou abiotiques complexes constituant des processus écosystémiques (*i.e.* transfert d'énergie ou de matière ; Lyons et al., 2005; Wallace, 2007). Ces processus écosystémiques ne sont pas spécifiquement liés à un seul service mais peuvent contribuer à plusieurs d'entre eux (Wallace, 2007). Les services écosystémiques sont généralement répartis en quatre catégories (Fig. 1) : des services d'approvisionnement (*e.g.* eau douce, ressources génétiques, aliments, matériaux…), des services de régulation (*e.g.* climat, qualité de l'eau, cycles biogéochimiques…), des services culturels (*e.g.* esthétiques, spirituels, éducatifs…) et des services de soutien aux conditions favorables à la vie sur terre (*e.g.* production de dioxygène, d'habitats…). Les services appartenant à cette dernière catégorie de service sont nécessaires à la production des autres services. Leurs effets sont donc souvent indirects ou apparaissent sur le long terme.

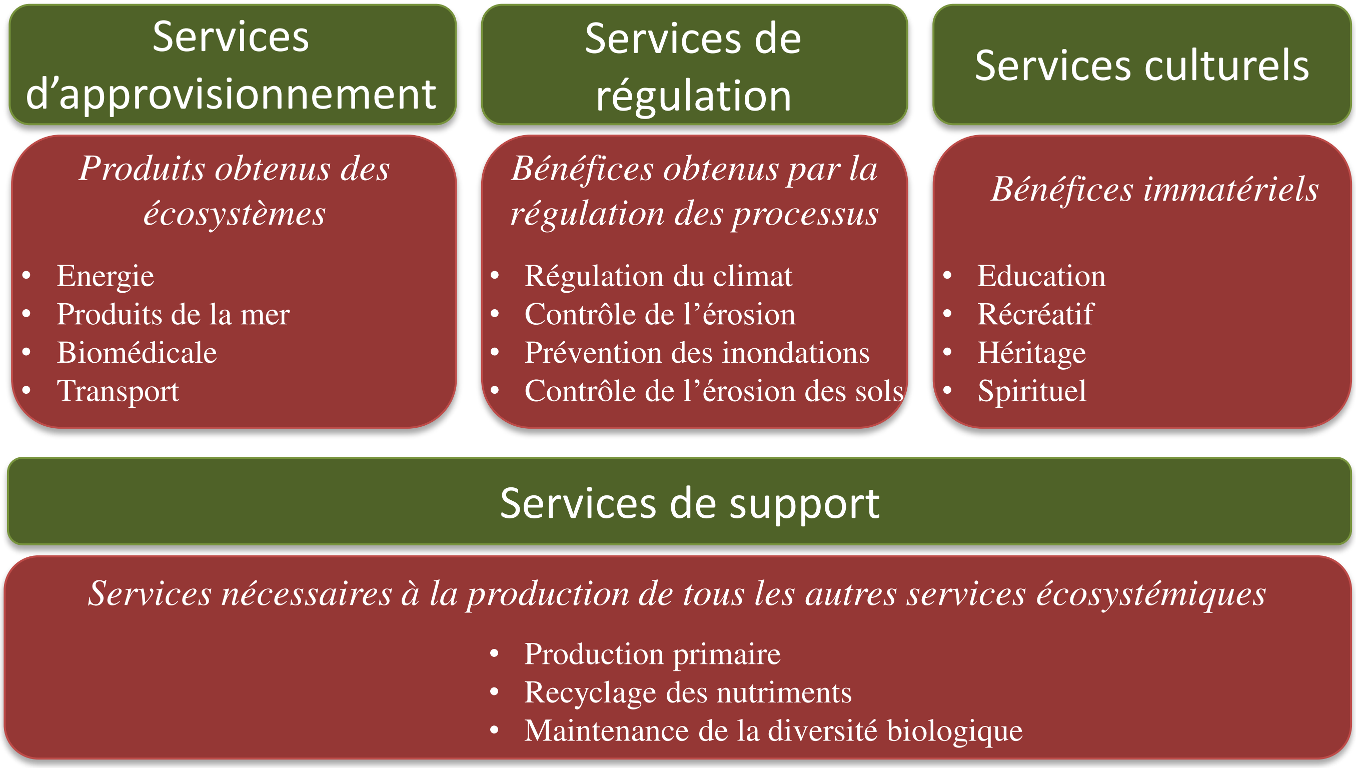

Figure 1. Description des services écosystémiques d'après le Millenium Ecosystem Assessment (2005).

A titre d'exemple, en étant le support de la production végétale, les sols contribuent à la production agricole (végétale et animale) qui couvre 90% des besoins nutritionnels de la population humaine (FAOSTAT, 2004). Les sols sont également des régulateurs majeurs du climat car ils contiennent environ 2.344 Gt de carbone organique dans leurs trois premiers mètres de profondeur dont 1.500 Gt dans le premier mètre (Jobbágy and Jackson, 2000; Guo and Gifford, 2002). Les sols sont ainsi le deuxième plus grand réservoir de carbone derrière les océans (38.400 Gt), contenant presque quatre fois plus de carbone que la biosphère (560 Gt) ou l'atmosphère (760 Gt ; Lal, 2004; Stockmann et al., 2013). De faibles variations des stocks de carbone organique des sols peuvent donc influencer significativement la concentration en CO_2 de l'atmosphère et, par conséquent, le réchauffement climatique (Kirschbaum, 2000). Par exemple, une libération de 10% du carbone organique des sols serait l'équivalent de 30 ans d'émission de gaz à effet de serre d'origine humaine (Kirschbaum, 2000).

Les sols sont composés d'une fraction minérale et d'une fraction organique qui définissent ses propriétés physiques (*e.g.* texture, types d'argiles, profondeur…), chimiques (*e.g.* concentration en nutriments, carbone, pH…) et biologiques (*i.e.* flore, faune, microorganismes ; Lavelle and Spain, 2001). Les propriétés des sols se forment également sous l'influence conjointe de facteurs lithologiques, topographiques, climatiques et biologiques qui interagissent au fil du temps pour conditionner la nature, la direction et la vitesse des processus de formation des sols (Jenny, 1980, 1994; Lavelle and Spain, 2001). L'expression variée et complexe de ces facteurs de formation des sols a conduit au développement de 32 grands types de sol à l'échelle du globe, chacun compartimenté en sous catégories avec des propriétés uniques (IUSS Working Group, 2015). Les processus qui mènent à la fourniture de services écosystémiques des sols sont en grande partie déterminés par ces propriétés (Fig. 2 ; de Groot et al., 2010; Dominati et al., 2010). Ainsi, il existe une diversité de services écosystémiques spécifique à chaque sol.

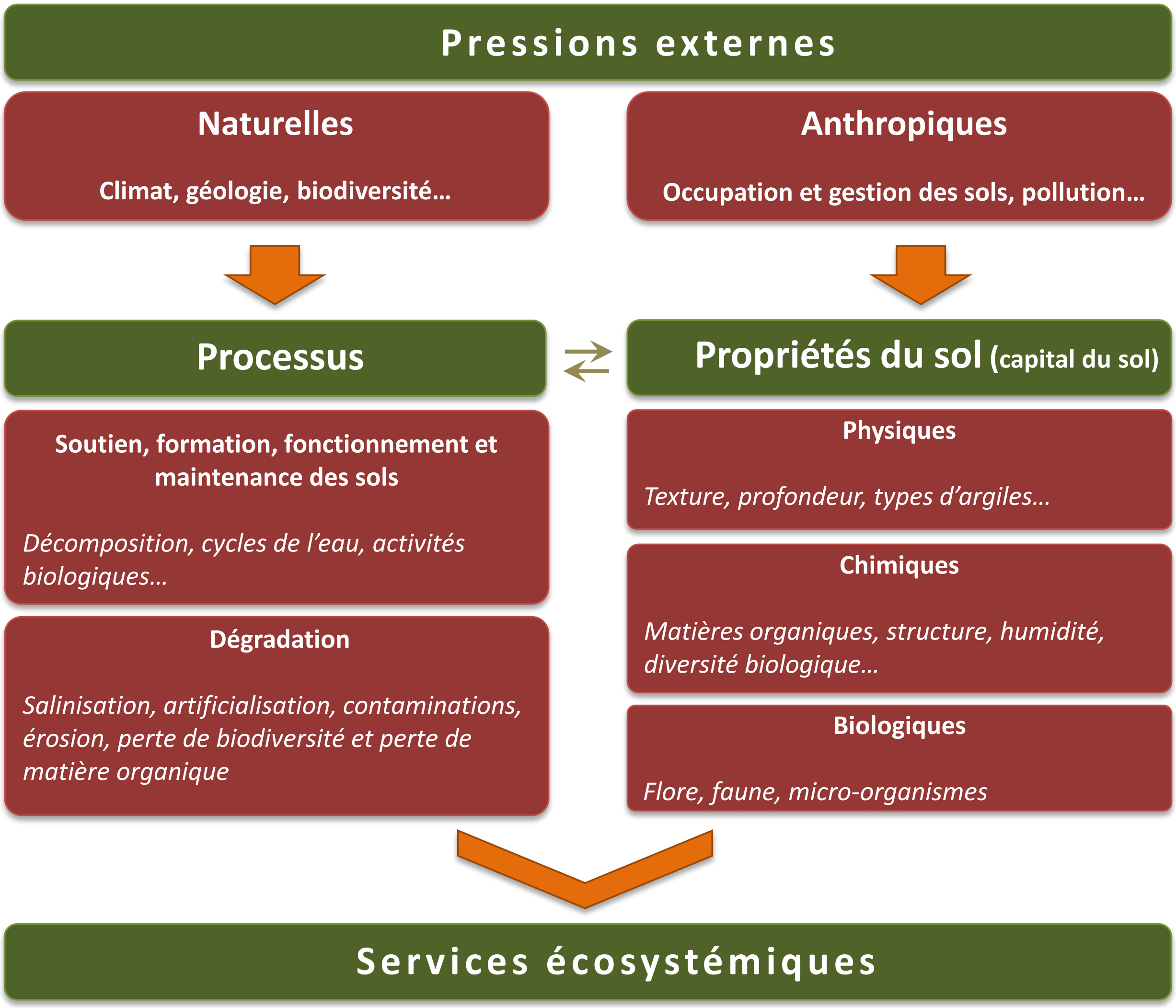

Figure 2. Processus et services écosystémiques fournis par les sols, d'après Dominati et al. (2010).

2. Les menaces sur les sols

La vitesse de formation des sols est très lente, de l'ordre de 114.3 ± 10.9 mm. an^{-1} à l'échelle du globe (Stockmann et al., 2014). Les sols sont donc non-renouvelables à l'échelle humaine puisqu'en en climat tempéré, leur formation prend entre 10 000 et 100 000 ans (Duchaufour, 1982). Les sols sont cependant exposés à huit menaces (Jones et al., 2012; Stolte et al., 2015; Orgiazzi et al., 2016) : la salinisation, l'artificialisation, les contaminations, l'érosion, la perte de biodiversité et la perte de matière organique. Ces pressions sur les sols sont de plus en plus importantes sous l'effet du réchauffement climatique et de l'accroissement de la population mondiale dont les besoins basiques augmentent (nourriture, eau potable, fibres, air, infrastructures). Compte tenu de ces menaces, les politiques publiques s'orientent de plus en plus à se saisir des enjeux de préservation des sols. Cependant, à l'échelle internationale, la seule protection qui intègre les sols est la convention Ramsar adoptée en 1971 pour la conservation et l'utilisation durable des zones humides, ces dernières étant définies en partie par la nature de leurs sols. Ratifiée par 170 pays, la convention constitue cependant davantage un engagement des Etats vers le développement de politiques de conservation des zones humides qu'une contrainte juridique forte sur l'utilisation des sols. Depuis 2002, les Nations Unies célèbrent tous les 5 décembre la Journée Mondiale des Sols afin de sensibiliser la population mondiale à l'importance des services écosystémiques rendus par les sols et d'accroître leur visibilité et leur préservation. Dans cette lignée, l'Organisation des Nations Unies pour l'alimentation et l'agriculture (FAO) a célébré en 2015 l'année internationale des sols. Au niveau européen, la Charte des Sols de 1972 a posé les bases d'une approche multifonctionnelle des sols et expose les premiers éléments d'une potentielle protection mais sans réelles contraintes juridiques. De plus, le retrait du projet de la Directive Cadre sur la protection des sols en 2014 par la Commission Européenne a annulé durablement la reconnaissance des sols comme ressource à protéger au même titre que l'eau (Directive Cadre sur l'Eau) et l'air (Directive Cadre sur l'Air). Lors de la COP 21 et des accords de Paris sur le climat de 2015, la France a lancé l'initiative « 4pour1000, les sols pour la sécurité alimentaire et le climat » qui a pour objectif de compenser les émissions mondiales de gaz à effet de serre liées à l'utilisation des énergies fossiles, en augmentant la quantité de carbone stockée dans les sols de 4‰ chaque année (Lal, 2016; Minasny et al., 2017). Cette petite augmentation suffirait à accroître le pouvoir tampon des sols vis-à-vis du réchauffement climatique.

Les processus associés au sol (*e.g.* formation des sols, cycles biogéochimiques, activité biologique…) sont donc à la base des services écosystémiques fournis par les sols. Ainsi, mieux connaître, préserver, voire accroître ces processus apparaît comme un des enjeux planétaire majeurs.

3. La matière organique des sols

La matière organique des sols (MOS) est composée de différents compartiments : la matière organique vivante (MOV, *i.e.* flore, faune et micro-organismes vivants), la matière organique particulaire (MOP, dont l'origine peut encore être identifiée, *e.g.* débris de végétaux ou d'animaux) et l'humus (matière organique stabilisée ; Bachelier, 1978; Duchaufour, 1982). La matière organique vivante ne représente que 2 % à 12% de la MOS (Bachelier, 1978; Gobat et al., 2010). Toute cette biomasse vivante finit par mourir et est alors soumise aux processus de décomposition. La majorité des entrées de la MOS est issue de la végétation (parties aériennes et souterraines ; Kögel-Knabner, 2002). Au sein des écosystèmes terrestres, jusqu'à 90% de la production primaire tombe à la surface du sol sous forme de litière végétale (Cebrian, 1999), ce qui peut atteindre jusqu'à 3.5 x 10^{10} tonnes par an (Meentemeyer et al., 1982). Les litières végétales sont donc des éléments incontournables d'apport de matière organique dans les sols. Au cours de leur décomposition, des éléments nutritifs (*e.g.* N, P, K…) sont libérés et contribuent à la formation de la MOP et à l'humus. Ce dernier compartiment constitue la matière organique la plus stable et qui est généralement associée aux argiles pour former le complexe argilo-humique (Stevenson, 1994). La taille des compartiments de MOP et d'humus résulte d'un équilibre entre les entrées (*e.g.* litière, racines, animaux morts…) et les sorties (*e.g.* minéralisation par les microorganismes, lessivage…) de matières organiques (Swift et al., 1979; Scholes et al., 1997).

De manière générale, les MOS sont impliquées dans de nombreux services écosystémiques (Calvet et al., 2011). En effet, elles modifient les propriétés physiques des sols, contribuant à sa perméabilité, son aération et sa stabilité (Tisdall and Oades, 1982; Chaney and Swift, 1984; Chenu and Guérif, 1991; Chenu et al., 2000). Les MOS interviennent également dans divers cycles biogéochimiques (*e.g.* C, N, P…) et dans la régulation du climat (Frank et al., 2017; Zomer et al., 2017). Les flux de carbone échangés en permanence entre les sols et l'atmosphère (*e.g.* CO_2, CH_4, COVs…) contribuent soit au stockage du carbone (stabilisation de la MOS) soit au contraire à l'émission de gaz à effet de serre (accessible aux micro-organismes ; Lal, 2004; Stockmann et al., 2013). Le bilan de ces flux peut affecter le climat (Heimann and Reichstein, 2008; Ahlström et al., 2012). La quantité de carbone organique dans les sols peut être évaluée en utilisant la quantité de MOS, puisqu'il est généralement admis que 1 g de carbone organique équivaut à 1.73 g de MOS (Stevenson, 1994). Ces matières organiques des sols constituant également un réservoir nutritif (ressources trophiques) à la base de plusieurs réseaux trophiques, elles sont donc impliquées dans le maintien de la biodiversité dans ces écosystèmes terrestres (Leroy et al., 2008; Birkhofer et al., 2011). De plus, l'interaction entre la MOS et les organismes du sol libère des éléments nutritifs à partir des compartiments de la MOS. L'ensemble des propriétés de la MOS lui confère un rôle majeur dans le contrôle de la fertilité des sols et de la production alimentaire (Tiessen et al., 1994).

La décomposition des litières est un processus essentiel permettant la création des différents compartiments de MOS qui, en interagissant ensemble, sont à la base de différents services écosystémiques fournis par les sols. Ainsi, une bonne compréhension du processus de décomposition des litières est un enjeu majeur scientifique (conception du fonctionnement du sol) et sociétal (contribution aux services écosystémiques).

4. Les sols prairiaux

En 2015, les écosystèmes prairiaux couvraient 21 % des terres européennes, soit autant que les champs cultivés (22 %) et presque deux fois moins que les forêts (38 % ; Eurostat, 2015). L'occupation relative des sols par des prairies est très variable selon les pays Européens allant de 4 % en Finlande à 56 % en Irlande en passant par 27% en France (Eurostat, 2015). Selon les organismes considérés, les prairies présentent généralement une diversité faunistique et floristique plus importante que les champs cultivés (Giulio et al., 2001; Krauss et al., 2004; Decaëns et al., 2008; Werling et al., 2014). Par exemple, en climat tempéré, l'abondance et la richesse des vers de terre sont plus élevées en système prairial comparé aux cultures (de 23 % et 39 % respectivement ; Cluzeau et al., 2012; Ponge et al., 2013). Selon l'intensité de leur gestion, les prairies et leurs sols contribuent différemment aux services écosystémiques (approvisionnement, régulation, culturel et de support ; Costanza et al., 1997; Sala and Paruelo, 1997; van Eekeren et al., 2010; Pilgrim et al., 2010; Isbell et al., 2011). Par exemple, les sols de prairies ont un plus grand potentiel de stockage de carbone que les champs cultivés, parfois même plus élevé que les forêts (Franzluebbers et al., 2000; Conant et al., 2001; Conant and Paustian, 2002; Eze et al., 2018; Stockmann et al., 2013). En prairie, la matière organique des sols (MOS) représente, selon le contexte pedo-climatique, entre 3 et 6% du poids total des sols à la différence des champs cultivés où elle en représente 1.5 à 2.5 % (Allison, 1973; Bachelier, 1978).

B. Décomposition des litières

1. Définition et étapes de la décomposition

La décomposition des litières aboutit au recyclage de la matière organique morte d'origine végétale, *i.e.* à la libération de nutriments, au cycle du carbone (Swift et al., 1979; Bardgett et al., 2005), et à la création des MOP et de l'humus. La décomposition peut se diviser en cinq étapes : le lessivage, la fragmentation, l'incorporation dans le profil de sol, la minéralisation et la stabilisation (Swift et al., 1979; Curry, 1994; Coûteaux et al., 1995). Le **lessivage** est dû aux flux d'eau qui traversent les litières et les sols et qui entraînent avec eux des ions minéraux mais aussi des molécules organiques. La **fragmentation** correspond soit à une action mécanique abiotique (température, humidité) soit à l'action de différents organismes des sols qui découpent les litières avec leurs pièces buccales et/ou par broyage dans leur tube digestif. **L'incorporation** dans les sols par la faune, est, soit directe par enfouissement ou soit indirecte via l'ingestion puis l'excrétion dans la profondeur des sols (Don et al., 2008). **La minéralisation** correspond à l'activité des microorganismes qui dégradent les molécules organiques en molécules organiques de plus faibles poids moléculaire jusqu'à leur minéralisation (CO_2, CH_4, nutriments…). Aux différentes étapes de la décomposition, des matières organiques en décomposition peuvent être **stabilisées** à plus ou moins long terme en se liant à d'autres molécules organiques ou aux minéraux des sols. Leur minéralisation est alors freinée, et elles constituent ainsi la matière organique humifiée (Kögel-Knabner et al., 2008; Cotrufo et al., 2015).

De manière générale, le processus de décomposition peut être divisé en deux grandes phases (Berg and McClaugherty, 2014) : un stade précoce de décomposition correspondant à une perte de masse d'environ 0 à 40 % et caractérisé par le lessivage des composés solubles (*e.g.* acides aminés, sucres simples, tanins…) et la décomposition de la cellulose et de l'hémicellulose (Coûteaux et al., 1995; Heim and Frey, 2004) ; un stade tardif représentant une perte de masse d'environ 40 à 100% et se rapportant à la dégradation des tissus lignifiés.

2. Régulation du processus de décomposition des litières

Le processus de décomposition des litières est régulé par le climat, la nature des litières et les communautés de décomposeurs (Swift et al., 1979; Duchaufour, 1982; Coûteaux et al., 1995; Cadisch and Giller, 1997; Aerts, 1997). A une échelle spatiale relativement fine (quelques km²), les traits des litières et les communautés de décomposeurs sont les principaux responsables de la vitesse de décomposition alors qu'à une échelle plus large, elle est contrôlée par le climat (Bradford et al., 2014, 2016; Djukic et al., 2018).

2.1. Facteurs édaphiques et microclimatiques

En régulant le métabolisme et l'activité des micro-organismes, l'humidité et la température sont les principaux paramètres abiotiques contrôlant le processus de décomposition des litières dans les sols. En effet, l'activité des microorganismes, principaux acteurs de la minéralisation, peut être optimisée par une stimulation de leur métabolisme suivant des changements du taux d'humidité et de la température des sols (Killham, 1994; Lavelle and Spain, 2001; Chapin et al., 2002; Allison et al., 2013). Ainsi, des températures annuelles inférieures à 10°C et une humidité annuelle inférieure à 30% peuvent limiter la décomposition des litières (Prescott, 2010). De plus, au-delà de 80% d'humidité, la porosité du sol est remplacé par de l'eau ce qui peut également conduire à des conditions anoxiques et limiter le développement de certains microorganismes décomposeurs. Outre ces facteurs microclimatiques, certaines propriétés édaphiques contrôlent également la décomposition des litières. Ainsi, au travers d'une méta-analyse de 376 expérimentations, Xu et al. (2016) ont pu observer que le taux de décomposition était plus important dans les sols avec une forte teneur en argile, une faible capacité de rétention en eau et un ratio C/N élevé.

2.2. Traits physico-chimiques des litières

Les traits physico-chimiques des litières sont les premiers paramètres d'importance pour le processus de décomposition puisque ce sont eux qui définissent la qualité des litières pour les décomposeurs (Cadisch and Giller, 1997; Cornelissen and Thompson, 1997; Cornwell et al., 2008; Makkonen et al., 2012; Pey et al., 2018). Ces traits sont susceptibles de varier au sein d'une même espèce de plante et par conséquent influencer le processus de décomposition (Lecerf and Chauvet, 2008; Albert et al., 2010, 2011).

Les traits chimiques des litières qui impactent sa qualité pour les décomposeurs sont majoritairement ses teneurs en carbone, azote et phosphore. Il est communément admis qu'une litière fortement concentrée en azote ou en phosphore se décomposera rapidement sous l'action des microorganismes (Wardle et al., 1997; Cornwell et al., 2008; Cotrufo et al., 2015). A l'inverse, les litières pauvre en azote et phosphore et essentiellement constituées de carbone seront plus lentes à se décomposer en raison de la présence de composés difficilement dégradables tels que la cellulose et la lignine (Meentemeyer, 1978; Kögel, 1986; Rutigliano et al., 1996). Le ratio entre ces différents éléments (stœchiométrie) est également très important car il conditionne la spécificité ou non des décomposeurs (*e.g.* bactérie *vs* champignons ; Sinsabaugh et al., 2008; Burns et al., 2013; Barantal et al., 2014). De plus, les litières riches en métabolites secondaires (*e.g.* composés phénoliques, terpènes…) se décomposent plus lentement (Harrison, 1971; Hättenschwiler and Vitousek, 2000; Ormeño et al., 2006; Chomel et al., 2016). En effet ces métabolites peuvent avoir des propriétés bactéricides ou fongicides et diminuer la palatabilité des litières pour la faune du sol (Chomel et al., 2016).

Les traits physiques des litières qui impactent sa qualité sont majoritairement la surface foliaire spécifique, la capacité de rétention en eau, l'épaisseur de la cuticule ou la dureté des litières (Pérez-Harguindeguy et al., 2000; Quested et al., 2007; Makkonen et al., 2012; Zukswert and Prescott, 2017). Par exemple, la cuticule de la litière agit comme une barrière pour les décomposeurs, plus cette cuticule est épaisse plus elle ralentit la décomposition (Ponge, 1991; Tian et al., 1997).

2.3. Décomposition des litières plurispécifiques

La décomposition d'une seule litière peut être expliquée par ses traits physico-chimiques mais lorsqu'elle est mélangée avec d'autres litières (ce qui se passe principalement en milieu naturel), la valeur de ses traits ne suffit plus pour expliquer le processus de décomposition (Wardle et al., 1997, 2003; Gartner and Cardon, 2004; Hättenschwiler et al., 2005; Gessner et al., 2010). Dans le cas des litières plurispécifiques, la similarité ou la dis-similarité de leurs traits détermine l'activité et la diversité des décomposeurs et donc le processus de décomposition (Gartner and Cardon, 2004; Pérez Harguindeguy et al., 2008; Tardif and Shipley, 2015). Par exemple, la complémentarité des ressources via le transfert de nutriments (*e.g.* N, P, Ca, K…) d'une litière à une autre, augmente l'activité et la diversité des décomposeurs généralistes et donc le processus de décomposition (Wardle et al., 1999; Coulis et al., 2015). De plus, différentes litières ayant des traits très similaires peuvent permettre le développement d'une communauté de décomposeurs spécialistes d'un type de ressource donné et ainsi modifier le processus de décomposition (Pan et al., 2015). Pour finir, les litières plurispécifiques ayant des traits physiques très contrastés (taille, forme, dureté…) peuvent se structurer ensemble modifiant le microclimat (humidité, oxygénation) au sein du mélange de litières et par conséquent altérer le processus de décomposition (Hättenschwiler et al., 2005).

2.4. Les communautés de décomposeurs

Les décomposeurs sont les organismes vivants qui participent à une ou plusieurs étapes du processus de décomposition. Dans le sol, les décomposeurs sont très abondants et diversifiés, appartenant à la fois à la faune (macro-, meso- et micro-) et aux microorganismes (bactéries, champignons, archées ; Anderson, 1975; Bachelier, 1978; Coûteaux et al., 1995). Lorsqu'une litière tombe au sol, différentes communautés de décomposeurs se succèdent généralement au cours du processus de décomposition (Anderson, 1975; Ponge, 1991).

Les décomposeurs se rapportant à la faune du sol (*e.g.* collemboles, cloportes…) ont la capacité de fragmenter la litière, ce qui diminue sa taille et augmente les surfaces de colonisation disponible pour les microorganismes (Scheu and Wolters, 1991; Wolters, 2001). Ces décomposeurs ingèrent la litière, qui sera soumise à diverses transformations chimiques, physiques et biologiques au cours du transit intestinal et permettra à l'organisme en question d'en assimiler une partie et d'en rejeter une autre sous forme de fèces (Laverack, 1963; Ineson and Anderson, 1985; Brauman, 2000; Zimmer and Topp, 2002). La faune du sol contribue également à l'enfouissement de la litière dans le profil de sol, soit directement (incorporation), soit indirectement en déposant des fèces dans le profil de sol. Cette litière enfouie est plus facilement colonisée par les microorganismes (Hoang et al., 2016, 2017) ce qui en cascade améliore la palatabilité de la litière pour la faune du sol (Wright, 1972; Moody et al., 1995). Au sein des décomposeurs de la faune du sol, les vers de terre, fourmis et termites interviennent conséquemment dans la décomposition des litières (*i.e.* fragmentation, incorporation, digestion et déjéctions ; Bruyn and Conacher, 1990; Brauman, 2000; Curry and Schmidt, 2007; Frouz and Jilková, 2008). Par ailleurs, ces organismes sont capables de modifier les propriétés physiques, chimiques et biologiques des sols et sont donc appelés ingénieurs de l'écosystème (Jones et al., 1994; Lavelle et al., 1997).

Les microorganismes interviennent spécifiquement dans l'étape de minéralisation (Coûteaux et al., 1995; Lavelle and Spain, 2001; Adl, 2003; Allison et al., 2013; Coleman et al., 2017). Globalement, les microorganismes sont très diversifiés, ubiquistes et capables de s'adapter rapidement (Adl, 2003; Bardgett et al., 2005; Berg and McClaugherty, 2014; Coleman et al., 2017). La minéralisation se produit en différentes phases, qui feront intervenir différentes guildes bactériennes et fongiques (Moorhead and Sinsabaugh, 2006). Bactéries et champignons sécrètent des enzymes (Dick and Tabatabai, 1992; Dick et al., 2000; Tabatabai, 2003) capables d'hydrolyser des liaisons chimiques de grosses molécules et polymères pour les transformer en composés à plus faible poids moléculaire (Burns and Dick, 2002). Ces composés seront soit assimilés par ces microorganismes pour leurs besoins métaboliques libérant des éléments minéraux (*e.g.* CO_2, CH_4, nutriments…) soit libérés dans les sols et accessibles à d'autres organismes (co-métabolisme).

Les bactéries sont le plus souvent immobiles (Bardy et al., 2003) et ne sécrètent à un temps donné qu'un type d'enzyme à la fois (Adl, 2003; Burns et al., 2013). Elles forment probablement le groupe le plus diversifié et le plus abondant des organismes sur Terre (Torsvik and Øvreås, 2002). Il est estimé que le nombre de bactérie dans un gramme de sol sec peut atteindre de 2×10^9 de cellules (Whitman et al., 1998). Il y a en moyenne 10.000 génomes de bactéries différents dans un gramme de sol (Torsvik and Øvreås, 2002), et leur biomasse peut atteindre jusqu'à 2 t .ha^{-1} dans un sol prairial (Killham, 1994), soit quasiment 3 à 5 % de la MOS. De par leur immobilité, une grande partie des bactéries sont inactives dans les sols par manque de ressource organique à proximité (Lavelle, 2002). La mise en contact avec un substrat, grâce à un organisme vivant (racines, faune) ou un processus hydrique, leur permet alors de s'activer (Lussenhop, 1992; Lavelle et al., 1995; Brown et al., 2000; Mikola et al., 2002).

Les champignons sont également très diversifiés : les estimations font état de 1.5 (Hawksworth, 1991) à 5.1 (Blackwell, 2011) millions d'espèces différentes dans le monde. Un gramme de sol contient environ un million d'individus et leur biomasse peut atteindre 2 à 5 t. ha^{-1} en climat tempéré (Killham 1994). Comparés aux bactéries, les champignons sécrètent différentes enzymes capables d'hydrolyser plusieurs types de molécules en même temps (Adl, 2003; Burns et al., 2013). Ces enzymes leur permettent de dégrader la matière organique difficilement hydrolysable ou récalcitrante (*e.g.* lignine, cutine… ; Osono, 2007; Money, 2008; Berg and McClaugherty, 2014; Dighton, 2016). Les champignons forment des hyphes, *i.e.* des filaments pluricellulaires, dont l'ensemble constitue le mycélium. Ces hyphes sont capables d'explorer le sol à la recherche de ressources nutritives ce qui permet aux champignons de conserver une activité prolongée dans le temps contrairement aux bactéries (Boddy, 1999; Frey and Reed, 2000). Le mycélium peut atteindre 200 m par gramme de sol (Bardgett and Wardle, 2010).

2.5. Lien entre diversité des décomposeurs et décomposition

Malgré leur importance fonctionnelle, les interactions entre les différents groupes de décomposeurs sont encore mal comprises (Bardgett and van der Putten, 2014), les études qui ont investigué le rôle de la diversité fonctionnelle au sein de la macrofaune étant rares (Gessner et al., 2010; Wall, 2012). Le lien entre la diversité des décomposeurs et le taux de décomposition repose sur l'hypothèse que la diversité taxonomique se traduit par une diversité fonctionnelle (Gessner et al., 2010) mais cette hypothèse n'est que très peu validée ou limitée à de faibles niveaux de diversité. Comme pour les litières (*cf.* 2.2 Traits physico-chimiques des litières), l'étude des traits fonctionnels des décomposeurs permet de comprendre le rôle de leur diversité dans le processus de décomposition. Par exemple, une expérimentation utilisant trois lombriciens, trois isopodes et deux diplopodes a mis en évidence que la richesse spécifique n'avait pas d'influence sur le taux de décomposition de la litière (Heemsbergen et al., 2004). Seule la dissimilarité fonctionnelle entre les espèces de décomposeurs permettait d'expliquer le taux de décomposition (Heemsbergen et al., 2004; Patoine et al., 2017). Dans l'expérimentation de Heemsbergen et al. (2004) cette dissimilarité fonctionnelle était notamment liée à la présence d'une espèce de vers de terre (*Lumbricus rubellus rubellus*) capable d'enfouir la litière, ce qui n'était pas le cas des sept autres espèces de décomposeurs présentes. De plus, il a été observé qu'au sein d'un même groupe fonctionnel de collemboles, le taux de décomposition était lié à la présence d'une espèce spécifique et non à la diversité de l'ensemble de la communauté (Cragg and Bardgett, 2001). La taille ou la biomasse des organismes sont des traits fonctionnels importants qui varient parmi les décomposeurs (Petchey and Gaston, 2006; Reiss et al., 2009) et qui facilitent les différents modes d'utilisation des ressources (*e.g.* fragmentation, incorporation, minéralisation ; Bardgett et al., 2005; Tonin et al., 2018).

Les interactions entre la diversité des décomposeurs et la décomposition de la litière ont principalement été testées indépendamment à l'échelle soit de la litière, soit des micro-organismes ou soit d'autres décomposeurs. Cependant, dans le contexte d'un réseau trophique, il est important de prendre en compte la diversité horizontale (au sein d'un niveau trophique), dont l'étude doit être complétée par des recherches sur l'importance de la diversité verticale (entre différents niveaux trophiques ; Fig. 3 ; Gessner et al., 2010). En effet, des changements au sein de la diversité verticale pourraient accroître ou réduire les effets de la diversité horizontale sur la décomposition. Ces différentes échelles de diversité sont donc cruciales pour le fonctionnement des écosystèmes (Duffy et al., 2007) et leur contribution aux services écosystémiques (Hines et al., 2016; Soliveres et al., 2016).

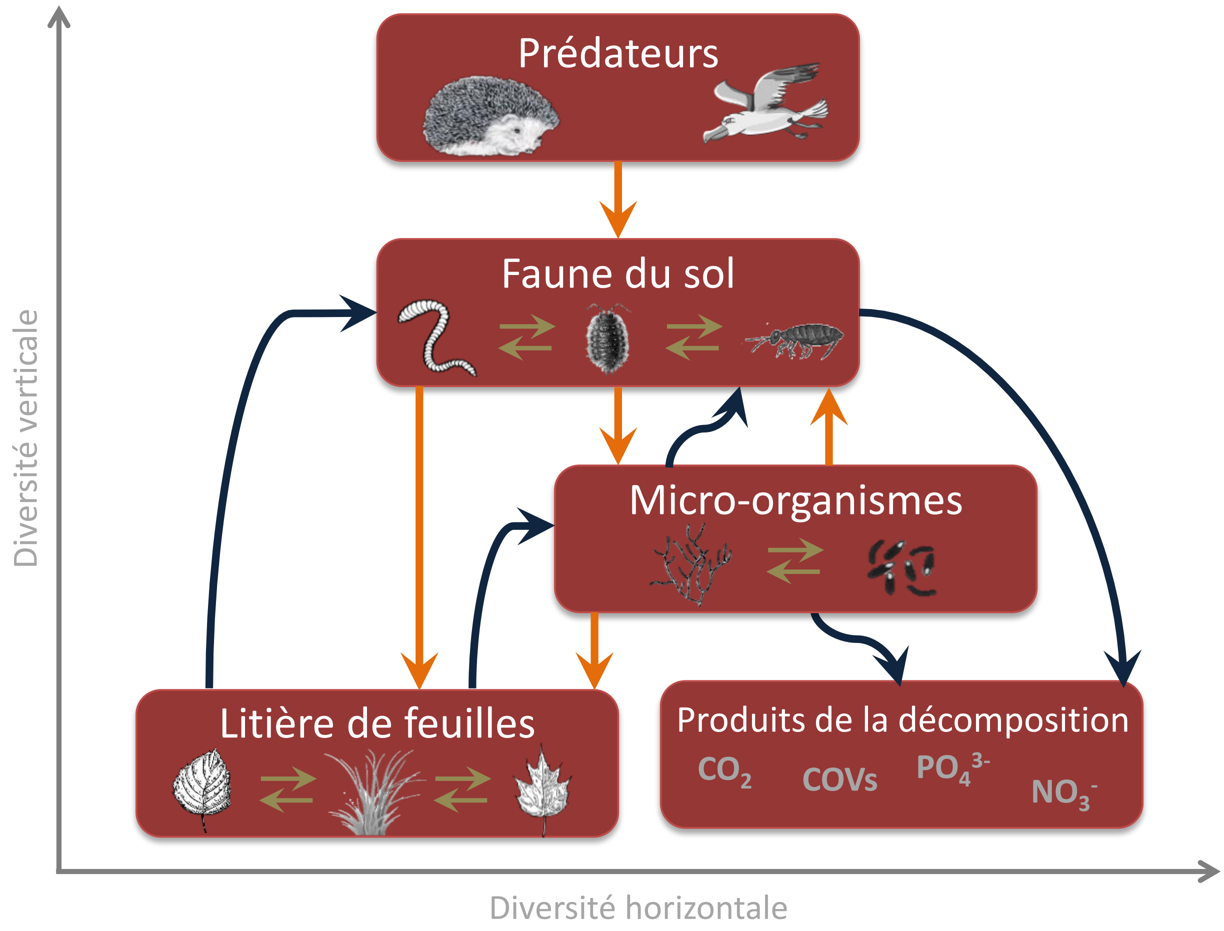

Figure 3. Schéma conceptuel illustrant les relations entre les niveaux trophiques (axe vertical) et la diversité (axe horizontal) au cours de la décomposition des litières (extrait de Gessner et al., 2010). Les flèches vertes représentent les interactions entre décomposeurs (faune ou micro-organismes) ou entre litières au travers de l'activité des décomposeurs. Les flèches orange indiquent des interactions entre niveaux trophiques et les flèches bleues indiquent le chemin principal du carbone de la litière.

C. Rôles des lombriciens dans la décomposition des litières

Les vers de terre font partie de la sous-classe des Oligochètes au sein du phylum des Annélides et sont représentés par 3700 espèces dans le monde (Decaëns et al., 2013). Cependant, il a récemment été estimé que seulement 23% des espèces de vers de terre ont été décrites (Orgiazzi et al., 2016). Les vers de terre sont très répandus dans l'ensemble des sols excepté en régions arides et gelées et représentent la biomasse animale terrestre la plus importante en climat tempéré (Hole, 1981; Lavelle, 1983; Brockie and Moeed, 1986; Bar-On et al., 2018). En France, les vers de terre sont représentés par plus de 110 espèces (Bouché, 1972) majoritairement de la famille des Lumbricidea mais les nouveaux outils moléculaires ont permis d'identifier un certain nombre d'espèces cryptiques supplémentaires (Decaëns et al., 2013). Tout au long de cette introduction, le terme « vers de terre » fera référence à des généralités alors que le terme « lombriciens » fera référence à la famille européenne des Lumbricidae.

Ils sont qualifiés d'espèces « clés de voûte » dans le fonctionnement des sols (*i.e.* qui a un effet disproportionné sur son environnement au regard de ses effectifs ou de sa biomasse ; Davis and Richardson, 1995; Heemsbergen et al., 2004), notamment dans le processus de décomposition de la matière organique (Edwards and Bohlen, 1996; Curry and Schmidt, 2007). Plusieurs auteurs ont déterminé des catégories écologiques regroupant les espèces lombriciennes en climat tempéré (Bouché, 1977; Perel, 1977; Satchell, 1980; Lee, 1985).

1. Les catégories écologiques lombriciennes

Le regroupement d'espèces lombriciennes le plus utilisé est celui de Bouché (1977) qui distingue trois catégories écologiques lombriciennes en se basant sur des traits morphologiques (taille, couleur), physiologiques (résistance à la sécheresse, durée de vie) et comportementaux (mobilité, milieu de vie ; Fig. 4 et 5) : (i) les épigés, (ii) les anéciques et (iii) les endogés.

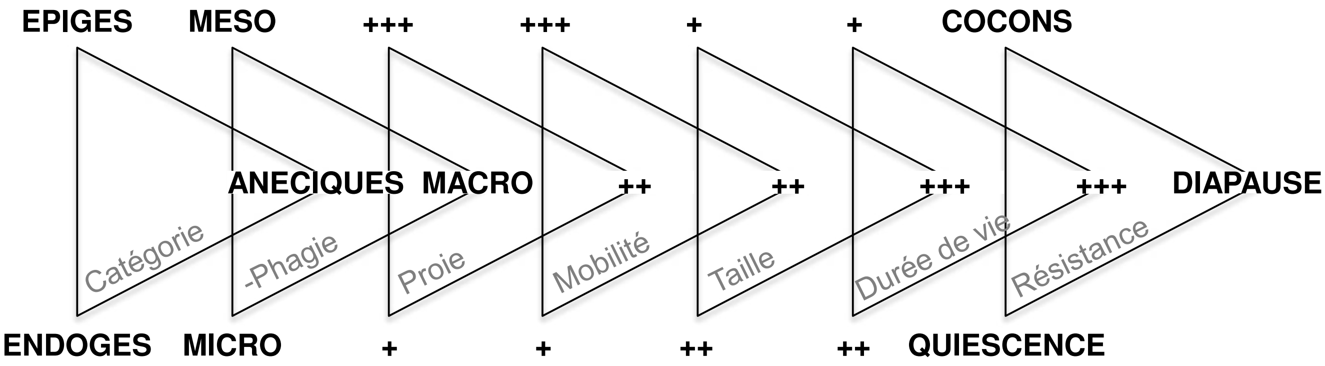

Figure 4. Paramètres morphologiques, physiologiques et comportementaux des trois catégories écologiques lombriciennes (Bouché, 1977).

(i) Les épigés vivent à la surface du sol et ne creusent pas de galeries en profondeur. Leur épiderme est généralement foncé, de couleur rouge et au stade adulte, sauf exception, leur taille n'excède pas 5 cm (Bouché, 1972). Ils sont méso-phages (Bouché, 1977; Satchell, 1980), consomment toutes les matières organiques à la surface du sol (Bouché and Kretzschmar, 1974; Kretzschmar, 1977; Piearce, 1978; Ferrière, 1980) et certaines espèces sont aussi coprophages (Bouché, 1972). La majorité des épigés contribue à la décomposition des litières (Lavelle, 1997). Leur impact sur le fonctionnement du sol se confine à l'horizon organique. Bouché (1972) précise que certaines espèces d'épigés sont commensales et empruntent des galeries creusées par des endogés ou des anéciques. Leur taux de reproduction est généralement assez élevé et leur durée de vie assez courte (quelques mois), suivant donc une stratégie démographique de type « r » (MacArthur and Wilson, 1967; Satchell, 1980; Cluzeau, 1992). Lorsque les conditions climatiques sont défavorables, ces espèces épigées peuvent survivre sous forme de cocons contenant 4 à 7 embryons (Cluzeau, 1992).

(ii) Les endogés vivent dans les horizons organo-minéraux et minéraux du sol et creusent des galeries horizontales à sub-horizontales (Capowiez et al., 1998, 2001; Bastardie et al., 2003; Capowiez et al., 2014, 2015). Leur épiderme est généralement dépourvu de pigment, ainsi ils sont souvent de couleur claire dans les tons vert, rose ou blanc (Bouché, 1972). Ils sont microphages (Bouché, 1977; Satchell, 1980) et consomment essentiellement de la matière organique humifiée présente dans les horizons organo-mineraux et minéraux. Leur impact sur la litière fraichement déposée est donc limité (Bouché and Kretzschmar, 1974; Kretzschmar, 1977; Piearce, 1978; Ferrière, 1980). Leur stratégie démographique se situe entre les stratégies « r » et « K » (Satchell, 1980). Lorsque les conditions climatiques sont défavorables, ces espèces endogées peuvent s'enfouir dans le sol pour chercher l'humidité ou la température adéquate pour créer des chambres d'estivation.

(iii) Les anéciques creusent des galeries verticales à sub-verticales, quasi permanentes et ouvertes à la surface du sol traversant ainsi divers horizons du sol (Jégou et al., 2000; Pérès, 2003; Bastardie et al., 2003, 2005). Leur épiderme possède un gradient antéro-postérieur de décoloration et au stade adulte, sauf exception leur taille peut varier entre 10 et 25 cm (Bouché, 1972). Ils sont macrophages (Bouché, 1977; Satchell, 1980) et consomment de la litière à la surface du sol et de la matière minérale des horizons sous-jacents (Bouché and Kretzschmar, 1974; Kretzschmar, 1977; Piearce, 1978; Ferrière, 1980). Ces matières organiques et minérales sont mélangées et brassées lors du transit digestif. Leur stratégie démographique est de type « K » (Satchell, 1980; Cluzeau, 1992). Lorsque les conditions climatiques sont défavorables, les populations lombriciennes anéciques peuvent s'enfouir dans le sol pour chercher l'humidité ou la température adéquate. Certaines espèces présentent des diapauses ou seulement des phases de quiescence en attendant que les conditions s'améliorent, les individus créant alors des chambres d'estivation.

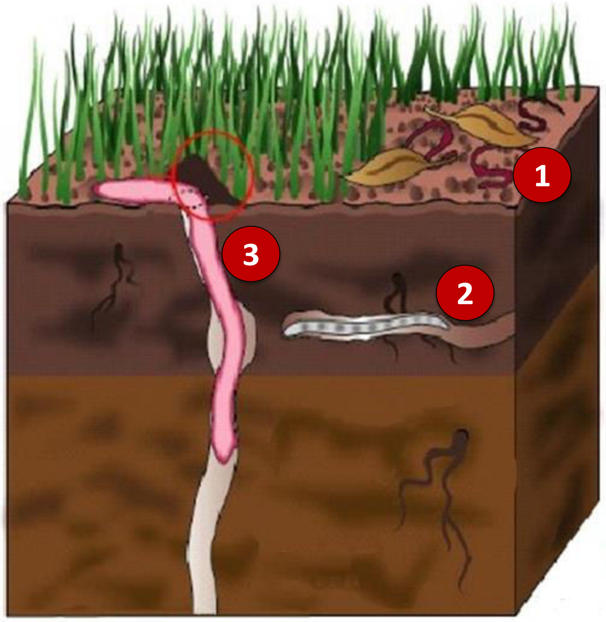

Figure 5. Illustration de la position dans le profil de sol des trois catégories écologiques lombriciennes (1) Epigé, (2) Endogé et (3) Anécique. © Damien Viel

Les anéciques et les endogés sont les deux catégories écologiques lombriciennes qui produisent le plus de structures dans les sols (cabanes, galeries, chambres d'estivation…) et qui ont donc des impacts importants sur les propriétés des sols et sur les processus qui en découlent (Brown, 2000). Cependant, les espèces anéciques sont celles qui interagissent le plus avec la litière (Brown, 2000) et, en prairie tempérée, la biomasse des lombriciens anéciques peut dominer jusqu'à 72% de la biomasse totale de la communauté lombricienne (Lavelle, 1983). Jégou et al. (1998a) ont ainsi observé que le transfert de carbone provenant de la litière de la surface vers la profondeur du sol était doublé en présence de *Lumbricus terrestris* (anécique) par rapport à une présence de *Eisenia foetida* ou d'*Aporrectodea caliginosa* (respectivement épigés et endogés). Groenigen et al. (2014), en effectuant une méta-analyse, ont observé que la production de biomasse végétale, en général, était augmentée de 18% en présence d'épigés, de 20% en présence d'endogés et de 25% en présence d'anéciques. Les auteurs ont suggéré que cette augmentation pouvait être liée à une stimulation de la minéralisation de la matière organique.

Bouché (1977) précise que le classement des espèces lombriciennes en trois catégories écologiques est relatif car bien que certaines espèces lombriciennes se positionnent comme étant strictement épigées, anéciques ou endogées, d'autres se situent entre ces trois catégories écologiques (Fig. 6). Ainsi, des sous-catégories écologiques lombriciennes peuvent être proposées, *i.e.* épi-endogés, épi-anéciques, endo-anéciques. Concernant les endogés, Lavelle (1983, 1988) a défini trois sous catégories écologiques selon la quantité et la qualité des matières organiques ingérées : les endogés poly-, méso- et oligo-humiques. Cette distinction apporte une précision sur la profondeur potentielle d'activité de ces espèces lombriciennes endogées. Au sein des lombriciens anéciques, plusieurs études ont mis en évidence deux sous-catégories écologiques selon des traits morphologiques (Briones and Álvarez-Otero, 2018), des comportements fouisseurs (Jégou et al., 1998a, 2000, 2001; Bastardie et al., 2003, 2005) et alimentaires (Ferrière, 1980; Eisenhauer et al., 2008; Andriuzzi et al., 2015; Larsen et al., 2016) : les anéciques stricts et les épi-anéciques.

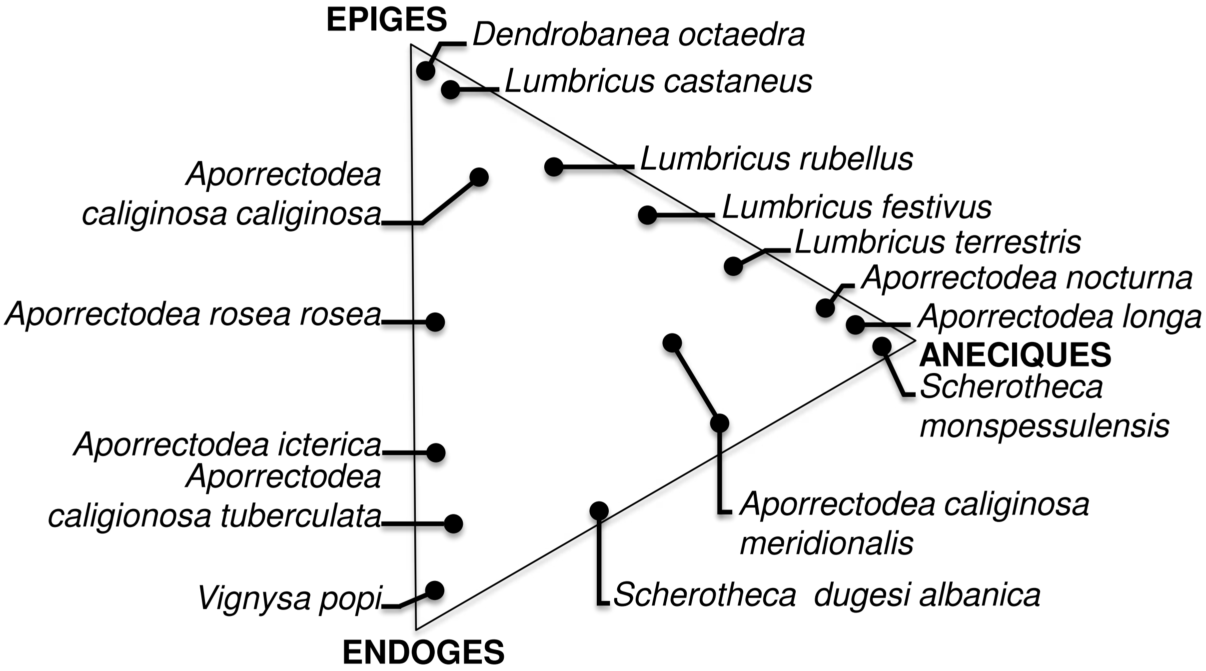

Figure 6. Diagramme situant approximativement quelques espèces lombriciennes communes dans le système tri-pôle des catégories écologiques (Bouché, 1977).

Il y a encore un manque évident de connaissances concernant l'écologie des espèces lombriciennes les plus communes (Edwards, 2004a; Curry and Schmidt, 2007). Très peu d'études se sont penchées sur le lien entre les espèces lombriciennes, les microorganismes, le type de litière et sa décomposition (Edwards, 2004a). Tant que les connaissances fondamentales sur la biologie et l'écologie des espèces lombriciennes ne seront pas suffisamment connues, il sera impossible d'évaluer de manière précise leur contribution aux services écosystémiques fournis par les sols. De plus, au sein des lombriciens anéciques stricts et épi-anéciques il existe une forte variabilité morphologique qui pourrait induire des différences en termes de besoins métaboliques et de sensibilité vis-à-vis des traits physico-chimique des litières, influençant ainsi leur contribution spécifique à la décomposition des litières.

2. La drilosphère des lombriciens anéciques : interaction avec les microorganismes du sol

La drilosphère représente tout le volume de sol sous l'influence des lombriciens (Bouché, 1975; Lavelle, 1988; Brown et al., 2000). La drilosphère inclut donc (i) l'environnement interne des lombriciens (tube digestif), (ii) la partie externe des lombriciens, (iii) les fèces (en surface et dans le sol), (iv) les cabanes, (v) les galeries et (vi) les chambres d'estivation. Chacun de ces compartiments a des effets contrastés sur l'activité des microorganismes et sur la dynamique temporelle de décomposition des litières (Brown et al., 2000). Les cabanes ou « middens » sont des structures à la surface du sol et à l'entrée des galeries de lombriciens composées d'un mélange de sol, de fèces, de mucus et de litières en cours d'enfouissement (Nielsen and Hole, 1964).

Les anéciques ont la capacité d'incorporer les litière de surface dans le profil de sol (Bouché, 1977) ce qui stimule sa décomposition par les microorganismes, la rendant plus assimilable pour les lombriciens par la suite (Brown et al., 2000). Ce processus d'incorporation de litière a également pour conséquence d'incorporer les microorganismes présents sur la litière de surface en profondeur dans les sols (Thorpe et al., 1996). Une fois ingérée, la matière organique, au cours du transit intestinal subir des transformations mécaniques sous l'action du gésier et des minéraux ingérés (Marhan and Scheu, 2005) et biologiques sous l'action des microorganismes (Laverack, 1963; Parle, 1963). De nombreux auteurs ont observé une forte augmentation de l'abondance et de la biomasse microbienne du sol à l'entrée du tube digestif, qui ont tendance à décroître au fur et à mesure de l'avancée dans le tube digestif (Brown, 1995; Tiunov and Scheu, 2000a; Drake and Horn, 2007; Knapp et al., 2009). L'origine de ces microorganismes fait encore débat. Ils peuvent provenir des litières ingérées ou du sol mais ils peuvent également faire partie du microbiote intestinal variant selon les différentes parties du tube digestif (*e.g.* pharynx, œsophage, gésier, intestin… ; Drake and Horn, 2007). Ainsi, le mucus produit par les vers de terre, qui est humide et enrichi en carbone soluble par rapport au sol environnant (Parle, 1963; Trigo and Lavelle, 1993, 1995; Sampedro et al., 2006; Sampedro and Whalen, 2007), peut activer les microorganismes en dormance dans les sols et initier un « priming effect » en stimulant la décomposition de la matière organique du sol dans un court espace-temps (Jenkinson et al., 1985; Lavelle et al., 1995; Kuzyakov et al., 2000). Pendant le passage dans le tube digestif, les organismes de la micro-faune (*e.g.* protozoaire, nématodes…) peuvent aussi décliner avec la digestion mais ils ne constituent probablement qu'une part mineure de l'assimilation des lombriciens contrairement aux micro-organismes (Larsen et al., 2016; Schmidt et al., 2016). De nombreuses enzymes ont été détectées dans le tube digestif des lombriciens (cellulases, chitinases, phosphatases, …) participant à la libération de molécules à faible poids moléculaire et donc de nutriments dont une partie peut être assimilée par les lombriciens (Tracey, 1951; Laverack, 1963; Prentø, 1987). L'origine de ces enzymes fait toujours débat entre une production directe par les lombriciens ou une synthèse par les microorganismes ingérés (Schönholzer et al., 1999, 2002; Egert et al., 2004; Drake and Horn, 2007).

Les excrétions des anéciques se font sous différentes formes : le mucus, l'urine et les déjections. Très peu d'études ont été réalisées pour apprécier la composition chimique des urines et du mucus (Needham, 1957; Tillinghast, 1967; Scheu, 1991; Schmidt et al., 1999). Les déjections sont de taille, stabilité, durabilité, et concentrations en nutriments très variables. Dans les déjections fraîches, la minéralisation continue mais diminue avec le temps (Scheu, 1987a). Les fèces peuvent être déposées à la surface du sol ou dans les galeries. A la surface, les déjections contribuent à la formation des cabanes créées essentiellement par les anéciques. Ces cabanes sont considérées comme des « hot-spots » pour les microorganismes et la faune du sol au sein desquels la décomposition des litières est stimulée (Hamilton and Sillman, 1989; Maraun, 1999; Aira et al., 2009). Les galeries des lombriciens anéciques sont généralement constituées d'un manchon de matière organique, *i.e* litières en cours d'enfouissement ou enfouies depuis un certain temps avec un mélange de fèces (Jégou et al., 1998b, 1998a; Binet and Curmi, 1992; Pey et al., 2013; Andriuzzi et al., 2015). Ainsi les galeries de lombriciens anéciques sont également des « hot-spots » de biodiversité et d'activité de microorganismes (Tiunov, 1997; Jégou et al., 2001; Tiunov et al., 2001; Tiunov and Dobrovolskaya, 2002; Stromberger et al., 2012).

3. Interactions entre espèces lombriciennes anéciques

Sous un m² de sol, il est possible d'observer jusqu'à treize espèces lombriciennes différentes (Cluzeau et al., 2012), dont une à six espèces anéciques (Gerard, 1967; Poier and Richter, 1992; Boag et al., 1997; Margerie et al., 2001; Decaëns et al., 2008). Les différentes populations lombriciennes d'anéciques qui coexistent dans un même sol développent des interactions susceptibles de modifier leur organisation, leur dynamique et leur activité (Butt et al., 1994b; Lowe and Butt, 1999, 2002a; Uvarov, 2009; Frazão et al., 2018). Ces interactions peuvent être de différents types (Wootton and Emmerson, 2005), par exemple, la compétition vis à vis de la ressource trophique, entraîne la dominance de 1 ou 2 espèces parmi le pool d'espèces. D'autre part, les espèces peuvent trouver un avantage à leur association (*e.g.* apport de ressources, dispersion…): il s'agit alors de mutualisme ou de commensalisme (Stachowicz, 2001).

L'interaction entre les espèces anéciques lombriciennes peut également modifier leurs impacts fonctionnels au cours du processus de décomposition des litières (Postma-Blaauw et al., 2006; Xia et al., 2011). Suite à ces interactions, trois types d'effets sur le processus de décomposition des litières peuvent être observés (Wardle et al., 1997; Gessner et al., 2010) :

- Effet additif (ou nul), si le taux de décomposition observé en présence de différentes espèces correspond à la somme des taux mesurés en présence de chaque espèce isolée.

- **Effet non-additif synergique (ou positif**), si le taux de décomposition observé en présence de différentes espèces est supérieur à la somme des taux mesurés en présence de chaque espèce isolée.

- **Effet non-additif antagoniste (ou négatif),** si le taux de décomposition observé en présence de différentes espèces est inférieur à la somme des taux mesurés en présence de chaque espèce isolée.

D. Règles d'assemblage des communautés lombriciennes

1. Contexte

De par l'importante diversité génétique et spécifique qu'ils contiennent, les sols sont considérés comme des réservoirs de biodiversité (Lavelle and Spain, 2001; Decaëns et al., 2006) abritant un quart de la biodiversité animale mondiale décrite à ce jour (Decaëns et al., 2006). En supportant la majorité des processus s'accomplissant dans les sols (*e.g.* structuration des sols, décomposition…) cette biodiversité contribue aux services écosystémiques rendus par ces écosystèmes (Decaëns et al., 2006; Barrios, 2007; Wall, 2012; Cardinale et al., 2012; Blouin et al., 2013). Les connaissances concernant l'écologie des sols sont donc essentielles à leur gestion durable (protection et conservation) et au maintien des services écosystémiques qu'ils rendent (Wall et al., 2015; Keith et al., 2016; Eisenhauer et al., 2017). Pour mieux comprendre ces contributions, l'écologie des sols attirent de plus en plus l'attention des scientifiques et des décisionnaires (Bardgett and van der Putten, 2014; Wall et al., 2015; Eisenhauer et al., 2017).

Actuellement, un effort de recherche considérable et très dynamique est réalisé pour comprendre la dynamique de la biodiversité des sols (Dornelas et al., 2014; Gonzalez et al., 2016; Vellend et al., 2017; Eisenhauer et al., 2017). Cet effort de recherche est notamment tourné vers la quantification des principaux facteurs qui déterminent et régulent les assemblages des communautés et la dynamique de la biodiversité des sols (Powell et al., 2015) face aux changements environnementaux globaux (Maestre et al., 2015; Veresoglou et al., 2015). La notion d'assemblage de communauté fait référence au processus de formation d'une communauté locale d'espèces à partir d'un pool régional (Weiher and Keddy, 2001; Weiher et al., 2011). Une communauté est donc un assemblage d'espèces dont l'organisation (distribution, composition, structure) est régie par des filtres environnementaux (*cf.* Filtres environnementaux ; Lortie et al., 2004). Les processus responsables de la constitution d'une communauté sont appelés règles d'assemblages (Keddy, 1992).

Afin de mieux appréhender le rôle des lombriciens dans le fonctionnement des sols et leur contribution aux services écosystémiques, il est nécessaire de comprendre l'assemblage des communautés lombriciennes. La formalisation de cet assemblage s'appuie sur le concept de niche écologique comme étant un hyper-volume à n dimensions qui représente n gradients environnementaux (*e.g.* texture, humidité, ressource alimentaire…) à l'intérieur desquels les lombriciens peuvent survivre, croître et se reproduire (Hutchinson, 1957).

2. Filtres environnementaux

L'assemblage des communautés lombriciennes est régi par cinq principaux filtres environnementaux (Fig. 7 ; Decaëns et al., 2008) : (1) le filtre biogéographique, (2) le filtre des propriétés des sols et du climat (habitat), (3) le filtre d'occupation et de gestion des sols et (4 et 5) les filtres d'interactions entre espèces. Chacun de ces filtres est associé à un ensemble spécifique de contraintes, qui sélectionnent hiérarchiquement les espèces, partant d'un pool total d'espèces jusqu'à un pool local.

Le premier filtre biogéographique qui régit l'assemblage des communautés lombriciennes correspond par exemple, aux glaciations, à la dérive des continents ou à l'orogénèse (Bouché, 1972). C'est ainsi qu'en Europe, le nombre d'espèces lombriciennes augmente selon un gradient longitudinal du nord vers le sud (Lavelle, 1983; Mathieu and Davies, 2014). En Islande, il y environ 8 espèces lombriciennes (Bengtson et al., 1975), 30 au Royaume-Uni (Gerard, 1967; Sims and Gerard, 1999) et 110 en France (Bouché, 1972). En France, seulement 30 espèces sont présentes dans le moitié nord du pays (Margerie et al., 2001; Decaëns et al., 2008; Cluzeau et al., 2012; Ponge et al., 2013). L'histoire biogéographique est pertinente pour comprendre la composition du bassin total d'espèces (Decaëns et al., 2008). Le deuxième filtre de l'habitat ou des propriétés du sol détermine les espèces lombriciennes ayant la capacité à s'installer localement en fonction des conditions environnementales (texture, pH, humidité, ressources ; Bouché, 1972; Lee, 1985; Eggleton et al., 2009). Le troisième filtre d'occupation et de gestion des sols permet de sélectionner des espèces à des échelles plus petites allant de la région aux paysages (Decaëns et al., 2003, 2008; Rutgers et al., 2009, 2016). Par exemple, sur un réseau de 100 parcelles en région Bretagne, Ponge et al. (2013) ont observé que les lombriciens étaient plus abondants en prairie qu'en culture. D'autres facteurs comme le travail du sol en culture (Chan, 2001; Briones and Schmidt, 2017), la fertilisation (Timmerman et al., 2006; Ponge et al., 2013), l'utilisation de pesticides (Pelosi et al., 2013, 2014) ou le chargement en bétail en prairie (Piearce, 1984; Cluzeau et al., 1992) contribuent également à sélectionner certaines espèces. Les espèces sélectionnées par ces filtres représentent le pool d'espèces potentiel pour la zone en question, adaptées à différentes contraintes (pédoclimatiques, gestion et occupation du sol). Cependant, toutes ces espèces lombriciennes ne peuvent cohabiter dans un même sol car il existe un quatrième filtre d'interactions interspécifiques ou intraspécifiques lié par exemple à de la compétition ou de la facilitation pour la ressource trophique (Butt et al., 1994b; Lowe and Butt, 1999, 2002a; Uvarov, 2009) qui s'applique pour aboutir à une communauté lombricienne locale. Cette communauté lombricienne locale peut être légèrement modifiée à l'échelle infra-parcellaire entre des patchs conditionnés par des variables physique, chimique et biologique ponctuelles (*i.e* humidité, ressource, prédateur… ; Cuendet, 1983; Cannavacciuolo et al., 1998; Margerie et al., 2001; Decaëns et al., 2008, 2011).

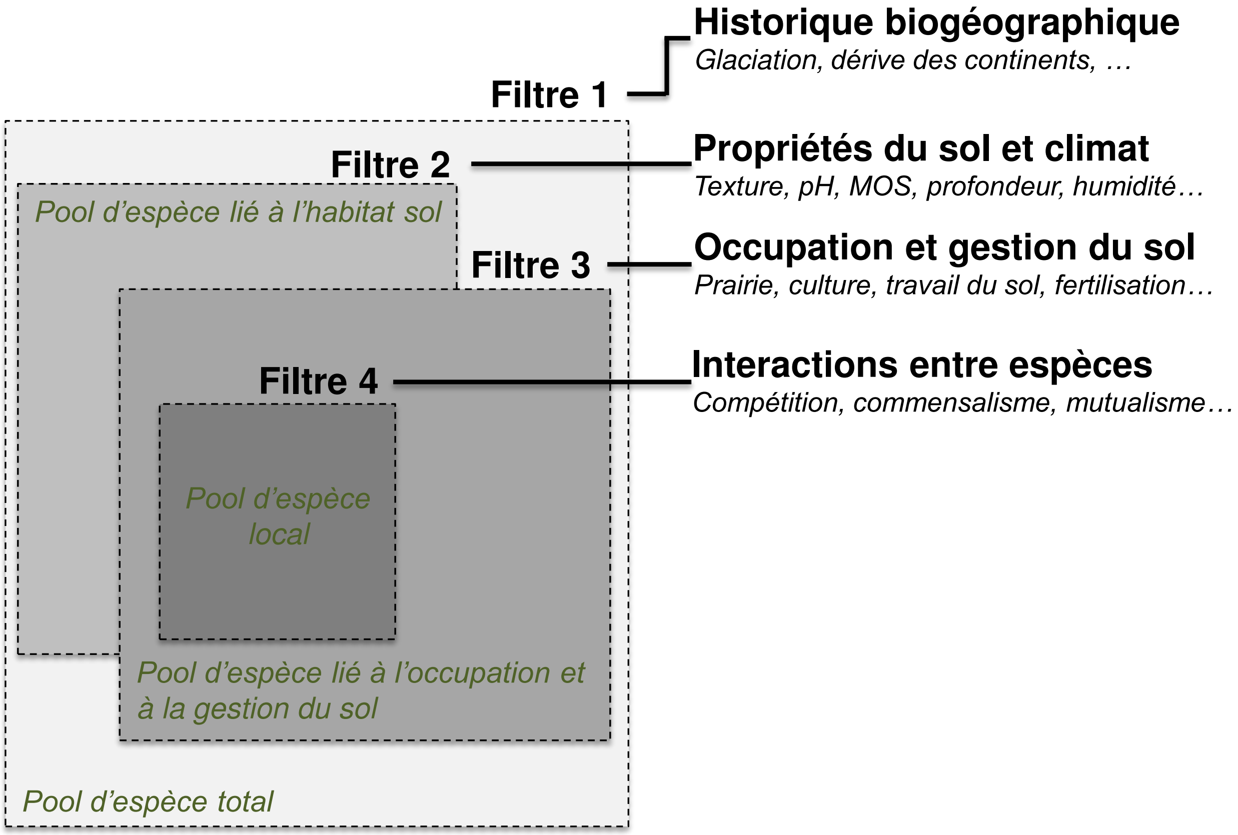

Figure 7. Représentation schématique des interactions entre les pools d'espèces et les filtres environnementaux qui opèrent sur l'assemblage des communautés de vers de terre (modifié d'après Decaëns et al., 2008). Les boîtes représentent les différents pools d'espèces. Pour chaque filtre environnemental, les contraintes correspondantes sont détaillées (caractères italiques).

3. Influence du paysage

L'écologie du paysage met l'accent sur l'interaction entre la configuration spatiale et les processus écologiques, c'est-à-dire, les causes et les conséquences de l'hétérogénéité spatiale à différentes échelles (Turner and Gardner, 2015). L'écologie du paysage est une discipline qui s'est considérablement développée ces dernières années (Turner and Gardner, 2015). L'hypothèse de l'hétérogénéité de l'habitat est l'un des concepts de base de l'écologie du paysage (Hutchinson, 1957). Elle suppose que des habitats structurellement complexes peuvent fournir plus de niches, augmenter les ressources disponibles, et ainsi accroître la diversité des espèces présentes (Bazzaz, 1975; Tews et al., 2004). Cette hypothèse a été démontrée pour une diversité d'organismes, par exemple, chez les oiseaux (MacArthur and MacArthur, 1961; Berry and Bock, 1998; Kissling et al., 2008), les Mammifères (Ceballos et al., 1999), les araignées (Dennis et al., 1998) et les fourmis (Perfecto and Snelling, 1995). Ainsi, des zones qui, à tous les autres égards sont écologiquement équivalentes, mais qui ont une plus grande variété d'habitats peuvent fournir un plus grand nombre de niches écologiques aux espèces d'une communauté, favorisant ainsi la biodiversité (Warfe et al., 2008). Tews et al. (2004) dans leur méta-analyse portant sur 85 articles, ont pu valider cette relation entre l'hétérogénéité de l'habitat et la diversité des espèces animales dans 85% des études. De plus, les auteurs précisent que cette relation dépend généralement de (i) la façon dont l'hétérogénéité de l'habitat est perçue par l'organisme étudié, (ii) la mesure de la diversité des espèces, (iii) la définition (*i.e.* la typologie) et de la (iv) la mesure de l'habitat et de l'échelle (v) temporelle et (vi) spatiale de l'étude. Par ailleurs cette relation peut parfois dépendre de structures « clé de voûte » (arbres isolé, haie, cours d'eau…) qui déterminent la diversité animale par leur présence. Ainsi la détection des structures clés de voûte de la végétation peut avoir de profondes implications pour la conservation et la gestion de la biodiversité animale.

Ainsi, plusieurs études ont été réalisées à différentes échelles pour évaluer l'effet du paysage sur les communautés lombriciennes dans les champs cultivés (Decaëns et al., 2008; Roarty and Schmidt, 2013; Regulska and Kolaczkowska, 2015; Frazão et al., 2017). La relation entre l'hétérogénéité de l'habitat et la richesse lombricienne a été mise en évidence à large échelle spatiale (1 km²) mais pas à plus petite échelle (200 m² ; Vanbergen et al., 2007). De plus, dans une étude de Regulska and Kolaczkowska (2015) la comparaison de deux sites cultivés, entourés d'un paysage complexe, a permis d'observer une diversité, une densité et une biomasse de lombriciens supérieures à celles de sites gérés de manière similaire mais entourés d'un paysage simple. Néanmoins, d'autres études n'ont pas mis en évidence d'effets du paysage avoisinant sur les communautés lombriciennes dans les parcelles cultivés (Kovács-Hostyánszki et al., 2013; Lüscher et al., 2014; Buchholz et al., 2017; Frazão et al., 2017). Afin d’expliquer de telles différences, des auteurs ont proposé l’hypothèse que l’assemblage des communautés lombriciennes dans les sols cultivés soit régi par les pratiques agricoles répétitives (Roarty and Schmidt, 2013; Frazão et al., 2017) telle que le labour (Chan, 2001; Briones and Schmidt, 2017) ou l'application de pesticides (Pelosi et al., 2014). A l’inverse, dans les prairies, la faible perturbation du sol et sa couverture permanente pourraient favoriser l’influence du paysage plutôt que celle des pratiques agricoles sur les communautés lombriciennes.

E. Objectifs de la thèse

La décomposition des litières est un processus clé du fonctionnement des sols et est largement étudiée en écologie fonctionnelle (Adl, 2003; Hättenschwiler and Gasser, 2005; Berg and McClaugherty, 2014; Djukic et al., 2018). Parmi les décomposeurs, les lombriciens en interaction avec les microorganismes du sol sont des acteurs majeurs de ce processus (Brown et al., 2000; Heemsbergen et al., 2004; Edwards, 2004b; Curry and Schmidt, 2007) et cela particulièrement dans les sols prairiaux où la richesse des communautés lombriciennes peut atteindre jusqu'à 13 espèces (Cluzeau et al., 2012). Pourtant, les connaissances sur la contribution des lombriciens à la décomposition des litières sont le plus souvent limitées aux trois catégories écologiques : les épigés, les endogés et les anéciques (Bouché, 1972, 1977). Cependant, les interactions des principales espèces lombriciennes constituant ces catégories écologiques entre elles et avec les communautés microbiennes ainsi que les conséquences sur les activités de ces dernières au cours du processus de décomposition, restent largement méconnues (Edwards, 2004b). Au sein des lombriciens, parmi les trois catégories écologiques, les anéciques constituent la biomasse la plus importante et leur spectre de ressources est majoritairement constitué de litières (Bouché, 1972; Lavelle, 1983; Lee, 1985). De plus, compte tenu de leur importance dans le processus de décomposition (Brown et al., 2000) mais également de leur contribution à de nombreux processus dans les sols, il apparaît nécessaire de mieux connaître les règles d'assemblage des communautés lombriciennes appartenant à cette catégorie écologique dans les sols de prairie. Si de telles règles existent, il est fort probable qu'elles soient multifactorielles (Decaëns et al., 2008; Frazão et al., 2017).

Le premier objectif de ce travail a été d'identifier les règles d'assemblage des communautés lombriciennes dans des sols prairiaux. Ainsi, nous avons cherché à évaluer la contribution des propriétés des sols, de la gestion des prairies et du paysage environnant dans l'assemblage des communautés lombriciennes (chapitre 1). Une des hypothèses était que les populations d'espèces épi-anéciques et anéciques stricts répondent différement aux filtres environnementaux sélectionnés. Les espèces lombriciennes anéciques observées en prairie ont été utilisées au cours des expérimentations en conditions contrôlées réalisées durant ce travail de thèse.

Le second objectif de la thèse a été de vérifier la distinction entre des espèces lombriciennes anéciques stricts et épi-anéciques et d'évaluer i) la contribution de ces espèces au sein de chaque catégorie écologique dans la décomposition des litières et ii) leur interaction avec les communautés microbiennes du sol. Dans un premier temps, nous avons établi une synthèse bibliographique des connaissances actuelles validant ou non cette distinction entre lombriciens épi-anéciques et anéciques stricts concernant leurs impacts sur la décomposition des litières (chapitre 2). Dans un second temps, nous avons cherché à déterminer comment l'identité des espèces épi-anéciques et le type de litières influençait la décomposition des litières et la structure des communautés microbiennes associées aux galeries de lombriciens (chapitre 3). Dans un troisième temps, nous avons évalué comment les différents assemblages d'espèces épi-anéciques et anéciques stricts contribuaient à la décomposition des litières et aux activités enzymatiques des micro-organismes associés à leurs cabanes et galeries (chapitre 4). Enfin, dans un quatrième temps nous avons évalué les impacts du type de litière et de l'interaction entre espèces sur l'assimilation des litières et donc le changement de biomasse individuelle des adultes de ces espèces anéciques (chapitres 3 et 5). Dans l'ensemble des travaux expérimentaux menés dans cette thèse, la perte de masse des litières a été utilisée comme proxy de la contribution des lombriciens à leur décomposition. Une présentation schématique du contexte et des chapitres de la thèse est présentée figure 8.

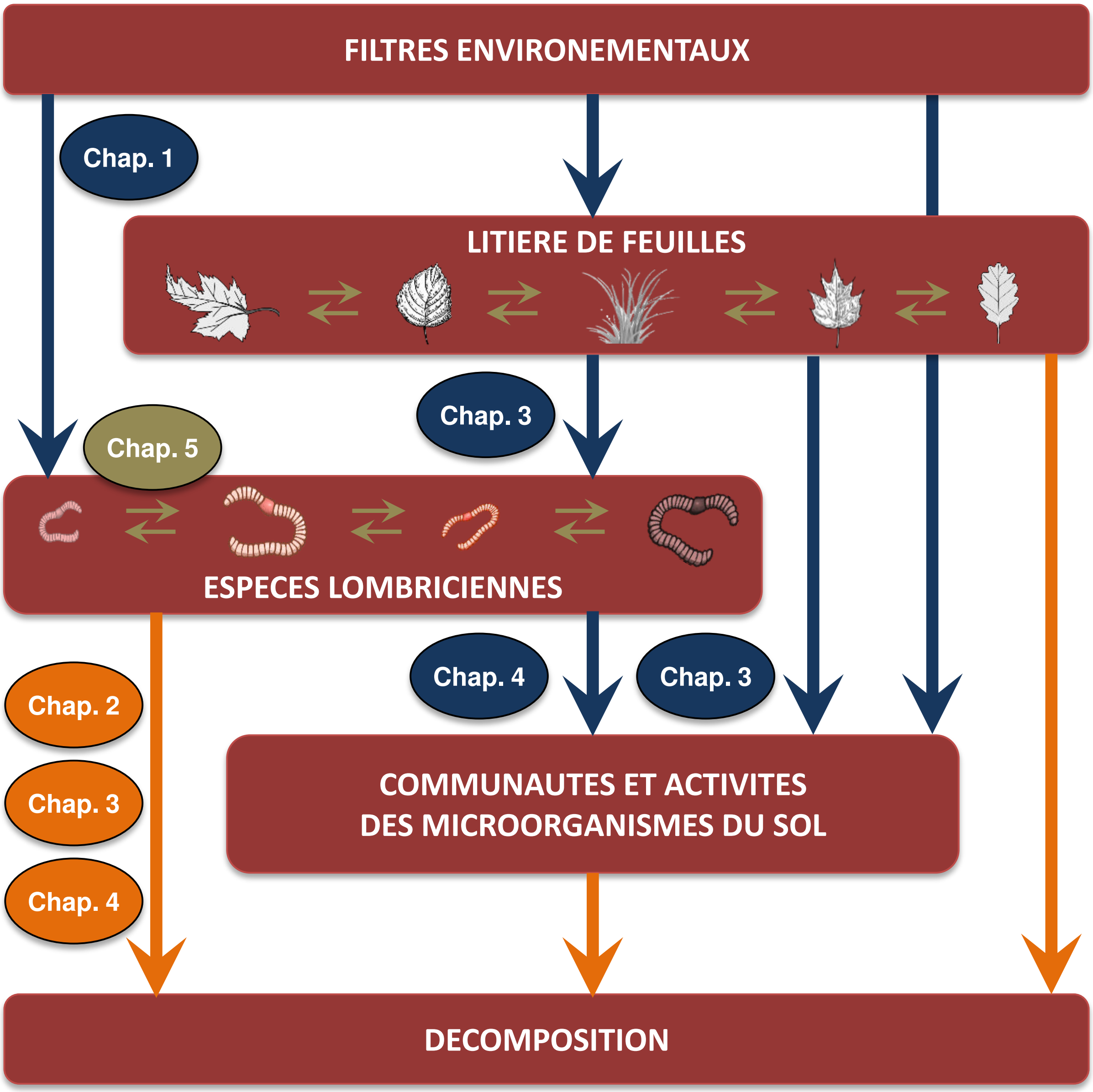

Figure 8. Présentation schématique du contexte et des chapitres de la thèse. Les flèches bleues représentent les facteurs qui régissent l'assemblage des organismes et des litières ; les flèches orange représentent les facteurs qui régulent le processus de décomposition et les flèches vertes représentent les interactions entre les espèces lombriciennes et au sein des litières. Les chapitres de la thèse (Chap.) s'insèrent dans chacune de ces trois parties avec les couleurs relatives aux flèches.

Ainsi, cette thèse s'articule autour de cinq chapitres, correspondant à autant d'articles dont deux sont publiés dans Soil Biology and Biochemitry (SBB) et deux autres sont en révisions dans des revues internationales :

Chapitre 1 : Règles d'assemblage des communautés lombriciennes dans les sols prairiaux (article en révision dans Pedosphere)

Chapitre 2 : Etat des connaissances sur le rôle des lombriciens anéciques dans le processus de décomposition de la litière (review en préparation pour soumission)

Chapitre 3 : Comportement alimentaire des espèces lombriciennes épi-anéciques et impacts sur les communautés microbiennes du sol (article publié dans SBB)

Chapitre 4 : Influence des lombriciens anéciques stricts et épi-anéciques et de leurs interactions sur les activités enzymatiques du sol (article publié dans SBB)

Chapitre 5 : Réponse de la biomasse individuelle des lombriciens anéciques à l'interaction entre deux espèces (article en révision dans Applied Soil Ecology)

CHAPITRE 1

Règles d'assemblage des communautés lombriciennes dans les sols prairiaux

Le premier chapitre correspond à un article en révision dans la revue internationale Pedosphere - Special Issue of the 1st International Earthworm Congress (IEC 1).

Objectifs de l'étude

Le but de ces observations de terrain était de tester l'hypothèse de « l'hétérogénéité de l'habitat » sur les communautés lombriciennes en prairie qui suppose que des habitats structurellement complexes peuvent fournir une plus grande diversité de niches et ainsi accroître la diversité des espèces. Pour cela nous avons évalué la contribution (i) des propriétés du sol (teneur en matière organique, pH, texture), (ii) de la gestion des prairies (âge des prairies, chargement en bétail, mode de fertilisation) et (iii) du paysage environnant (richesse et diversité du paysage, diversité des communautés végétales) sur l'assemblage des communautés lombriciennes dans les sols des prairies étudiées. De plus, à l'échelle des parcelles, nous avons testé l'hypothèse de « l'hétérogénéité de l'habitat » en comparant les communautés lombriciennes présentes à proximité de deux types de délimitation de parcelles, une haie et un fossé.

Méthodologie

Nous avons sélectionné 24 prairies selon un gradient de complexité de paysage au sein de la Zone Atelier Armorique (Long Term Ecological Research). Dans l'ensemble de ces prairies, nous avons échantillonné les lombriciens à 30 mètres de toute bordure de champs et sélectionné différents indicateurs des communautés lombriciennes à expliquer : l'abondance, la biomasse et la diversité lombricienne totale ainsi que celles de chaque sous-catégorie écologique. De plus, trois de ces prairies ont été ciblées pour sur-échantillonner les communautés lombriciennes se trouvant soit à proximité d'une haie et soit d'un fossé (contrôle sans haie).

Principaux résultats

Nos résultats indiquent que l'assemblage des communautés lombriciennes dans les sols de prairie était régie à la fois par les propriétés du sol, la gestion des prairies et la diversité du paysage environnant. Une teneur en matière organique importante et un pH du sol alcalin étaient les plus favorables à l'abondance et à la biomasse totale lombricienne, en particulier pour les lombriciens endogés. En ce qui concerne la gestion des prairies, l'augmentation du chargement en bétail était le facteur le plus nuisible aux communautés lombriciennes, en particulier pour la biomasse des anéciques et la richesse des endogés. La diversité des paysages a eu un impact négatif sur l'abondance lombricienne totale et sur la biomasse lombricienne des épigés, mais elle était corrélée positivement à la richesse des lombriciens anéciques. À une échelle plus fine, nous avons également démontré que la présence de haies entourant les prairies augmentait la richesse lombricienne et notamment celles des lombriciens épigés et épi-anéciques.

Soil properties, grassland management and landscape diversity drive the assembly of earthworm communities in temperate grassland.

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Abstract

Earthworms are widespread soil organisms contributing to a wide range of ecosystem services. As such, it is important to improve our knowledge, still scanty, of the rules driving the assembly of earthworm communities. The aim of the present study was to conjointly evaluate the effects of (i) soil properties, (ii) grassland management, (iii) landscape diversity, and (iv) presence of hedgerows on the assembly of earthworm communities. The study was conducted in temperate grasslands of Brittany, France. Earthworms were sampled in 24 grasslands, and among three grasslands earthworms were oversampled near a hedgerow and a ditch (control without hedgerow). Soil properties explained the larger part of the variation in earthworm community indicators compared to grassland management or landscape diversity. Increasing soil organic matter content and pH were the most favorable factors for earthworm abundance and biomass, and in particular for endogeic species. Regarding grassland management, increasing livestock unit was the most damaging factor for earthworm communities, in particular for anecic earthworm biomass and endogeic species richness. Surprisingly, landscape diversity negatively affected the total earthworm abundance and epigeic earthworm biomass, but landscape diversity was related to an increase of epi-anecic species. At a finer scale, we also demonstrated that the presence of hedgerows surrounding grasslands enhanced the earthworm species richness, especially within the epigeic and anecic ecological categories. As our study highlights that earthworm ecological categories respond specifically to environmental filters, the understanding of assembly rules of earthworm communities should be done at this ecological category level. In addition, our study points out key factors favoring or disfavoring earthworms communities, which could potentially help policymaker decisions for landscape management.

1. Introduction

Earthworms are widespread soil organisms constituting the most important terrestrial biomass under temperate climates (Hole, 1981; Bar-On et al., 2018). They are usually classified into three main ecological categories depending on their physiology, morphology and behaviour: epigeic, anecic and endogeic species (Bouché, 1972, 1977). Briefly, epigeic earthworms live in and consume surface organic matter, anecic earthworms burrow vertical galleries to feed on a mixture of surface and soil organic matter, and endogeic earthworms burrow horizontal galleries to feed on soil organic matter (Bouché and Kretzschmar, 1974; Bouché, 1977; Jégou et al., 1998a). Additionally, within the anecic earthworms, epi-anecic species feeding preferentially on fresh surface organic matter (*e.g.* leaf litter) are distinguished to strict-anecic species feeding preferentially on humified organic matter already incorporated into the soil (Ferrière, 1980; Jégou et al., 1998a; Bastardie et al., 2003; Hoeffner et al., 2018). Depending on their ecological categories and associated feeding or burrowing behaviour, earthworms are qualified as ecosystem engineers (Lavelle, 1988; Jones et al., 1994; Lavelle et al., 1997) contributing to distinct ecosystem services provided by the soil (Blouin et al., 2013; Bertrand et al., 2015). For example, van Groenigen et al. (2014) reported in a meta-analysis that an increase in crop production was observed in presence of earthworms, this increase ranging from 18% in presence of epigeic species to 32% in presence of anecic species.

Earthworm communities are governed by different environmental filters, including biogeographical history, soil properties, land use and management as well as species interactions within the community (*e.g.* competition, facilitation ; Lavelle, 1983; Curry, 2004; Decaëns et al., 2008). Previous studies focusing on the impact of soil properties on earthworms communities highlighted the key role played by soil pH, soil organic matter content and soil texture (Bouché, 1972; Joschko et al., 2006; Lee, 1985; Decaëns et al., 2008). Other studies focused on the impact of land use on these earthworm communities (Boag et al., 1997; Decaëns et al., 2003, 2008; Cluzeau et al., 2012). For example, Ponge et al. (2013) reported that grasslands exhibited a higher anecic earthworm abundance than croplands. In addition, Zaller and Arnone (1999) observed a positive correlation between the density and the biomass of earthworm communities and the plant species richness of grasslands, and in particular for endogeic species. Concerning land management, previous studies reported that ploughing (Chan, 2001; Briones and Schmidt, 2017), pesticide application (Pelosi et al., 2014) and low permanent cover (Vršic, 2011) negatively impact earthworm communities. Inter or intra-specific interactions may also shape earthworms communities: for instance, Butt et al. (1994) observed that the biomass as well as the growth of hatchling of *Lumbricus terrestris* was negatively impacted by the density of its population. However, these biotic interactions remain largely unstudied and only tested in laboratory experiments (Neuhauser et al., 1980; Hartenstein and Amico, 1983; Cluzeau, 1992; Butt et al., 1994).

Other studies have been undertaken at a greater scale to evaluate the impact of landscape diversity on earthworm communities within croplands (Vanbergen et al., 2007; Decaëns et al., 2008; Roarty and Schmidt, 2013; Lüscher et al., 2014; Frazão et al., 2017). For example, Flohre et al. (2011) observed that the earthworm species richness in croplands decreased with the percentage of surrounding agricultural fields. Regulska and Kolaczkowska, (2015) also reported that a cropland surrounded by a diverse landscape supported a higher earthworm diversity, density and biomass than the same type of cropland surrounded by a simple landscape. However, the majority of the previous studies reported no effect of landscape diversity on earthworms communities of croplands and vineyards (Decaëns et al., 2008; Kovács-Hostyánszki et al., 2013; Lüscher et al., 2014; Frazão et al., 2017; Buchholz et al., 2017). Moreover, field margins of croplands were reported to exhibit higher abundance and diversity of earthworms than in the croplands but, surprisingly, these field margins were not reported to enhance earthworm populations of these croplands (Smith et al., 2008; Roarty and Schmidt, 2013; Crittenden et al., 2015). Whether and how earthworms disperse within agricultural landscapes hence remains an unresolved issue.

A strong research effort has been done in the past decades to study the earthworm communities of croplands. However, grasslands remain poorly studied. Grasslands are the largest terrestrial ecosystem in the globe and produce many key ecosystem services, such as carbon storage, soil erosion mitigation or support for pollinators (Costanza et al., 1997; Conant and Paustian, 2002; Werling et al., 2014). The main objective of the present study was to conjointly evaluate the effects of soil properties, grassland management and landscape diversity on the assembly of grassland earthworm communities. Specifically, we hypothesized that the intensity of grassland management would negatively affect earthworm community indicators while the landscape diversity surrounding the grasslands would increase the earthworm community parameters. The second objective was to evaluate the effect of hedgerows on these earthworm communities. We hypothesized that the presence of a hedgerow in the grassland edge would increase earthworm community indicators. We conducted the study in an agricultural landscape of Brittany, France. Earthworms were sampled in 24 grasslands and among three grasslands earthworms were oversampled near a hedgerow and a ditch (control without hedgerow). Several indicators of the earthworm communities were evaluated including (i) the total abundance, total biomass, species richness and species diversity, (ii) the earthworm ecological categories abundance, biomass and richness and (iii) the earthworm individual biomass within these ecological categories.

2. Materials and methods

2.1. Study site

The study site covers 10 km2 and is a part of the Long Term Ecological Research (LTER) ‘‘Zone Atelier Armorique’’, located in Brittany, France (48°50’ N, -1°58’ W). The climate of the area is oceanic with a mean annual temperature of 11.7 °C, a mean annual rainfall of 815.0 mm and a mean annual relative humidity of 80.9 % (mean values over the period 2010-2016, data from Météo France). The main soil types encountered are Cambisols (IUSS Working Group, 2015) with high bedrock heterogeneity (granite, soft schist and aeolian loam). Moreover, the study area presents a substantial micro-topography, mainly due to a high variability of landscape structures (*e.g.* hedges and ditches as field margins) with a hedge density ranging from 50 to 100 m.ha-1 (Baudry et al., 2000; Thomas et al., 2016). Land use comprises mainly annual crops (corn, wheat, barley) and temporary or permanent grasslands, forest and unmanaged areas.

We used ground-truth aerial photos, which were taken every year since 1990, to construct a detailed land-use history for all grasslands, allowing us to precisely determine the age of each grassland. Based on this land-use history and verification with grassland owners, we selected 24 grasslands ranging from 1 to 25 years since the last crop. Among them, three grasslands with an age gradient of 1, 2 and 7 years old were selected and oversampled from a hedgerow and a ditch to take into account spatial effect of hedgerow on soil properties (Marshall and Moonen, 2002; Walter et al., 2003).

2.2. Earthworm sampling and laboratory analyses

Earthworms were sampled in 2016 first within the overall 24 grasslands at a 30 m distance from any grassland edges and second in the 3 selected grasslands at 1 m, 5 m and 10 m (i) from a hedgerow at the grassland edge and (ii) from a ditch at the grassland edge consisting a control treatment (*i.e* absence of a hedgerow).

Earthworms sampling followed the normalized protocol ISO 23 611-1, that was modified and validated during the RMQS BioDiv program (Cluzeau et al., 2012) combining chemical and physical extractions. Briefly, each earthworm sampling was characterised by a mean of three earthworms sub-sampling spaced of 10 m in line. Earthworms sub-sampling consisted of three watering of 10 L with a gradient concentration of formaldehyde (0.25, 0.25 and 0.4%) on one square meter. After each watering, earthworms were collected for 15 min. Afterwards, a block of soil (25 × 25 × 20 cm, length × width × depth) was excavated within each sub-sampling area and earthworms were hand-sorted. Earthworms' hand-sorted (HS) number was multiplied by 16 to obtain an estimation per square meter. This number was then added to the number of earthworms counted with the formaldehyde extraction (F) to obtain the total number of earthworms per square meter (FHS): $FHS = F + (16 \times HS)$.

In the laboratory, each earthworm individual was counted, weighted, assigned to a stage of development (juvenile, sub-adult and adult), identified at the sub-species level and assigned to its ecological categories: epigeic, anecic or endogeic (Bouché, 1972, 1977). Additionally, we distinguished within anecic earthworms, the epi-anecic (genus *Lumbricus*) from the strict-anecic earthworms (genus *Aporrectodea* ; Ferrière, 1980; Jégou et al., 1998a). For juvenile individuals, identification was first limited to the genus and thereafter they were identified at the species level according to the proportions of sub-adults and adults present on each square meter. Earthworm diversity was analyzed through three levels: total species richness, Shannon diversity index and species evenness index. In addition, we calculated the individual biomass of each adult within each grassland for the earthworm species represented by at least three adults in more than 10 grasslands.

Due to the low number of epigeic individual adults, we only focused on anecic and endogeic species for these individual biomass measurements.

2.3. Environmental filters

We selected three environmental filters to explain earthworm community indicators: soil properties, grassland management and landscape diversity.

Soil properties were characterized by the soil texture, organic matter content and pH that were assessed by sampling 10 bulk soil samples (20 cm depth) in each grassland. These 10 soil samples were pooled and homogenized to account for small-scale variations and sent to the analytical laboratory of LABOCEA (Combourg, France). Briefly, clay content ranged from 9.5% to 19.7%, sand content from 13.3% to 68.9%, organic matter content from 1.8% to 5.2% and soil pH from 5.5 to 6.7 (Supplementary Table S1).

Grassland management was assessed with farmers' interviews (Supplementary Table S1) and from ground-truth aerial photos. The grassland age ranged from 1 to 25 years since last row cropping with quite similar sowing (*Lolium perenne* and *Trifolium repens* or *pratensis*). In addition, livestock unit per hectare varied from 0 to 4.3. Fertilisation rate was declarative so we used only the distinction between organic and mineral input.

Landscape structure within 100 m radius around the sampled fields was classified into 9 habitats based on aerial photos. The radius of 100 m was chosen to reflect the overall low mobility of earthworms (Bardgett et al., 2005; Eijsackers, 2010, 2011). Landscape diversity was characterized by two indexes: total richness of habitats within the radius and Shannon Diversity Index of habitats (hereafter called SHDI). Mapping and analysis were done using the softwares QGis 2.8.1 and FRAGSTATS 4.296. In addition, we characterized the plant community of the 24 grasslands in Spring 2015 using 10 quadrats (1 × 1 m) evenly distributed in each grassland, characterizing for each plant species its covering percentage. Between the 24 grasslands selected, landscape richness varied from to 1 to 7 habitats, SHDI from 0.1 to 1.6 and plant Shannon index (hereafter called Plant diversity) within grasslands from 1.2 to 3.2 (Supplementary Table S1).

2.4. Statistical analysis

First, we used a multiple linear regression models to test the effects of soil properties (decomposed in clay, sand, organic matter and pH contents), grassland management (decomposed in grassland age, livestock unit and fertilisation), and landscape diversity (decomposed in landscape richness, SHDI and plant diversity) on all earthworm community indicators (*i.e.* total earthworm abundance and biomass, total earthworm diversity indexes, earthworm ecological categories abundance and biomass). In addition, we used a multiple linear regression models to test the effects of soil properties, grassland management, landscape diversity and earthworm abundance on earthworm individual biomass. More precisely, we included this earthworm abundance factor in order to test if the increase in earthworm individuals within an earthworm ecological category impact the individual biomass of a selected earthworm species within this earthworm ecological category. We constructed a full model comprising all environmental filters, and then we selected the significant environmental filters using a backward stepwise selection procedure, that selects the best model using the AIC criterion (Crawley, 2012; stepAIC function of the “MASS” package). We also evaluated the variance inflation factor (VIF) of each variable selected by the previous procedure, to test for multicollinearity among environmental filters. We removed from the model the environmental filters, even significant, that showed a VIF > 5. Data met the conditions of normality and homoscedasticity.

Second, within each of the three selected grasslands (*i.e.* 1, 2 and 7 years old), we compared fields earthworm communities with and without hedgerow using the three sampling points per plot as replicates. We used separated *t*-tests within the three selected grassland to assess the differences on earthworm abundance, earthworm biomass, and species richness according to the presence or absence of a hedgerow.

Statistical analyses were performed with the R software 3.2.3 (R. Core Team, 2017). Significance was evaluated in all cases at $P < 0.05$.

3. Results

3.1. Impact of soil properties, grassland management and landscape diversity on earthworms communities

Over the 24 grasslands sampled, the average earthworm abundance and biomass were 517.0 ± 57 individual.m^{-2} and 219.4 ± 20 g.m^{-2}, respectively. The mean earthworm species richness was 10.8 ± 0.3. Eighteen species belonging the three ecological categories were identified (Supplementary Table S2). *Allolobophora chlorotica* and *Aporrectodea caliginosa* were the most abundant species whereas *Eisenia tetraedra* and *Dendrobaena rubida* were present only in one grassland (data not shown).

Higher soil organic matter content increased the total earthworm abundance ($F = 5.3$, $P = 0.033$, Table 1), the endogeic species abundance ($F = 5.7$, $P = 0.028$, Supplementary Table S3) and the endogeic species richness ($F = 5.4$, $P = 0.031$, Supplementary Table S5). In addition, the endogeic species abundance was negatively correlated to the increase in sand content ($F = 6.9$, $P = 0.017$, Supplementary Table S3), while the total earthworm abundance and biomass increased when soil pH was more alkaline ($F = 5.0$ and 6.8, $P < 0.05$, Fig. 1, Table 1).

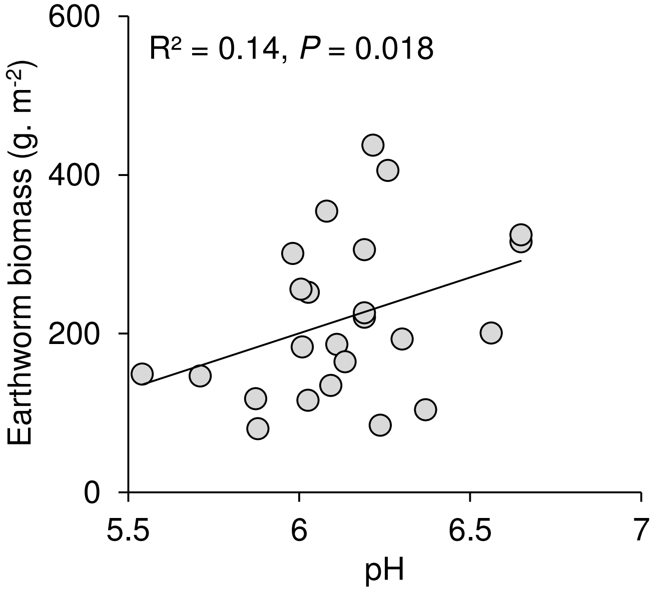

Fig. 1. Relationship between total earthworm biomass and soil pH. R² and associated *P*-value of the linear regression are indicated

The increase in livestock unit decreased total earthworm biomass ($F = 5.7$, $P = 0.028$, Table 1), and in particular anecic species biomass ($F = 9.6$, $P = 0.005$, Fig. 2a, Supplementary Table S4). However, this negative effect was confirmed only for epi-anecic species biomass ($F = 4.4$, $P = 0.049$, Fig. 2b, Supplementary Table S4). The increase in livestock unit also decreased total earthworm species richness, Shannon diversity index and species evenness ($F = 2.8$ to 9.6, $P < 0.05$, Fig. 2c, Table 1), and in particular endogeic species richness ($F = 9.5$, $P = 0.006$, Supplementary Table S5). Mineral fertilisation enhanced epigeic species abundance and biomass compared to organic fertilisation ($F = 6.6$ and 8.6, $P < 0.02$, Supplementary Table S3 and S4). Surprisingly, grassland age did not affect any of the indicators of the earthworm communities studied.

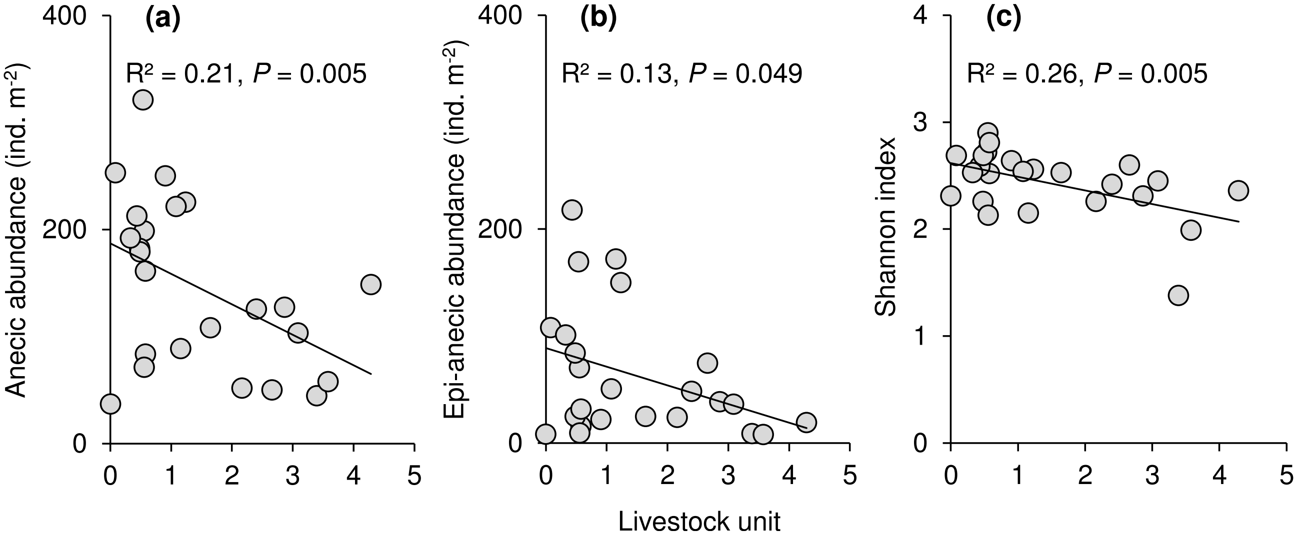

Fig. 2. Relationships between livestock unit and (a) anecic earthworm abundance, (b) epi-anecic earthworm abundance and (c) Shannon index. R² and associated *P*-values of linear regressions are indicated.

Landscape richness decreased epigeic species biomass ($F = 4.9$, $P = 0.041$, Supplementary Table S5) but enhanced epi-anecic species richness ($F = 6.6$, $P = 0.019$, Supplementary Table S5). The increase of SHDI decreased total earthworm abundance ($F = 4.6$, $P = 0.047$, Table 1). In addition, the increase in plant diversity was positively correlated to Shannon diversity index and species evenness ($F = 5.0$ and 4.8, $P < 0.04$, Table 1).

Interestingly, strict-anecic species abundance, biomass and richness were not affected by any of the environmental filters measured (Supplementary Table S3, S4 and S5).

3.2. Impact of soil properties, grassland management, landscape diversity and earthworm abundance on earthworm individual biomass

The individual biomass of earthworm species was highly variable across the 24 grasslands. For example, *Lumbricus rubellus rubellus* individual biomass ranged from 0.50 to 1.11 g (Fig. 3a) and the one of *Allolobophora icterica* varied from 0.24 to 0.97 g (Fig. 3c). Higher soil organic matter content increased the biomass of *Lumbricus festivus* ($F = 4.9$, $P = 0.049$, Table 2), whereas when soil was richer in sand and clay both *Lumbricus centralis* and *Lumbricus terrestris* biomasses decreased ($F = 12.1$ and 7.8, $P < 0.03$, Table 2). The increase of grassland age decreased the biomass of *Lumbricus rubellus rubellus*, *Lumbricus centralis* and *Lumbricus terrestris* ($F = 5.8$ to 14.6, $P < 0.04$, Table 2), but increased the biomass of *Aporrectodea nocturna* ($F = 5.2$, $P = 0.029$, Supplementary Table S6). The increase in plant diversity only enhanced the biomass of *Lumbricus centralis* ($F = 11.8$, $P = 0.026$, Table 2). Landscape richness only increased only the biomass of *Aporrectodea icterica* ($F = 12.3$, $P = 0.007$, Supplementary Table S6, Fig 3c). With increasing abundances of epi-anecic and strict-anecic earthworms, the individual mean mass of *Lumbricus rubellus* and *Aporrectodea nocturna* decreased, respectively ($F = 8.7$ and 5.2, $P < 0.04$, Table 2 and supplementary Table S6, Fig. 3a and b).

We noted that the biomass of *Aporrectodea giardi*, *Allolobophora chlorotica*, *Aporrectodea caliginosa* and *Allolobophora rosea* were not influenced by any environmental filters selected (data not shown).

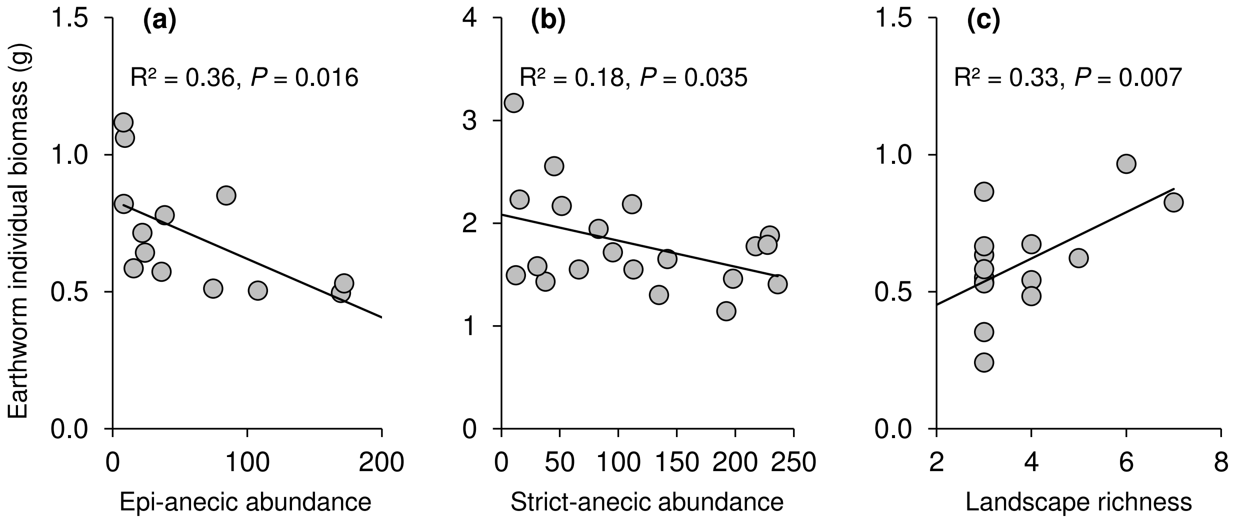

Fig. 3. Relationships between (a) individual adult biomass of *Lumbricus rubellus rubellus* and epi-anecic earthworm abundance, (b) individual adult biomass of *Aporrectodea nocturna* and strict-anecic earthworm abundance and (c) individual adult biomass of *Allolobophora icterica* and landscape richness. R² and associated *P*-values of linear regressions are indicated.

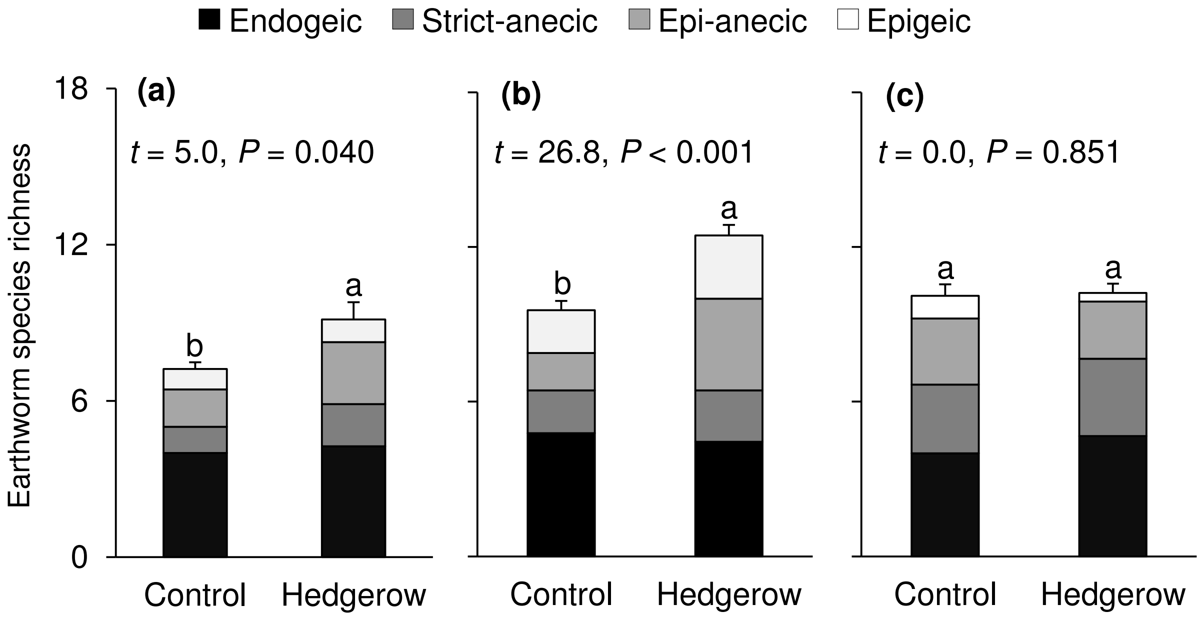

Fig. 4. Earthworm species richness in plots with a hedgerow or with a ditch (*i.e.* control plot without hedgerow) for grassland of (a) 1 year old, (b) 2 years old and (c) 7 years old. Values are means ± SD; n = 3. Different letters denote significant differences between the two plots with a > b (post hoc Tukey test results).

Table 1 ANOVA results of multiple linear models testing for the effects of soil properties, grassland management and landscape diversity on total earthworm abundance, total biomass, species richness and Shannon diversity index and evenness index. *F*-values and associated *P*-values are indicated. Significant *P*-values are indicated in bold ($P < 0.05$). df = degrees of freedom, %SS = percentage of sum of square.

	Total abundance				Total biomass				Total richness				Shannon				Equitability			
	df	%SS	*F*	*P*	df	%SS	*F*	*P*	df	%SS	*F*	*P*	df	%SS	*F*	*P*	df	%SS	*F*	*P*
Soil properties																				
Clay content	1	5.9	2.2	0.157																
Sand content	1	5.2	1.9	0.182	1	0.4	0.1	0.711	1	0.1	0.0	0.841								
Organic matter content	1	14.4	5.3	**0.033**	1	6.0	2.1	0.164												
pH	1	13.4	5.0	**0.039**	1	19.4	6.8	**0.018**												
Grassland management																				
Grassland age													1	7.5	9.9	0.107	1	7.3	2.4	0.141
Livestock unit					1	16.3	5.7	**0.028**	1	31.3	9.6	**0.005**	1	26.2	2.8	**0.005**	1	15.1	4.8	**0.040**
Fertilisation																				
Landscape diversity																				
Landscape Richness																				
SHDI	1	12.3	4.6	**0.047**	1	6.4	2.2	0.153												
Plant diversity													1	13.2	5.0	**0.037**	1	15.1	4.8	**0.040**
Residuals	18	48.7			18	51.5			21	68.6			20	53.1			20	62.5		

Table 2 ANOVA results of multiple linear models testing for the effects of soil properties, grassland management, landscape diversity and abundance of the corresponding earthworm ecological category on individual biomass of *Lumbricus rubellus rubellus*, *Lumbricus festivus*, *Lumbricus centralis*, *Lumbricus terrestris, Allolobophora icterica*. *F*-values and associated *P*-values are indicated. Significant *P*-values are indicated in bold (P < 0.05). df = degrees of freedom, %SS = percentage of sum of square.

	L. rubellus rubellus				*L. festivus*				*L. centralis*				*L. terrestris*			
	df	%SS	*F*	*P*	df	%SS	*F*	*P*	df	%SS	*F*	*P*	df	%SS	*F*	*P*
Soil properties																
Clay content					1	1.1	0.2	0.688	1	3.6	2.3	0.203	1	23.9	7.8	**0.013**
Sand content									1	19.1	12.1	**0.025**				
Organic matter content					1	30.3	4.9	**0.049**	1	4.4	2.8	0.169				
pH	1	0.5	0.1	0.730					1	6.2	3.9	0.119				
Grassland management																
Grassland age	1	26.6	6.5	**0.032**					1	23.0	14.6	**0.019**	1	17.8	5.8	**0.028**
Livestock unit									1	11.2	7.1	0.056				
Fertilisation																
Landscape diversity																
Landscape Richness																
SHDI																
Plant diversity									1	18.6	11.8	**0.026**	1	6.0	2.0	0.179
EC abundance																
Epi-anecic or endogeic	1	35.9	8.7	**0.016**					1	7.4	4.7	0.096				
Residuals	9	37.0			11	68.6			4	6.3			17	52.3		

3.3. Impact of hedgerow presence on earthworms communities

The three grasslands selected to test the effect of hedgerow presence on earthworm communities showed distinct earthworm community indicators. Briefly, the earthworm abundance was higher in the 2-years old grassland (834 ± 76 individuals.m^{-2}) compared to the 1-year old (306 ± 32 individuals.m^{-2}) and 7-years old grasslands (385 ± 32 individuals.m^{-2}). Earthworm species richness was higher in the 2- and 7-years old grasslands (11.0 ± 0.4 and 10.2 ± 0.3, respectively) compared to the 1-year old grassland (7.9 ± 0.4). Earthworm species composition was also strongly different between these three grasslands. For example *Eisenia tetraedra* occurred only in the 2-years old grassland and *Aporrectodea caliginosa meridionalis* occurred only in the 7-years-old grassland.

Earthworm abundance was not affected by the presence of a hedgerow in the three selected grasslands (t = 0.0 to 0.03, P > 0.865). In contrast, earthworm species richness was 21.0% and 23.2 % higher in presence of a hedgerow than in presence of a ditch for the grassland of 1- and 2-years old, respectively (t = 5.8 and 13.9, P < 0.03, Fig. 4a and b). Earthworm species richness was not affected by the presence of a hedgerow in the 7-years old grassland (t = 0.0, P = 0.851, Fig. 4c).

Overall, except *Allolobophora icterica* and *Aporrectodea nocturna*, strict-anecic and endogeic species were evenly distributed between the plots with and without a hedgerow. Epi-anecic earthworm species distribution was heterogeneous, but *Lumbricus rubellus rubellus* and *Lumbricus terrestris* were more often observed within the plot with a hedgerow than within the control plot. The distribution of epigeic earthworm species was species dependent: *Dendrobaena mammalis* occurrence was higher in the plot with a hedgerow and *Eisenia tetraedra* was observed in the plot with a hedgerow only in the 2-years old grassland. *Lumbricus castaneus* and *Lumbricus rubellus castanoïdes* occurrences were overall similar between the plots with and without a hedgerow.

4. Discussion

In the present study, we clearly demonstrated that the selected indicators of the grassland earthworm communities were conjointly impacted by soil properties, grassland management and landscape diversity. Our findings hence contrast with those of Frazão et al. (2017) that reported that earthworm communities of croplands were impacted only by agricultural practices and not by soil properties nor landscape diversity.

Contrary to previous studies that observed a soil property effect only at the regional scale (Decaëns et al., 2003; Vanbergen et al., 2007; Decaëns et al., 2008), here we observed that both total earthworm abundance, earthworm biomass and species richness were strongly influenced by soil properties at a finer scale, *i.e.* 10 km². This result might be due to the strong spatial heterogeneity of the soil properties in the studied region (Jamagne, 2011). In agreement with previous studies, we observed that the increase in soil sand content decreased total earthworm abundance (Hendrix et al., 1992; Lapied et al., 2009), which could be due to the low capacity of sandy soils to hold water leading to an unfavorable habitat for earthworms (Lee, 1985). In addition, the increase in soil pH was positively correlated to both earthworm species richness (Joschko et al., 2006) and total earthworm abundance (Ma et al., 1990). Nonetheless, several reviews have shown that earthworm sensibility to soil pH was species dependent (Bouché, 1972; Edwards and Lofty, 1977; Lee, 1985). In line with their feeding behavior that consists in consuming mainly humified organic matter, endogeic earthworm communities were more abundant and diversified in grasslands presenting high soil organic matter content (Bouché, 1977; Piearce, 1978; Bouché and Kretzschmar, 1974; Ferrière, 1980).

Regarding grassland management, increasing livestock unit was the most damaging factor for earthworm communities as it decreased both total earthworm biomass, earthworm species richness, Shannon diversity index and species evenness. This strong negative effect could be associated to the fact that trampling at high stocking levels can damage soil structure and thus adversely affect earthworm communities and burrows (Hutchinson and King, 1980; Cluzeau et al., 1992; Ligthart, 1997; Pietola et al., 2005; Chan and Barchia, 2007). Interestingly, earthworm response to livestock unit was almost entirely confined to the larger anecic and endogeic species and only earthworm biomass was affected compared to earthworm abundance, suggesting a decrease in mean body size rather than in individuals' number. Surprisingly, mineral fertilisation enhanced epigeic species abundance and biomass, but this finding is nonetheless in line with some previous studies that reported an increase in earthworm abundance according to N mineral fertilisation (Muldowney et al., 2003; King and Hutchinson, 2007; Curry et al., 2008). Overall, we observed that within grasslands, pasturage pressure led to smaller and less-diversified earthworm communities, but mineral fertilization could counteract this effect.

To our knowledge, for the first time, we observed a negative effect of increasing landscape diversity on total earthworm abundance and epigeic biomass in grasslands. A negative correlation between total earthworm abundance and landscape diversity was also observed by Flohre et al., (2011) in croplands, and the authors hypothesized that landscape diversity increases the number of earthworm predators. Indeed, several studies highlighted that landscape diversity could enhance the abundances of invertebrates, mammals and birds that are potential predators for earthworms (Granval and Aliaga, 1988; Marshall and Moonen, 2002; Vickery et al., 2002; Maudsley et al., 2002; O'Brien et al., 2016). In contrast, epi-anecic earthworm richness was enhanced by the landscape diversity. As epi-anecic earthworm species have a great mobility (Hoogerkamp et al., 1983; Eijsackers, 2010, 2011; Nuutinen et al., 2014), thus increasing landscape diversity around grasslands could enhance their areas of emigration. In addition, increasing landscape diversity can also enhance litter quantity and diversity deposited at the soil surface and thus favours epi-anecic earthworms that have a diet mainly composed of fresh organic matter (Bouché and Kretzschmar, 1974; Piearce, 1978; Ferrière, 1980). Endogeic earthworm species were not impacted by landscape diversity and there were highly abundant within each grassland as previously reported (Lavelle, 1983; Decaëns et al., 2008). Overall, it is possible that low agricultural practices within grasslands, compared to croplands or vineyard, could increase the effect of the surrounding landscape diversity on earthworm communities (Flohre et al., 2011; Roarty and Schmidt, 2013; Lüscher et al., 2014; Frazão et al., 2017; Buchholz et al., 2017).

In addition to the landscape diversity effect, we highlighted the importance of hedgerow surrounding the grassland, especially in the young grasslands (*i.e.* 1- and 2-years old grassland). It is well known that hedgerow locally modify soil properties (Marshall and Moonen, 2002), and especially the amount and type of litter deposited every year on the soil surface (Walter et al., 2003). This litter input is a key factor for earthworm communities development (Lee, 1985; Edwards, 2004a), and in particular for epigeic and epi-anecic species that have a diet mainly composed of fresh organic matter (Bouché and Kretzschmar, 1974; Piearce, 1978; Ferrière, 1980). In-field earthworm communities surrounded by a hedgerow contained higher earthworm species richness than the communities surrounded by a ditch, especially for epigeic and epi-anecic earthworm species. Thus hedgerows presence could favour more diverse grassland earthworm communities.

Finally, we also demonstrated for the first time that morphological variability of several earthworm species was strongly impacted by the environmental filters selected. Surprisingly, the study of earthworm morphological variability is poorly developed, and it is commonly accepted that it is mainly controlled by soil properties (Satchell 1967, Lavelle 1983). In addition, several laboratory studies reported that earthworm population density could also negatively affect the growth rate of earthworm species (Neuhauser et al., 1980; Hartenstein, 1984; Cluzeau, 1992; Butt et al., 1994; Mathieu, 2018). For example, with an increasing density varying from 1 to 12 individuals of *L. terrestris* in the same soil volume, Butt et al., (1994) observed a decrease in their individual biomass of approximately 74%. Our study conducted at the field scale confirmed these previous laboratory observations for both *L. rubellus* and *A. icterica*. We indeed observed a 55% decrease in the individual biomass of *L. rubellus* between grasslands with a low density and a high density of epi-anecic earthworms and, similarly, the individual biomass of *A. icterica* decreased by 75% between grasslands containing a low density and a high density of endogeic earthworms. These first results based on field observations are of great importance given the key role played by individual earthworm biomass since this biomass is directly linked to population dynamics (Michon, 1954; Butt et al., 1994) and to their impact on soil functioning (Hoeffner et al 2018).

5. Conclusion

Our study clearly illustrated that earthworm communities of grasslands were affected by the three environmental filters considered: soil properties, grassland management and landscape diversity. Soil properties were the main environmental filters controlling earthworm communities. However, we also highlighted important effects of grassland management, for instance a strong decrease in abundance of earthworms with large livestock unit. We observed various effects of landscape diversity, such as a surprising overall decrease of earthworm abundance in diverse landscapes. Therefore, our findings demonstrated conjoint effects of various environmental filters as drivers of earthworm communities. Taken together, our results suggest a strong context dependency in the assembly rules of earthworm communities, despite these communities are well known to be ubiquitous and resilient.

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SUPPORTING INFORMATION

Table S1 Mean value and rage of soil properties, grassland management and landscape diversity paramaters selected in the study.

	Mean ± SE	Range
Soil properties		
Clay content	13.3 ± 0.5	9.5 - 19.7
Sand content	42.5 ± 3.6	13.3 - 68.9
Organic matter content	3.1 ± 0.1	1.8 - 5.2
pH	6.1 ± 0.1	5.5 - 6.6
Grassland management		
Grassland age	8.5 ± 1.6	1 - 25
Livestock unit	1.5 ± 0.3	0 - 4.3
Fertilisation	Organic	10
	Mineral	14
Landscape diversity		
Landscape Richness	3.8 ± 0.3	2 - 7
SHDI	0.7 ± 0.1	0.1 - 1.6
Plant diversity	2.0 ± 0.1	1.2 - 3.2

Table S2 Names and associated ecological characteristics of the 18 earthworm species of study site.

Earthworm species or sub-species	Description	Ecological category
Eisenia tetraedra	Savigny (1826)	Epigeic
Dendrobaena rubida rubida	Savigny (1826)	Epigeic
Dendrobaena mammalis	Savigny (1826)	Epigeic
Lumbricus castaneus	Savigny (1826)	Epigeic
Lumbricus rubellus castanoides	Bouché (1972)	Epigeic
Lumbricus rubellus rubellus	Hoffmeister (1843)	Epi-anecic
Lumbricus terrestris	Linné (1758)	Epi-anecic
Lumbricus centralis	Bouché (1972)	Epi-anecic
Lumbricus festivus	Savigny (1826)	Epi-anecic
Aporrectodea giardi	Savigny (1826)	Strict-anecic
Aporrectodea nocturna	Ude (1885)	Strict-anecic
Aporrectodea caliginosa meridionalis	Bouché (1972)	Strict-anecic
Aporrectodea caliginosa caliginosa	Savigny (1826)	Endogeic
Allolobophora chlorotica chlorotica	Savigny (1826)	Endogeic
Allolobophora icterica	Savigny (1826)	Endogeic
Allolobophora rosea rosea	Savigny (1826)	Endogeic
Octalasium cyaneum	Savigny (1826)	Endogeic
Octalasium lacteum	Savigny (1826)	Endogeic

Table S3 ANOVA results of multiple linear models testing for the effects of soil properties, soil management and landscape diversity on epigeic, anecic, epi-anecic, strict-anecic and endogeic earthworm abundance. *F*-values and associated *P*-values are indicated. Significant *P*-values are indicated in bold (P < 0.05). df = degrees of freedom, %SS = percentage of sum of square.

	Epigeic abundance				Anecic abundance				Epi-anecic abundance				Strict-anecic abundance				Endogeic abundance			
	df	%SS	*F*	*P*	df	%SS	*F*	*P*	df	%SS	*F*	*P*	df	%SS	*F*	*P*	df	%SS	*F*	*P*
Soil properties																				
Clay content																	1	6.5	2.6	0.124
Sand content	1	1.5	0.5	0.500													1	17.3	6.9	**0.017**
Organic matter content	1	6.7	2.1	0.167	1	18.5	2.3	0.143	1	11.3	3.1	0.095					1	14.3	5.7	**0.028**
pH					1	27.3	3.4	0.079					1	12.0	3.2	0.090	1	9.6	3.8	0.066
Grassland management																				
Grassland age																				
Livestock unit																				
Fertilisation	1	21.6	6.6	**0.0191**																
Landscape diversity																				
Landscape Richness	1	11.6	3.6	0.075																
SHDI					1	27.1	3.4	0.079	1	10.9	2.9	0.101	1	8.4	2.2	0.150	1	7.1	2.9	0.109
Plant diversity																				
Residuals	18	58.5			20	27.1			21	77.8			21	79.6			18	45.1		

Table S4 ANOVA results of multiple linear models testing for the effects of soil properties, soil management and landscape diversity on epigeic, anecic, epi-anecic, strict-anecic and endogeic earthworm mass. *F*-values and associated *P*-values are indicated. Significant *P*-values are indicated in bold (P < 0.05). df = degrees of freedom, %SS = percentage of sum of square.

	Epigeic biomass				Anecic biomass				Epi-anecic biomass				Strict-anecic biomass				Endogeic biomass			
	df	%SS	*F*	*P*	df	%SS	*F*	*P*	df	%SS	*F*	*P*	df	%SS	*F*	*P*	df	%SS	*F*	*P*
Soil properties																				
Clay content																	1	1.7	0.5	0.477
Sand content	1	0.2	0.1	0.777	1	0.6	0.2	0.666	1	0.0	0.0	0.986					1	13.6	4.2	0.055
Organic matter content	1	11.3	4.0	0.060													1	12.7	3.9	0.062
pH													1	12.2	3.3	0.084	1	10.2	3.1	0.093
Grassland management																				
Grassland age																				
Livestock unit					1	31.6	9.6	**0.005**	1	17.2	4.4	**0.049**	1	9.6	2.6	0.123				
Fertilisation	1	24.2	8.6	**0.009**																
Landscape diversity																				
Landscape Richness	1	13.6	4.7	**0.041**																
SHDI																				
Plant diversity																				
Residuals	18	50.7			21	69.1			21	82.8			21	78.2			19	61.8		

Table S5 ANOVA results of multiple linear models testing for the effects of soil properties, soil management and landscape diversity on epigeic, anecic, epi-anecic, strict-anecic and endogeic earthworm richness. *F*-values and associated *P*-values are indicated. Significant *P*-values are indicated in bold (P < 0.05). df = degrees of freedom, %SS = percentage of sum of square.

	Epigeic richness				Anecic richness				Epi-anecic richness				Strict-anecic richnesss				Endogeic richness			
	df	%SS	*F*	*P*	df	%SS	*F*	*P*	df	%SS	*F*	*P*	df	%SS	*F*	*P*	df	%SS	*F*	*P*
Soil properties																				
Clay content													1	2.8	0.8	0.383				
Sand content	1	11.0	2.7	0.113													1	0.3	0.1	0.748
Organic matter content																	1	15.4	5.4	**0.031**
pH					1	16.2	4.2	0.051	1	4.5	1.3	0.269								
Grassland management																				
Grassland age									1	5.0	1.4	0.246	1	14.3	4.0	0.059				
Livestock unit													1	6.7	1.9	0.187	1	27.1	9.5	**0.006**
Fertilisation																				
Landscape diversity																				
Landscape Richness									1	22.9	6.6	**0.019**								
SHDI									1	1.4	0.4	0.537								
Plant diversity													1	8.6	2.4	0.138				
Residuals	22	89.0			22	83.8			19	66.2			19	67.6			20	57.2		

Table S6. ANOVA results of multiple linear models testing for the effects of soil properties, grassland management, landscape diversity and abundance of the corresponding earthworm ecological category on individual biomass of *Aporrectodea nocturna* and *Allolobophora icterica*. *F*-values and associated *P*-values are indicated. Significant *P*-values are indicated in bold (P < 0.05). df = degrees of freedom, SS = percentage of sum of square.

	Aporrectodea nocturna				*Allolobophora icterica*			
	df	%SS	*F*	*P*	df	%SS	*F*	*P*
Soil properties								
Clay content					1	5.4	1.5	0.254
Sand content								
Organic matter content	1	9.3	3.0	0.103				
pH					1	0.1	0.0	0.896
Grassland management								
Grassland age	1	17.7	5.6	**0.029**				
Livestock unit					1	1.9	0.5	0.487
Fertilisation								
Landscape diversity								
Landscape Richness					1	44.9	12.3	**0.007**
SHDI								
Plant diversity					1	15.0	4.1	0.073
EC abundance								
Strict-anecic or endogeic	1	16.3	5.2	**0.035**				
Residuals	18	57.7			9	32.7		

CHAPITRE 2

Etat des connaissances sur le rôle des lombriciens anéciques dans le processus de décomposition de la litière

Le deuxième chapitre correspond à une review qui sera soumise à la revue internationale Pedobiologia.

Objectifs et méthodologie de la synthèse bibliographique

Le but de cette synthèse bibliographique était de faire un état de l'art des connaissances actuelles sur le comportement alimentaire des lombriciens anéciques notamment en renforçant la distinction entre les lombriciens anéciques stricts et les épi-anéciques. Pour cela nous avons (i) qualifié et (ii) quantifié le spectre des ressources des lombriciens anéciques mais également (iii) évalué les propriétés de la matière organique influençant leur appétence, (iv) résumé leur digestion et leur assimilation de la matière organique et (v) évalué les conséquences de leur spectre de ressources sur les cycles du carbone et de l'azote dans leur fèces.

Principaux résultats

Dans cette synthèse nous avons souligné qu'au sein des lombriciens anéciques, il est possible de distinguer deux sous-catégories écologiques lombriciennes en se basant sur leur comportement alimentaire : les lombriciens épi-anéciques et les anéciques stricts. Les lombriciens épi-anéciques (genre *Lumbricus*) consomment principalement de la litière végétale fraîche à la surface du sol. Leur tube digestif est alors fortement concentré en matière organique, ce qui semble stimuler l'abondance et modifier les communautés de microorganismes y habitant. Les fèces des lombriciens épi-anéciques sont donc très riches en carbone et azote d'origine végétale. En revanche, les lombriciens anéciques stricts (genre *Aporrectodea*) consomment à la fois de la matière organique humifiée dans le sol (augmentant leur comportement fouisseur par rapport aux lombriciens épi-anéciques) et une légère proportion de litière végétale fraîche. Leur tube digestif est alors essentiellement composé de matière organique humifiée. Par conséquent, leurs fèces sont également plus riches en carbone et en azote par rapport au sol environnant, mais moins que celles des lombriciens épi-anéciques. Cette distinction entre lombriciens anéciques stricts et épi-anéciques est donc nécessaire à prendre en compte dans la décomposition des litières végétales puisqu'ils ont des spectres de ressources différents avec des conséquences sur les cycles du carbone et de l'azote contrastés.

A review of interactions between temperate earthworms and organic matter with a focus on sub-divisions of the anecic earthworm ecological category.

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Keywords:

Lumbricidae, SOM, resource spectrum, palatability, ecological category, cast, faeces

Abstract

Soil organic matter (SOM) has a crucial role in soil functioning and earthworms, widespread in temperate climates, interact extensively with SOM which they ingest, digest, assimilate and excrete. Usually, earthworm species are classified into three ecological categories, epigeic, anecic and endogeic earthworms with a particular interaction with SOM. In this review, we highlighted that within anecic earthworms, two sub-categories can be distinguished on their feeding behaviour: epi-anecic from the strict-anecic earthworms. Epi-anecic earthworms (genus *Lumbricus*) consume mainly fresh plant litter on the soil surface. Their digestive tract is then highly filled with material concentrated in organic matter, which seems to stimulate the abundance and modify communities of microorganisms. Consequently, their faeces are very rich in C and N of relatively recent plant origin. By contrast, strict-anecic earthworms (genus *Aporrectodea*) consume both humified organic matter already incorporated into the soil and a slight proportion of fresh plant litter with a more important burrowing behaviour than epi-anecic earthworms. Their digestive tract is then essentially composed of humified organic matter. Consequently, their faeces are also richer in C and N compared to the surrounding soil but less when compared with epi-anecic earthworms. This distinction between epi- and strict- anecic earthworms leads to different impacts on the overall turnover and the different pools of the SOM.

1. Introduction

Soil organic matter (SOM) plays a central role in provisioning, regulating, cultural and supporting ecosystem services (Wall, 2012; Adhikari and Hartemink, 2016). In terrestrial ecosystems, SOM is composed of all living and dead organisms in the soil (animal or plant origin) which, post-death, are subjected to decomposition processes that determine the nature of the SOM. The decomposition process consists of the following steps: leaching, fragmentation, incorporation, microbial catabolism, and stabilization (Curry, 1994; Coûteaux et al., 1995). Decomposition is driven by the initial physical and chemical organic matter composition, its initial distribution within the profile and the temperature and moisture regimes of the soil and soil organisms (Stout and Goh, 1980; Coûteaux et al., 1995).

Among soil decomposers in temperate climates earthworms are widespread, present in many ecosystems (Boag et al., 1997; Decaëns et al., 2008; Cluzeau et al., 2012) are considered as ecosystem engineers, modifying soil physical, chemical and biological composition (Jones et al., 1994; Blouin et al., 2013) and are key organisms (Blondel and Aronson, 1995) especially in organic matter decomposition (Edwards and Bohlen, 1996). They are mainly found in the subsurface horizon (0 to 20 cm) and due to their widespread distribution in temperate climates and the data available, this review will only consider the Lumbricidae family. Interaction between earthworms and organic matter remains one of the most important, challenging questions due to its further impact in the SOM decomposition process. Earthworms are heterotrophic organisms that feed on SOM and many studies have been undertaken to more fully understand earthworms feeding behaviour and consequences on soil ecosystem (Perel, 1977; Satchell, 1983; Lee, 1985; Edwards and Bohlen, 1996; Edwards, 2004b; Curry and Schmidt, 2007). Overall, these studies neglected that earthworm species have been classified into three main ecological categories; epigeic, endogeic and anecic (Bouché, 1972, 1977). Depending on the earthworm ecological category, SOM will be incorporated into the soil, ingested, digested, absorbed, utilized (metabolism) and excreted differently (Laverack, 1963; Edwards, 2004b; Curry and Schmidt, 2007). According to Bouché, (1977), epigeic earthworms have a high metabolism, live on the soil surface with no real ability to burrow into the soil and feed on surface organic matter; endogeic earthworms feed on humified organic matter accompanied with more or less mineral soil, with horizontal or near-horizontal burrows; anecic earthworms feed on soil surface organic matter and they are able to incorporate it into the soil in their vertical or near-vertical permanent burrows.

Anecic earthworms incorporate SOM into the soil either directly or via casting deposited in their burrows forming the drilosphere (Lavelle, 1988; Brown et al., 2000). Consequently, this behaviour modifies the surface available resource for surface decomposers (Rämert et al., 2001) and in their burrows, anecic earthworms modify activities and communities of soil decomposers (Görres et al., 1997; Schrader and Seibel, 2001; Tiunov et al., 2001) and notably soil microorganisms (Brown, 1995; Brown et al., 2000; Tiunov and Dobrovolskaya, 2002), which at a fine scale, are the main actors of organic matter decomposition (Chapin et al., 2002). Consequently, the drilosphere generated by anecic earthworms is often rich in C and N compared with the surrounding soil (Syers et al., 1979). However, Bouché (1977) specified that a continuum exists between the three main earthworm ecological categories, *i.e.* there are sub-categories. Since Bouché (1972, 1977), several authors have specified or qualified these ecological categories (Perel, 1977; Lavelle, 1983; Lee, 1985). As there are differences in feeding behaviour between the three earthworm ecological categories, it can be expected that there will also be some slight differences between earthworm ecological sub-categories. For example, (Lavelle, 1983, 1988) proposed the distinction within the endogeic category; euryhumic, oligohumic and polyhumic endogeic earthworms, based on the organic content of material ingested and therefore partly on endogeic location in the soil profile.

Further studies on feeding behaviour of anecic earthworms sub-divided epi-anecic earthworms from strict-anecic earthworms (Ferrière, 1980; Cortez et al., 1989; Jégou et al., 2000, 2001; Andriuzzi et al., 2015; Larsen et al., 2016). As a consequence, Andriuzzi et al. (2016) observed that *L. terrestris* increased root biomass, whereas *A. longa* increased shoot biomass. Moreover, several studies have also confirmed this possible distinction between epi- and strict-anecic earthworms in their burrowing behaviour (Jégou et al., 1998b, 2001; Bastardie et al., 2005), obligatory diapause (Michon, 1954; Satchell and Lowe, 1967) or the tegument thickness (Briones and Álvarez-Otero, 2018). Thus, these differences in physiology, anatomy and behaviour could mean ecological sub-categories within anecic earthworms and so different contributions to organic matter decomposition. In addition, feeding behaviour may play a major part in determining the structure of earthworm communities (Lavelle, 1983, 1985; Decaëns et al., 2008). Overall, very few studies have compared or used epi- and strict- anecic earthworm designations at the same time in the decomposition process of organic matter, despite the fact that several authors reported that epi- or strict-anecic earthworm communities could be composed from 1 to 6 species each living together in the same area (Gerard, 1967; Poier and Richter, 1992; Boag et al., 1997; Margerie et al., 2001; Decaëns et al., 2008).

Different factors can influence the palatability of organic matter or the resource spectrum of anecic earthworms: (1) species that evolve on a physiological, anatomical and ecological gradient (Bouché, 1972, 1977); (2) soil properties (Daniel and Anderson, 1992; Wolters and Joergensen, 1992; Neilson et al., 2000; Koutika et al., 2001; Clause et al., 2014; Ashwood et al., 2017); (3) soil moisture and temperature (Butt, 1991; Daniel, 1991; Daniel et al., 1996; Cortez, 1998; Berry and Jordan, 2001; Rämert et al., 2001; Butt and Lowe, 2011; Butt and Briones, 2011); (4) time or photoperiod (Curry and Byrne, 1997; Cortez, 1998); (5) earthworm stage of development (Bouché and Kretzschmar, 1974; Schmidt et al., 1997b; Schmidt, 1999; Whalen et al., 2000); or (6) their repleteness (Edwards and Bohlen, 1996).

For this review, we propose to strengthen this distinction between epi- and strict-anecic earthworms on their interaction with SOM by (i) qualifying and (ii) quantifying the resource spectrum of anecic earthworms, as well as (iii) assessing the mechanisms influencing palatability of SOM, (iv) summarizing the action of anecic earthworms during digestion and assimilation of SOM and (v) evaluating the direct consequences on the future of SOM (C and N) in their fresh casts.

2. Procedure

A literature search of peer-reviewed article up until August 2018 was made using Web of Science using English and French languages. For this review, we decided to distinguished epi- and strict-anecic earthworms on their (i) resource spectrum, (ii) mechanisms that modify organic matter during intestinal transit and (iii) their egestate in terms of C and N. For each part, an overview was constructed on what is known about anecic earthworms, to better understand the differences between epi- and strict-anecic sub-categories. Based on the anecic species placement on the earthworm ecological categories triangle proposed by Bouché (1977), we classified the anecic earthworm species (presented in this review) into epi- and strict-anecic earthworms (Table 1). When data in original publications were presented graphically, we estimated values from manually digitized figures.

For the resource spectrum, we used studies using (i) the litter mass loss observed between before and after the experiment, (ii) the digestive tract content, (iii) mass change and (iv) isotopic markers of organic matter that can be found either in the soil or in the composition of the anecic earthworm body. These four observational methods are complementary and do not have the same constraints. The quantification of the digestive tract gives an indication of the resource spectrum proportion ingested by anecic earthworms because it can be undertaken on individuals *in situ* with a wide variety of organic matter available. However, it does not reflect what anecic earthworms actually assimilate or that the parts of the digestive tract observed differ according to the authors, thus, authors' observations cannot be strictly compared.

For organic matter removal, we used data of litter mass loss (mg. g^{-1} day^{-1}) published by different authors. We excluded studies that did not include controls (without earthworms), otherwise it is impossible to distinguish earthworm activity from microorganism activity. Organic matter burial by earthworms in the soil is either by mechanical action or by ingestion and egestion into the soil profile. Nevertheless, it is difficult to know in what proportion this organic matter is buried or ingested by earthworms.

Table 1: Proposed classification of the major anecic earthworm species in temperate climates (featured in this review) according to Bouché (1977) and the sub-division between epi-anecic and strict anecic earthworms.

Epi-anecic earthworms	Strict-anecic earthworms
Lumbricus terrestris (Linné, 1758)	*Aporrectodea giardi* (Savigny, 1826)
Lumbricus centralis (Bouché, 1972)	*Aporrectodea longa* (Ude, 1885)
Lumbricus friendi (Cognetti de Martiis, 1904)	*Aporrectodea longa ripicola* (Bouché, 1972)
Lumbricus festivus (Savigny, 1826)	*Aporrectodea nocturna* (Evans, 1946)
Lumbricus rubellus rubellus (Hoffmeister, 1843)	*Aporrectodea caliginosa meridionalis* (Bouché, 1972)
	Aporrectodea velox (Bouché, 1967)

Regarding the mechanisms that transform organic matter during intestinal transit, we have identified three sub-parts, the microorganisms and the enzymes observed it in the gut as well as the assimilation of organic matter by epi- and strict-anecic earthworms.

For chemical composition of faeces, we decided to focus only on C and N cycles due to their high consequences in ecosystem function. We are aware that earthworms excrete mucus or urine which can be very rich in N or organic compounds (Needham, 1957; Laverack, 1963; Tillinghast, 1967; Schmidt et al., 1999) but due to the lack of available studies to clarify this, we focused only on fresh casts.

3. Resource spectrum

3.1. Qualitative

Pokarzhevskii et al. (1997) proposed that earthworms are ecosystemivores not saprophagous animals due to the very large spectrum of resources that pass through their digestive tract, more simply they are omnivores (Laverack, 1963). Anecic earthworms, as already mentioned, feed on mineral soil, different parts of plants (leaves, roots and seeds), fungi, bacteria, protozoa, algae and at different stages (alive or dead; Table 2). Experiments on the resource spectrum of anecic earthworms are often reduced to a few organic materials proposed and do not cover the entire resource spectrum which can largely influence their diet or the techniques used do not allow precise identification. Animal dung (manure, slurry) are digested organic materials very frequently used for experiments under controlled conditions because of easy assimilation by earthworms (Schmidt and Ostle, 1999). It allows good growth and reproduction of anecic earthworms (Butt, 2011) and is recommended to encourage breeding (Lowe and Butt, 2005).

Distinction between the epi- and strict-anecic earthworm resource spectrum is not visible from a quantitative aspect, nevertheless only epi-anecic earthworms have been observed consuming or selecting live plants (Bouché, 1977; Sims and Gerard, 1999; Griffith et al., 2013; Kirchberger et al., 2015). Sims and Gerard (1999) proposed that this capacity is related to their tanylobic prostomium form which enables them to grasp an object by holding it against the upper lip or "roof" of the mouth by comparison to the epilobic prostomium form of strict-anecic earthworms. However, authors mentioned that within epi-anecic earthworms this behaviour occurs only rarely and plays a minor role in earthworm nutrition (see quantitative section). In the meantime, only strict-anecic earthworms were observed consuming earthworm cocoons (Dalby et al., 1998) which may be due to their higher digging behaviour compared to epi-anecic earthworms (Jégou et al., 2000; Bastardie et al., 2003). Within anecic earthworms, several cases of seed incorporation or digestion have been recorded, for further detail see the review of Clause et al. (2017).

3.2. Quantitative

Burial behaviour by anecic earthworms has been very widely observed in several ecosystems; crops (Perel and Sokolov, 1964; Mackay and Kladivko, 1985; Byrne, 1992), grasslands (Dickinson, 1983; Potter et al., 1990; Clements et al., 1991), and forest (Vimmerstedt and Finney, 1973). Data available on litter mass loss highlighted a preponderance of studies on epi-anecic earthworms compared to strict-anecic earthworms and could already be interpreted to be related to their resource spectrum. Epi-anecic earthworms contribute to surface litter mass loss, from 2.4 (Binet and Trehen, 1992) to 84 (Cortez and Hameed, 1988) mg g^{-1} day^{-1} and generally occurs during the night (Nuutinen et al., 2014). Moreover, it has been observed that within epi-anecic earthworms, relating to four epi-anecic species, surface litter mass loss was strongly correlated with the initial biomass (Hoeffner et al., 2018). Then, several authors extrapolated their results, underestimating the activity of microorganisms, to highlight a litter mass loss with *L.*

terrestris which can reach from 840 kg ha^{-1} yr^{-1} of corn litter (Bohlen et al., 1997), 3000 kg ha^{-1} over 3 months in deciduous forest (Satchell and Lowe, 1967) to 5000 kg ha^{-1} yr^{-1} of oak litter (Benham et al., 2012; Rajapaksha et al., 2013a). This enrichment of soil by litter incorporation is visible beyond 7 years (Potvin and Lilleskov, 2017) to 50 years (VandenBygaart et al., 1998).

By comparison, few studies have used strict-anecic earthworms as models, but where so, litter mass loss was quite low, from 4.1 (Ashwood et al., 2017) to 9.8 (Whalen and Parmelee, 1999) mg g^{-1} day^{-1}. Other studies have also reported quite high litter mass loss, from 7.1 with *A. giardi* fed with *Triticum aestivum* (Cortez et al., 1989) to 16.3 mg g^{-1} day^{-1} with *A. caliginosa meridionalis* fed with *Castanea sativa* (Cortez and Bouché, 2001), but these studies seem to have neglected to take into account the litter mass loss from microorganism activity. Eisenhauer et al. (2008) observed that compared with a control (without earthworms), *A. longa* did not contribute to surface litter mass loss or seed burial while at the same time, *L. rubellus friendoides* and *L. terrestris* strongly contributed to litter mass loss and seed burial. Moreover, Moody et al. (1995), using straw inoculated with different fungal species, showed that for the same fungal species inoculated, mean amount of straw loss was higher with *L. terrestris* than with *A. longa*. Overall, organic matter incorporation would allow pre-digestion of organic matter by soil microorganisms, reduce the exposure time to predators on the soil surface and increase the amount of food available for individual earthworms, if competitors feed on the same food resource (Satchell and Lowe, 1967; Brown, 1995; Kohli et al., 1999; Brown et al., 2000).

Table 2: Resource spectrum of epi-anecic and strict-anecic ecological groups found in this review (non-exhaustive table, useful for illustration purposes). The references indicated come from studies on the contents of the digestive tract, litter mass loss, earthworm mass change and isotopic markers.

Organic matter		Epi-anecic earthworms	Strict-anecic earthworms
PLANTS	Shoots	**Dead:** Van Rhee, 1963; Wright, 1972; Vimmerstedt and Finney, 1973; Piearce, 1978, 1989; Springett and Syers, 1984; Judas, 1992; Doube et al., 1997; Neilson and Boag, 2003; Eisenhauer et al., 2008; Stromberger et al., 2012; Rajapaksha et al., 2013b; Kirchberger et al., 2015; Andriuzzi et al., 2015; Larsen et al., 2016. **Alive:** Bouché, 1977; Shumway and Koide, 1994; Sims and Gerard, 1999; Eisenhauer et al., 2008; Griffith et al., 2013; Kirchberger et al., 2015.	**Dead:** Van Rhee, 1963; Piearce, 1978; Cortez et al., 1989; Doube et al., 1997; Cortez and Bouché, 2001; Neilson and Boag, 2003; Rajapaksha et al., 2013b; Andriuzzi et al., 2015; Larsen et al., 2016; Ashwood et al., 2017.
	Roots	Bouché and Kretzschmar, 1974; Piearce, 1978; Ferrière, 1980; Bernier, 1998; Uyl et al., 2002. **Alive:** (Cortez and Bouche, 1992)	Piearce, 1978; Bernier, 1998.
	Seeds	Mcrill and Sagar, 1973; Piearce, 1978; Shumway and Koide, 1994; Piearce et al., 1994; Eisenhauer et al., 2008, 2009; Forey et al., 2011; Clause et al., 2011; Quackenbush et al., 2012; Clause et al., 201.)	Van Tooren and During, 1988; Piearce, 1978; Eisenhauer et al., 2008.
	Other	**Pollen and moss :** Bernier, 1998.	**Pollen and moss:** Bernier, 1998.
Dung		Lee, 1985; Hendriksen, 1991a, 1991b, 1997; Doube et al., 1997; Lowe and Butt, 1999, 2002b, 2003.	Lee, 1985; Doube et al., 1997; Lowe and Butt, 1999; Svendsen and Baker, 2002; Lowe and Butt, 2002b, 2002a; Schon et al., 2016.
Soil		Hameed et al., 1994; Zhang and Hendrix, 1995; Doube et al., 1997; Schulmann and Tiunov, 1999; Neilson and Boag, 2003; Marhan and Scheu, 2005.	Piearce, 1978; Cortez et al., 1989; Doube et al., 1997; Neilson and Boag, 2003.
Micro-organisms		**Fungi:** Piearce, 1978; Cooke and Luxton, 1980; Cooke, 1983; Moody et al., 1995; Bonkowski et al., 2000; Larsen et al., 2016. **Bacteria:** Wright, 1972; Larsen et al., 2016. **Protozoa:** Piearce, 1972, 1978; Rouelle, 1983. **Algae:** Piearce, 1972, 1978; Bernier, 1998.	**Fungi:** Piearce, 1978; Moody et al., 1995; Larsen et al., 2016. **Bacteria:** Larsen et al., 2016. **Protozoa:** Piearce, 1972, 1978. **Algae:** Piearce, 1972, 1978; Bernier, 1998.
Others materials		**Paper sludge:** Butt, 1993a. **Sewage sludges:** Hamilton et al., 1988; Doube et al., 1994. **Nodes and arthropod cuticle:** Piearce, 1978.	**Earthworm cocoons:** Dalby et al., 1998. **Sewage sludges:** Doube et al., 1994. **Nodes and arthropod cuticle:** Piearce, 1978.

Even if anecic earthworms have different organic matter available, they will in most cases additionally ingest a given amount of mineral soil (Doube et al., 1997; Schulmann and Tiunov, 1999; Marhan and Scheu, 2005). *L. terrestris* gains more mass in the presence of sand grains than without ; this intentional ingestion of mineral soil is in order to facilitate the use of its gizzard and thus the assimilation of nutrients from organic matter (Marhan and Scheu, 2005). In addition, Doube et al. (1997) observed that *A. longa* consumed bulk soil alone first, followed by mixtures of organic matter and soil, while *L. terrestris* and *L. rubellus* had the same preference, with either a mixture of organic matter with soil or bulk soil alone. Neilson and Boag (2003) using the same choice chamber design of Doube et al. (1997), observed different results between *L. rubellus*, *L. terrestris* and *A. longa*: of the available food types, *L. rubellus* had no single preferred food choice, *L. terrestris* removed *Poa annua* the most but soil the least, whereas *A. longa* had no distinct food preference removing soil first followed by many plant species, although there was an apparent rejection of *Ranunculus repens* compared with soil and *Lolium perenne*. However, this choice chamber may not be suitable for earthworms, as authors introduced earthworms on damp filter papers which could over-explain the selection of soil for anecic earthworms perhaps in order to create their burrows.

Several studies have observed that the gut content of epi-anecic earthworms contains mainly organic matter (Piearce, 1972; Bouché and Kretzschmar, 1974; Piearce, 1978; Judas, 1992; Bernier, 1998), from 39% (Bouché and Kretzschmar 1974) up to 76 % (Judas, 1992; Bernier, 1998) for *L. terrestris*. This organic matter was essentially composed of leaf litter or roots (Piearce, 1972, 1978) from 50% (Bouché and Kretzschmar, 1974; Judas, 1992) to 65% (Ferrière, 1980). By comparison, authors agree that the digestive tract of strict-anecic earthworms was low in organic matter, up to only 49% (Bernier, 1998) and essentially composed of mineral material (Bouché and Kretzschmar, 1974; Piearce, 1978; Ferrière, 1980). This organic matter was essentially very degraded or humified (Ferrière, 1980; Bernier, 1998) and suggested a possible re-ingestion of faeces, as observed with *A. velox* (Bouché et al., 1983). One exception was observed with *A. longa ripicola* with a gut content still poor in organic matter compared to epi-anecic earthworms, but this organic matter was composed of 75% of fresh leaves (Ferrière, 1980). Organic matter differs according to and within epi- and strict-anecic earthworms in quantity, but also qualitatively with preferences for particular plant species (Ferrière, 1980).

Isotopic markers make it possible to specify that *L. terrestris* assimilate more C from recently deposited fractions of soil organic matter, composed of more readily decomposable substances, with a turnover time in soil of a few years (Martin et al., 1992). Thus, Zhang and Hendrix (1995) observed that *L. rubellus* principally assimilated C from leaf litter which was confirmed by Ferlian et al. (2014) with *L. rubellus* and *L. terrestris*. As strict-anecic earthworms have a higher digging behaviour than epi-anecic earthworms (Jégou et al., 2001; Bastardie et al., 2003), it is likely that the age of the C used could be slightly higher compared to epi-anecic earthworms. Using amino acid ^{13}C fingerprinting to identify protein sources of *L. terrestris* and *A. longa*, Larsen et al. (2016) found that their diet relied principally on plant-derived material, then bacteria and least on fungi. Furthermore, Larsen et al. (2016) also specified that *A. longa* fed preferentially on aged/composted soil C sources which supported findings of previous studies (Cortez et al., 1989; Cortez and Bouché, 2001; Schmidt and Ostle, 1999). For Schmidt et al. (1997b), *A. longa* appeared to take an intermediate position, feeding on both soil organic matter and plant litter which was confirmed by others studies observing a variable feeding behaviour relative to the other earthworm species (Schmidt, 1999; Neilson et al., 2000). Briones et al. (1999) observed that the C and N consumed by *A. longa* was on a gradient between *L. terrestris* and a number of endogeic species (among others, *Allolobophora chlorotica*, *Aporrectodea caliginosa* and *Allolobophora rosea*). Neilson et al. (2000), also using C and N isotopic markers of different earthworm species from six sites with different habitats, suggested that at one site, *A. longa* was similar to the endogeic species *A. rosea*, whereas at another site *A. longa* was isotopically similar to the epi-anecic species *L. terrestris*.

To summarize, strict-anecic earthworms seem to feed on a large proportion of humified organic matter already incorporated into the soil, with only a slight proportion of fresh organic matter. Directly opposed to this, epi-anecic earthworms seem to feed principally on fresh organic matter, opportunistically, mainly composed of plant leaves. Even within epi- and strict-anecic earthworm ecological categories, it would appear that there are slight variations in the resource spectrum of anecic species as observed by Ferrière (1980) and (Neilson and Boag, 2003). Many studies focused on qualitative aspects but less on the quantitative aspect, which means that the auto-ecology of these species is still largely misunderstood.

3.3. Factors influencing the palatability of the organic matter

Naturally, leaf litter is deposited on the soil surface, but it is possible that certain human activities (ploughing or soil creation) bury or mix fresh organic matter within the soil profile. Thus, litter position within the soil profile may be essential for better accessibility or palatability for anecic species. Hoeffner et al. (2018), in a laboratory experiment using soil from a grassland, observed that epi-anecic earthworms only consumed surface litter and none buried at 10 cm deep. Whalen and Parmelee (1999) observed that the growth of *L. terrestris* was probably limited by the absence of surface organic residues. Lowe and Butt (2002) in a laboratory experiment using a loam soil with 5% of organic matter, observed that *L. terrestris* and *A. longa* development was higher with solid cattle manure deposited at the soil surface compared to mixed within the soil, suggesting that for these two earthworm species, the surface position of the organic matter was preferred. However, authors have observed that *A. longa* is more capable of foraging within the soil profile.

Thus, it is also possible that the solid cattle manure mixed in the soil would have hindered the feeding behaviour of *A. longa* while on the surface *A. longa* could easily consume the soil organic matter of the soil.

The palatability of food is defined by its physical and chemical characteristics that act on the appetite (Cornelissen and Thompson, 1997). The most advanced understanding of the factors influencing the palatability of organic matter for earthworms has been made on leaf litter, as a consequence these studies mainly concern epi-anecic earthworms. Previous studies have been undertaken without specifying the species or ecological categories concerned (Edwards and Lofty, 1977; Lee, 1985) or have been carried out in the field without making it possible to separate the species or ecological category (Staaf, 1987; Hendriksen, 1990; Šlapokas and Granhall, 1991; Byrne, 1992; Curry and Byrne, 1997; Cortez and Bouché, 2001). Other studies have identified palatability gradients between different litters, for example Rajapaksha et al. (2013b) and Ashwood et al. (2017) reported a gradient of palatability for *A. longa* between leaf litter from eight trees, where: *Alnus glutinosa* = *Fraxinus excelsior* = *Betula pendula* = *Alnus cordata* > *Eucalyptus nitens* = *Acer platanoides* > *Acer pseudoplatanus* = *Castanea sativa*. Using webcams in choice chamber experiment, Rajapaksha et al. (2013b) observed that *L. terrestris* demonstrated clear leaf litter selection behaviour and it was not a random activity. From the data review, we observed that the quantity of litter mass loss by epi-anecic earthworms is very variable depending on species of leaf litter provided (Fig. 1). However, it is well known that there is a large intraspecific variability in litter traits (Albert et al., 2010, 2011) which can lead to changes in palatability to earthworms. Thus, contradictory results can be observed using plant species names (Satchell and Lowe, 1967; Rajapaksha et al., 2013b). Nevertheless, there are a large number of studies which do not measure physicochemical litter traits (Avel, 1929; Michon, 1954; Wright, 1972; Zicsi, 1983; Piearce, 1989; Hendriksen, 1990). Consistent studies are those where litter traits are measured. The information of which we have the most, concerns epi-anecic earthworms and more particularly *L. terrestris* with factor by factor correlations. Some studies didn't observe relationships between measured litter traits and litter mass loss, perhaps due to a lack of measured litter traits, or they highlight that epi- and strict-anecic earthworms are adaptable and able to feed on a variety of food sources differing in quality and palatability (Kohli et al., 1999; Neilson and Boag, 2003; Rief et al., 2012; Ashwood et al., 2017). Several studies have used choice chambers to try to specify the palatability of litters (Doube et al., 1997; Neilson and Boag, 2003; Rajapaksha et al., 2013b; Ashwood et al., 2017). Here we make the distinction between chemical and physical properties, but these obviously interact on the palatability for epi-anecic earthworms.

L. terrestris appeared to be negatively sensitive to phenolic compounds (Satchell and Lowe, 1967; Wright, 1972; Piearce, 1989), positively sensitive to N or low C/N ratios, soluble carbohydrates (Satchell and Lowe, 1967; Bohlen et al., 1999; Rajapaksha et al., 2013a) and calcium content (Rajapaksha et al., 2013a). Nevertheless, Wright (1972) observed that leaves with an artificially doubled phenolic compound content did not reduce *L. terrestris* feeding rate, but at five times the normal level of phenolic compound content, feeding rates were reduced by 50% compared with untreated disks. *L. rubellus* possess a class of unique surface-active metabolites in their gut (called drilodefensins), these compounds counteract the inhibitory effects of polyphenols on earthworm gut enzymes and so protect *L. rubellus* from the harmful effects of ingested polyphenols (Liebeke et al. 2015). In addition, several authors have been able to assess in other ecological categories of earthworms the effects of tannins (Hendriksen, 1990; Šlapokas and Granhall, 1991) or lignin (Hendriksen, 1990) on the palatability of organic matter.

Available information to highlight physical characteristic effect on litter palatability between epi- and strict-anecic were not sufficient and mainly concerned epi-anecic earthworms through studies focusing on *L. terrestris*. Darwin (1881), mentioned that the shape of the leaves impacts their selection by earthworms and globally, earthworms are more attracted to moist than dry material (Heath et al 1966). Epi-anecic earthworms appears to be sensitive to litter flexibility and size (Wright, 1972; Piearce, 1989). Some authors suggested that anecic earthworms are macrophages compared to endogeic earthworms which are microphages and epigeic earthworms which are mesophages (Bouché, 1977; Satchell, 1980). Epi-anecic earthworms, preferentially consume objects of 0.5 ± 1.0 mm (Shumway and Koide, 1994), 0.76 ± 0.06 in width and 7.22 ± 1.11 mm in length (Piearce, 1978), up to 3 mm in diameter and 6 mm in length (Piearce et al., 1994). Strict-anecic earthworms can consume spheres as large as 2.5 mm in diameter (Dalby et al. 1998) but can consume up to 0.7 mm in width and 9.1 mm in length (Piearce, 1978). Piearce (1978) observed that the particle size ingested does not appear to be correlated with fresh mass of earthworm species especially with organic fragments. Lowe and Butt (2003) observed that milled solid cattle manure (< 1 mm) enhanced the growth of *L. terrestris* compared to un-milled solid cattle manure. Nevertheless, *L. terrestris* actively searches grains of sand, preferably of small sizes (0.5-1 mm) over larger (1-2 mm and 2-3 mm) grains allowing it to fragment the size of the litter particle (Schulmann and Tiunov, 1999).

Nonetheless, univariate and multivariate analyses showed that seed length, width, mass and seed oil content could significantly affect the ingestion of seeds for *L. terrestris*. Seed width and seed oil content were the two traits that influenced the digestion of seeds the most, but only for *L. terrestris* (Clause et al., 2011).

3.4. Microorganisms as a trophic resource for earthworms

Microorganisms are either a food resource (direct effect) or they alter the physical and chemical properties of the given resource making it more palatable (indirect effect) (Brown et al., 2000; Cooke and Luxton, 1980), but it is difficult to separate the two effects. Previous studies have mentioned that microorganisms are important for earthworms diet because they prefer to consume organic matter inoculated with microorganisms than without and this effect depends on microorganism species presented (Wright, 1972; Cooke and Luxton, 1980; Cooke, 1983; Moody et al., 1995; Bonkowski et al., 2000). It was well established that, within microorganisms, fungi were a major source of food for earthworms (Cooke and Luxton, 1980; Cooke, 1983; Edwards and Fletcher, 1988; Schönholzer et al., 1999; Tiunov and Scheu, 2000b). For example, Cooke and Luxton (1980) using two fungal species- and one bacterial species-inoculated filter paper disks and observed a higher preference for fungal over bacterial discs. In another experiment, Cooke (1983) confirmed that *L. terrestris* was attracted by three fungal species and not by three bacterial species. These observations could partly explain the negative correlation in field studies between earthworm abundance and fungal abundance (Valchovski, 2016). Nevertheless, Wolter and Scheu (1999) with *L. terrestris* and Monroy et al. (2008) with *L. rubellus,* nuanced the fact that microorganisms are a major component of their diet. Thus, Larsen et al. (2016) observed that the food bolus of *L. terrestris*, consisted of 26% microorganisms against 74% leaves, and 30% vs 70% in *A. longa.* For these two anecic species, the proportion of bacteria was always higher than that of fungi moreover, this study did not give the age of litter consumed. Moody et al. (1995) observed that the preferences by *L. terrestris* and *A. longa* across seven fungal species studied, were very similar. The question of microorganism selection by anecic species remains controversial. Furthermore, within epigeic earthworms, mechanisms of detection of chemical molecules (Smith, 1902) or fungal colonies (Zirbes et al., 2011) have been observed, meaning that within anecic earthworms others mechanisms of organic matter detection could exist.

4. Organic matter transformation during gut transit

4.1. Gut microbiota

Most studies on anecic earthworms microorganism gut content have been made on epi-anecic earthworms, *L. rubellus* and *L. terrestris*. As previously described, epi-anecic and strict-anecic earthworms ingest voluntarily or involuntarily microorganisms present in soils or on plant litter. This external supply of microorganisms considerably modify epi-anecic earthworms gut content in term of communities and activities of microorganisms (Brown, 1995; Tiunov and Scheu, 2000a; Brown and Doube, 2004; Egert et al., 2004; Knapp et al., 2008, 2009); for more details see the review of Drake and Horn (2007). These previous studies suggest no existence of an indigenous earthworm microbial community. Nevertheless, some physical links were found between bacterial cells and epithelium in the hindgut of *L. terrestris* (Jolly et al., 1993) and Karsten and Drake (1997) observed within *L. rubellu*s and Sampedro et al. (2006) within *L. terrestris* some evidence of gut symbionts or specific gut microflora. Briefly, several studies observed that the numbers of soil bacteria do not change or even increase in number, and those of fungi decrease from food to faeces of earthworms during the passage of the digestive tract (Parle, 1963; Daniel and Anderson, 1992; Kristůfek et al., 1995; Schönholzer et al., 1999; Furlong et al., 2002; Byzov et al., 2007; Kurakov et al., 2016). Fungi can however, survive passage as cysts. Moreover, it is possible to observe some changes in microorganism communities (Tiunov and Scheu, 2000b; Furlong et al., 2002; Schönholzer et al., 2002; Nechitaylo et al., 2009): for example, large cells are mainly destroyed during gut transit (Schönholzer et al., 1999; Cai et al., 2002). High mechanical disintegration during passage through crop and gizzard can explain the differences between fungi and bacteria and shift in microorganisms communities (Schönholzer et al., 1999). Fungi are more sensitive to mechanical disturbance compared to bacteria, moreover bacteria absorb soluble and easily available substrates allowing them to develop quickly in substrate-rich habitats (Chapin et al., 2002). Consequently, anecic earthworms can be considered as vectors of microorganisms from the soil (indigenous) or the litter (Heijnen and Marinissen, 1995; Tiunov and Scheu, 2000b; Thimm et al., 2001) by carrying faeces into the soil profile or kill specific microbial species. Thus for example, anecics can destroy plant pathogens or enhanced plant promoters like mycorrhizal fungi (Gange, 1993; Stephens et al., 1993; Harinikumar and Bagyaraj, 1994; Zaller et al., 2013). It is also known that microorganisms are transferred or mixed into the soil by simple burial of the litter or by transportation on earthworm skin (Thorpe et al., 1996).

Few studies have highlighted different impacts between epi- and strict-anecic earthworms on microorganisms during the gut transit. For example, Parle (1963) counted yeasts, fungi, actionomycetes and bacteria in the gut of *L. terrestris* and *A. longa* collected from the same soil and compared with bulk soil. He observed that *A. longa* gut contained significantly more fungi and yeasts than soil compared to the bulk soil, whereas no difference was observed between *L. terrestris* gut and bulk soil. Parle (1963) suggested that the reasons for these small increases were not clear but may have come from differences between the soil sampled and the actual material eaten by the earthworms. Moreover, Schmidt et al. (1997a) measured *Pseudomonas corrugate* number in casts of separate individuals of *L. rubellus*, *L. terrestris*, *A. longa* and *A. caliginosa* after contact with soil containing *P. corrugate* and observed that *A. longa* produced casts containing higher numbers of *P. corrugata* than those of *L. rubellus* and *L. terrestris*. Thus, this result suggests that *A. longa* fed principally on soil, compared to *L. rubellus* and *L. terrestris*. In the same way, Moody et al. (1996) measured the effect of passage through *L. terrestris* and *A. longa* gut on the viability of spores of three saprotrophic fungi (*Fusarium lateritium*, *Mucor hiemalis* and *Chaetomium globosum*). At the end of the experiment, *F. lateritium* failed to germinate after gut passage of both *L. terrestris* and *A. longa*, however, *M. hiemalis* spores were more severely affected by the passage through the gut of *L. terrestris* than *A. longa* (10% and 36% survival respectively). *C. globosum* spore viability was slightly reduced (20%) after the passage through the gut of *L. terrestris,* whereas it was significantly increased after passage through the gut of *A. longa*. This selectivity between *L. terrestris* and *A. longa* observed by Moody et al. (1996) may indicate two different impacts between epi- and strict-anecic but would usefully be further investigated and confirmed using several anecic species to exclude interspecific variability.

4.2. Gut enzymes

Anecic earthworms need organic matter to be degraded into smaller molecules to be assimilated. Thus, microorganisms or animals secrete enzymes (Dick and Tabatabai, 1992; Dick et al., 2000; Tabatabai, 2003) to digest specific chemical bounds hydrolysing complex molecules and polymers into smaller molecules (Burns and Dick, 2002). Due to the high concentration of microorganisms present in the digestive tract of earthworms, there is a high concentration of enzymes. Thus, gut content of *L. terrestris* contains several enzymes involved in several biogeochemical cycles such as C, N and P (Laverack, 1963; Prentø, 1987; Urbasek, 1990). With all of these digestive enzymes, their presence seems to decrease along the gut transit with some exception (among others, phosphorylase and glutamate dehydrogenase) (Jeuniaux, 1969; Tillinghast and Macdonnell, 1973). This indicates that many organic matter transformations take place during intestinal transit in earthworms. The question that remains, supported by several authors (Laverack, 1963; Parle, 1963; Urbasek, 1990) is whether these enzymes are produced by anecic earthworm species or by microorganisms present in the digestive tract. However, each enzyme generally requires an optimal pH that is different from one enzyme to another while the pH is relatively stable from 6.3 to 6.6 along intestinal transit in *L. terrestris* (Laverack, 1963).

However, few studies comparing epi- and strict-anecic earthworms species have highlighted differences in the presence of specific enzymes (Tracey, 1951; Parle, 1963). Cellulose represents a high proportion of plant composition and as earthworms consume much plant material, it would be of considerable value to use the cellulose of these plants which is confirmed by the presence of cellulase in both epi- and strict-anecic earthworms (Tracey, 1951; Parle, 1963).

4.3. Mass change

Food ingested and decomposed during gut transit is then available for assimilation by anecic earthworms. It is then used for growth and reproduction, thus generating an increase in organic matter stabilisation in the short term. Average anecic earthworm biomass and thus short stabilisation are variable according to the species considered, from 0.5 g for *L. rubellus* to 15.0 g for *L. terrestris* within epi-anecic earthworms and from 0.3 g for *A. caliginosa meridionalis* to 3.2 g for *A. giardi* within strict-anecic earthworms (Bouché, 1972). Growth to maturity (days to reach clitellum development) is longer by +43% at 15°C and by +20% at 20°C for *L. terrestris* compared with *A. longa* (168, 112 and 120, 90 days respectively) (Butt, 1993b; Svendsen and Baker, 2002). In the same way, by comparing the growth of *L. terrestris* and *A. tuberculata*, Whalen and Parmelee (1999) observed that *L. terrestris* grew more rapidly than *A. tuberculata*. These observations could be a consequence of the nutrition (see resource spectrum) and the ability to assimilate resources. With a grass input gradient from 0 to 17 g. kg^{-1} dry soil, (Martin, 1982) observed that *L. rubellus* won mass only at the maximum amount of 17 g. kg^{-1} dry soil while *A. trapezoides* increased its mass from 4.4 g. kg^{-1} dry soil. Moreover, *L. rubellus* and *L. terrestris* raised in a soil containing 1.01% of C with either litter or seeds provided at the soil surface gained +67% and +70% of mass respectively, whereas *A. longa* lost 12% of mass (Eisenhauer et al. 2008). In the same way, *A. giardi* lost 38% mass in 31 days of an experiment with a soil containing 1.3% of C and fresh litter provided at the soil surface (Cortez et al., 1989). Thus, it could be hypothesized (following previous observations) that the low C content of the initial soil was not able to satisfy the needs of strict-anecic earthworms. Moreover, species interactions can modify the mass of earthworms due to competition for resources (feed and space) and it was proposed that the degree of niche overlap, therefore earthworm ecological category, may limit the intensity of interaction (Lowe and Butt, 2002b, 2002a, 1999). The greatest understanding on this subject relates to mass change; for more details on earthworms interactions see the review of Uvarov (2009).

5. Chemical composition of faeces (C and N)

There is a strong correlation between the loss of litter mass and the composition of surface casts (Syers 1979). Thus, within the anecic ecological category, several studies have focused on epi-anecic earthworms through analyses of C and N in casts of *L. terrestris* and *L. rubellus*. Several studies highlighted that C, N content in their casts were very high compared to the surrounding soil (Daniel and Anderson, 1992; Flegel et al., 1998; Buck et al., 1999; Tiunov and Scheu, 2000a; Clause et al., 2014). For example, within a soil of a beech forest, C/N ratio was up to 40% lower in casts of *L. terrestris* than surrounding soil (15.2 and 25.6 respectively) (Tiunov and Scheu, 2000a). Nevertheless, as described above, the quality of the organic matter ingested or the type of soil strongly impacts the quality of the casts compared to un-ingested soil (Shipitalo et al., 1988; Flegel et al., 1998; Clause et al., 2014). Overall, using isotopic markers, several studies observed a higher concentration of litter C in *L. terrestris* or *L. rubellus* casts which means that this litter is very new (Zhang and Hendrix, 1995; Jégou et al., 1998a, 2000; Don et al., 2008). When there is no litter on the soil surface, *L. terrestris* does not increase the amount of C in casts compared to the surrounding soil (Shipitalo et al., 1988; Farenhorst and Bowman, 2000; Görres et al., 2001; Marhan et al., 2007). Farenhorst and Bowman (2000) observed that casts of *L. terrestris* increased in organic C when plant litter was provided at the soil surface (2.63% for corn residues and 3.73% for soybean residues) compared to the initial soil (1.63%). Conversely, Cortez et al. (1989)

observed that after 31 days of an experiment with litter provided at the soil surface, C and N within *A. giardi* casts had originated from native soil organic matter about 6 and 11 times larger than ingested litter C and N, respectively. Similarly, Alekseeva et al. (2006) observed that organic C within casts of *A. giardi* and without litter provided at the soil surface was richer than the bulk soil (5.34 for *A. giardi* casts, 3.76 for soil 0-20 cm and 1.24 for soil 40-60 cm). This observation meant that C-enrichment in casts of *A. giardi* comes mainly from the soil. By comparing *L. terrestris* and *A. giardi*, Jégou et al. (1998a, 2001), observed both species enhanced total C and N compared to the surrounding soil, nevertheless, total C and N were higher in casts of the epi-anecic *L. terrestris* than with the strict-anecic *A. giardi*.

Consequently, the burrows of epi-anecics are often richer in C and N than the original surrounding soil (Jégou et al., 1998a; Tiunov and Scheu, 2000a; Jégou et al., 2001; Shuster et al., 2001; Don et al., 2008; Andriuzzi et al., 2015). For example, Shuster et al. (2001) observed that additions of *L. terrestris* significantly increased average soil organic C content from 16.1 to 17.9 g C kg^{-1} for the 0–10 cm soil, and from 12.4 to 14.7 g kg^{-1} at 10–20-cm depth, and changed the spatial distribution of soil organic C from uniform to patchy, compared with the ambient treatment. Stromberger et al. (2012) observed that *L. terrestris* contributed to enhanced recent grass C (8 years) by at least 26% of the drilosphere soil C. Overall, Jégou et al. (2001) observed that *L. terrestris*, built burrows more enriched in C litter compared to *A. giardi*. The permanent character of the *L. terrestris* burrow system could lead to a high and constant enrichment of the entire burrow system whereas the denser system of burrows developed by *A. giardi* resulted in C litter dilution in the whole of the formed structures. As a consequence, the percentage of C litter in the “burrow wall” and “burrow periphery” compartments of *L. terrestris* was important despite their low volume.

6. Conclusions and future approaches

Many reviews have shown that earthworms contribute significantly to litter decomposition, but these studies did not take into account that earthworms are classified into ecological categories with different impacts on soil organic matter decomposition. Here we report that within the category of anecic earthworms, it is possible to distinguish epi-anecic from strict-anecic species. Epi-anecic earthworms are opportunistic, saprophagous, consume mainly fresh plant litter on the soil surface and thus contribute strongly to the burial of organic matter from the soil surface into the soil profile. Their digestive tract is then highly filled with material concentrated in organic matter, which seems to stimulate the abundance and modify communities of microorganisms. Consequently, their faeces are very rich in C and N of relatively recent plant origin. By contrast, strict-anecic earthworms are geo-saprophagous and consume both surface litter but also humified organic matter already incorporated into the soil with a more important burrowing behaviour than epi-anecic earthworms. Their digestive tract is then essentially composed of humified organic matter. Consequently, their faeces are also richer in C and N compared to the surrounding soil but less when compared with epi-anecic earthworms. The two feeding behaviours for these widespread animals can have serious consequences on residual organic matter at the soil surface but also on C storage, availability or the turn-over of nutrients in soils and soil microorganism communities. Nevertheless, the distinction of feeding behaviour and consequences on the soil environment highlighted in this review have been observed often using the same earthworm species of epi-anecic (*L. rubellus* and *L. terrestris*) or strict-anecic (*A. longa* and *A. giardi*) earthworms. Furthers studies could be undertaken in other species within these ecological categories to better understand the ecology of species that are also nonetheless widespread. Moreover, this would allow an assessment of the homogeneity of these ecological categories and their interaction with organic matter and the consequences thereof on the soil ecosystem. Future studies should also consider that litter traits change during decomposition and earthworms feed preferentially on decaying litter and not fresh as is often used experimentally. Moreover, it seems important to take into account multiple litter trait effects on palatability but also consequences on microorganisms and faeces composition. Thus, there still remains a very important research gap regarding the auto-ecology of epi- and strict-anecic earthworms.

CHAPITRE 3

Comportement alimentaire des espèces lombriciennes épi-anéciques et impacts sur les communautés microbiennes du sol

Le troisième chapitre correspond à un article publié dans la revue internationale Soil Biology and Biochemistry 125 (2018) 1–9, DOI : 10.1016/j.soilbio.2018.06.017

Objectifs de l'étude

Le but de cette expérimentation était de déterminer si au sein des lombriciens épi-anéciques et en fonction de leur comportement alimentaire toutes les espèces (i) avaient le même rôle fonctionnel quant à la décomposition des litières et (ii) impactaient similairement la structure des communautés bactériennes et fongiques.

Méthodologie

Nous avons mené une expérimentation en conditions contrôlées au laboratoire et déterminé la contribution de quatre espèces lombriciennes épi-anéciques (*Lumbricus rubellus rubellus*, *Lumbricus festivus*, *Lumbricus centralis* et *Lumbricus terrestris*) à la perte de masse de litière de trois espèces végétales distinctes, avec des traits contrastés, deux herbacées (*Lolium perenne*, *Holcus lanatus*) et une arboricole (*Corylus avellana*). Chacune de ces litières était située à la surface et à 10 cm de profondeur pour étudier le comportement alimentaire des espèces lombriciennes épi-anéciques. La structure des communautés fongiques et bactériennes des galeries des différentes espèces lombriciennes épi-anéciques nourries avec les différentes litières a été analysée par 'Terminal Restriction Fragment Length Polymorphism' (T-RFLP) après extraction des ADNs de sol.

Principaux résultats

Les lombriciens épi-anéciques ont uniquement contribué à la perte de masse de la litière végétale à la surface du sol alors qu'à 10 cm de profondeur la perte de masse des litières était principalement due à l'activité microbienne. La perte de masse des litières végétales était corrélée positivement à la biomasse initiale des lombriciens épi-anéciques et l'intensité de cette relation dépendait de la nature des litières. Nous avons observé que, au regard de sa biomasse, *Lumbricus festivu*s contribuait le plus à la perte de masse des litières végétales en surface en lien avec une stimulation de la diversité fongique dans ses galeries. La structure des communautés fongiques dépendait à la fois du type de litière et de l'identité des espèces lombriciennes épi-anéciques. A l'inverse la structure des communautés bactériennes n'a pas été impactée par le type de litière et l'identité des espèces lombriciennes épi-anéciques. Cependant la richesse et la diversité bactérienne a été stimulée en présence de lombriciens épi-anéciques.

Feeding behaviour of epi-anecic earthworm species and their impacts on soil microbial communities

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Abstract

Earthworms contribute to numerous ecosystem services provided by soils. Most of the studies focusing on the contributions of earthworms on leaf litter decomposition were conducted by comparing distinct ecological categories (epigeic, epi-anecic, anecic strict and endogeic), whereas their specific contributions within a given ecological category remains largely unknown. In this context, the aim of this study was to determine the contribution of four epi-anecic earthworm species (*Lumbricus rubellus, Lumbricus festivus*, *Lumbricus centralis* and *Lumbricus terrestris)* to the leaf litter decomposition of three plant species (*Lolium perenne, Holcus lanatus* and *Corylus avellana*) with contrasted litter traits located at both the soil surface and at a depth of 10 cm. Fungal and bacterial communities inhabiting epi-anecic earthworm burrows were also assessed using T-RFLP analysis. Epi-anecic earthworms improved the leaf litter mass loss solely at the soil surface, while leaf litter mass loss was mainly due to microbial activity at 10 cm deep. Leaf litter mass loss was positively correlated to the initial biomass of the epi-anecic earthworms and the intensity of this relationship was dependent on litter type. Interestingly, *L. festivus* seemed to have a higher contribution to surface leaf litter mass loss that was linked to a stimulation of the fungal communities in its burrows. Fungal communities were thus impacted by both the litter type and the epi-anecic earthworm identity whereas soil bacterial diversity and richness were stimulated in the earthworm burrows whatever the epi-anecic earthworm species considered. Overall, epi-anecic earthworms contributed to enhance the diversity of the drilospheric microbiota.

1. Introduction

Earthworms constitute the dominant biomass of soil invertebrates (Lee, 1985; Curry, 1994) and are considered as both ecosystem engineers (Jones et al., 1994) and keystone species (Blondel and Aronson, 1995) due to their contribution to physical, chemical and biological modifications of soil properties, thus driving key ecosystem services provided by soils (Blouin et al., 2013). Earthworms are classified into three ecological categories (epigeic, anecic and endogeic species) based on their ecological behaviour, with a continuum between these three categories (Bouché, 1977). Briefly, epigeic earthworms live in and consume surface organic matter, endogeic earthworms burrow horizontal galleries to feed on soil organic matter, and anecic earthworms burrow vertical galleries to feed on a mixture of surface and soil organic matter. Within the anecic earthworms, Jégou et al. (1998, 2000), based on studies from Bouché (1972, 1977), distinguished the strict-anecic from the epi-anecic earthworms: strict-anecic earthworms construct a high density of semi-permanent burrows and have low surface litter incorporation rates whereas the epi-anecic earthworms build permanent burrows into which they incorporate high quantities of fresh litter from the soil surface.

Within the drilosphere, corresponding to the soil influenced by earthworm activities (Lavelle, 1988), this deep litter incorporation into their burrows enhances soil microbial development (Tiunov and Dobrovolskaya, 2002) and mineralization activity (Winding et al., 1997; Brown et al., 2000). Soil microorganisms are indeed responsible, at the fine scale, for soil organic matter decomposition (Coûteaux et al., 1995). Among them, fungi are commonly the main decomposers of fresh plant litter (Chapin et al., 2002) through the production of extracellular enzymes able to decompose complex material with low nutrient concentration such as lignocellulose compounds (Ingold and Hudson, 1993). Meanwhile, bacteria mainly absorb soluble and easily available substrates allowing them to develop quickly in substrate-rich habitats (Chapin et al., 2002).

In temperate grasslands, epi-anecic earthworms are subjected to leaf litter of various quality depending on the plant species composition. For example, *Lumbricus terrestris* preferentially selects leaves with high nitrogen content (Shipitalo et al., 1988). This feeding preference induces strong variations in litter feeding rates by epi-anecic earthworms according to leaf litter traits (Wright, 1972; Edwards and Lofty, 1977; Lee, 1985; Hendriksen, 1990; Šlapokas and Granhall, 1991). In addition, leaf litter can be located at different positions in the soil profile according to agricultural practices. Leaf litter is naturally deposited at the soil surface but, considering some specific agricultural practices, leaf litter can also be incorporated deeper in the soil through temporary grasslands ploughing. Compared to surface leaf litter, this buried leaf litter might thus alter epi-anecic earthworms' contribution to leaf litter decomposition.

The literature on epi-anecic earthworms is biased towards *L. terrestris* (Needham, 1957; Satchell and Lowe, 1967; Curry and Bolger, 1984; Shipitalo et al., 1988; Tiunov and Dobrovolskaya, 2002; Andriuzzi et al., 2016) due to its widespread distribution (Bouché, 1972) and breeding facility (Daniel, 1991; Butt et al., 1994a; Daniel et al., 1996). However, the epi-anecic ecological category includes several earthworm species (Diaz Cosin et al., 1992; Decaëns et al., 2008; Cluzeau et al., 2012) which might exhibit distinct feeding preferences and specific interactions with soil microorganisms. For example, the fresh biomass of epi-anecic earthworms can vary from 0.75 g for *Lumbricus festivus* up to 15 g for *L. terrestris* (Bouché, 1972), suggesting different metabolic needs depending on the species identity. Such high differences in earthworm biomass could lead to different quantity and/or quality of leaf litter transported in the burrows and thus to distinct impact on soil microorganisms. It has already been demonstrated that earthworm species from different ecological categories affected soil microbial abundance and activity differently with cascading effects on microbial transformation of labile carbon (Sheehan et al., 2008; Chang et al., 2016). However, to our knowledge, no previous study has been conducted in order to decipher the impact of different earthworm species on microbial communities within a given ecological category.

The purpose of this study was to determine whether, within the epi-anecic ecological category, different earthworm species specifically interact with leaf litter and microbial communities inhabiting their burrows leading to distinct rates of leaf litter mass loss. We conducted a laboratory mesocosm experiment and determined the contribution of four epi-anecic earthworm species (*Lumbricus rubellus, L. festivus*, *Lumbricus centralis* and *L. terrestris)* to the leaf litter mass loss of three distinct plant species, two grass (*Lolium perenne, Holcus lanatus*) and a tree species (*Corylus avellana*), located at both the soil surface and at a depth of 10 cm after 10 and 20 days of incubation. At the end of the experiment, the community structure of the bacteria and fungi inhabiting the epi-anecic earthworms' burrows were analyzed and compared using Terminal Restriction Fragment Length Polymorphism (T-RFLP). First, we hypothesized that leaf litter mass loss increases according to the initial biomass of the epi-anecic earthworms. Second, we hypothesized a higher epi-anecic earthworms' contribution to leaf litter mass loss at the soil surface compared to a 10 cm depth. Finally, we expected that both the litter type and the epi-anecic earthworm identity control microbial community structure in earthworm burrows.

2. Materials and methods

2.1. Material collection

Soil (deep to 5-20 cm) and epi-anecic earthworms were collected in a temporary grassland near Trans-La-Forêt, France (48°50’ N, -1°58’ W) in the Long Term Ecological Research (LTER) site ‘‘Zone Atelier Armorique’’. The climate of the region is oceanic with a mean annual temperature of 11.7 °C, a mean annual rainfall of 815.0 mm and a mean annual relative humidity of 80.9 % (mean values over the period 2010-2016, data from Météo France). Soil collected was hand sieved at 4 mm, homogenized, and a soil sample was sent to the central analytical laboratory of INRA (SAS, Arras, France) for texture, organic matter, C:N ratio and pH measurements. The soil was identified as a brown soil with 48.2% sand, 37.5% silt and 14.3% clay, characterized by 2.9 % of organic matter, a C:N ratio of 9.7 and a pH of 6.4. The soil was also pre-incubated for one week at 12 °C under a 12h:12h light: dark regime with a water content adjusted to 31% w/w by addition of deionized water prior to the experiment.

Fresh leaf litter of *Lolium perenne* and *Holcus lanatus* was collected from non-permanent grasslands close to the earthworm and soil sampling location. Freshly abscised leaves of *Corylus avellana* were collected from trees close to the Biological Station of Paimpont, France (48°01 N, -2°17 W). The three litter types were thereafter air-dried at room temperature and stored until the beginning of the experiment. *L. perenne* and *H. lanatus* leaves were cut into sections of approximately 7 cm length while *C. avellana* leaves were used intact. *H. lanatus* and *L. perenne* are two grass species typical of temporary grasslands, whereas *C. avellana* is a shrub frequently encountered in hedges surrounding these grasslands.

Four epi-anecic earthworm species were studied (Bouché, 1972, 1977): *L. rubellus rubellus* (Hoffmeister, 1843; hereafter referred to as LR), *L. festivus* (Savigny, 1826; hereafter referred to as LF), *L. centralis* (Bouché, 1972; hereafter referred to as LC) and *L. terrestris,* (Linné, 1758;

hereafter referred to as LT). Epi-anecic earthworms were hand collected a week before the experiment, placed in a sample of the hand-sieved soil and fed with litter composed of a mixture of air-dried leaves of the three plant species than the ones used in the experiment.

2.2. Initial litter characteristics

Carbon (C) and nitrogen (N) concentrations were determined by thermal combustion using a Vario Pyro cube CNS analyzer (Elementar France SARL, Lyon, France). Lignin, cellulose, hemicellulose and water soluble compound (WSC) concentrations were determined according to the Van Soest extraction protocol (Van Soest and Wine, 1967) using a fiber analyzer (Fibersac 24; Ankom, Macedon, NJ, USA). Phenolic concentrations were measured colorimetrically using the method described in Santonja et al. (2015) with gallic acid as a standard. To determine the water holding capacity (WHC), intact leaf litter samples were soaked in distilled water for 24 h, drained and had mass determined. The dry mass was determined after drying samples at 60 °C for 48 h. WHC was calculated as (moist mass / dry mass) × 100% (Santonja et al., 2015). Specific leaf area (SLA) was determined by using the Image J software (https://imagej.nih.gov/ij/, MA, USA). SLA was calculated as the ratio between leaf area and leaf dry mass. Initial litter traits were determined from four samples of each of the three litter types (except for WHC and SLA for which n = 10).
With the exception of cellulose, all of the initial litter characteristics varied between the three litter types (Table 1). Nitrogen and WSC concentrations decrease according to the gradient *L. perenne* > *H. lanatus* > *C. avellana*, whereas WHC and SLA followed the gradient *H. lanatus* > *L. perenne* > *C. avellana* (Table 1). Carbon, lignin and phenolic concentrations, in addition to C:N and lignin:N ratios, were higher in *C. avellana* litter than in *H. lanatus* and *L. perenne* (Table 1).

Table 1 Initial leaf litter characteristics of the three plant species. Values are mean ± standard error; n = 4 (except for specific leaf area and water holding capacity for which n = 10). Separated one-way ANOVAs were performed for differences among plant species. *F*-Ratios and *P*-values (with the respective symbols * for *P*<0.05, ** for *P*<0.01, and *** for *P*<0.001) are indicated. Different letters denote significant differences among litter types with a>b>c (post hoc Tukey test results).

	C. avellana			*H. lanatus*			*L. perenne*			one-way ANOVA	
Carbon (%)	43.68	± 0.03	a	43.23	± 0.01	b	42.57	± 0.02	c	978.91	***
Nitrogen (%)	1.18	± 0.01	c	2.33	± 0.01	b	3.48	± 0.01	a	12768.59	***
Lignin (%)	21.49	± 1.01	a	3.1	± 0.43	b	1.79	± 0.07	b	301.01	***
Cellulose (%)	15.37	± 1.28	a	16.42	± 0.51	a	14.52	± 0.82	a	1.06	NS
Hemicellulose (%)	24.05	± 1.50	a	25.43	± 0.93	a	18.46	± 1.23	b	8.83	**
Water Soluble Compounds (%)	39.09	± 2.69	c	55.05	± 1.49	b	65.23	± 1.55	a	43.95	***
Phenolics (%)	6.31	± 0.27	a	3.62	± 0.19	b	3.14	± 0.11	b	73.24	***
Water Holding Capacity (%)	347.18	± 6.36	c	431.73	± 11.61	a	401.27	± 6.57	b	25.19	***
Specific Leaf Area ($cm^2 g^{-1}$)	106.56	± 4.64	c	525.9	± 25.97	a	346.35	± 14.06	b	148.64	***
C/N ratio	36.99	± 0.32	a	18.11	± 0.07	b	12.25	± 0.03	c	4564.5	***
Lignin/N ratio	18.17	± 0.70	a	1.33	± 0.18	b	0.52	± 0.02	b	567.12	***

2.3. Experimental setup

A 5 × 3 factorial design was performed with 4 replicates and for two incubation times (10 and 20 days): with or without (control accounting for the litter mass loss due to microbial decomposition or leaching) one of the four epi-anecic earthworm species; with one of the three litter types placed at both the soil surface and at 10 cm deep. For each litter type, 30 litterbags (11 × 9.5 cm; 1.2 cm mesh size) were prepared and filled with 2 g of air-dried leaves re-humidified just before the experiment. In parallel, 2 g of leaves were dried at 72 °C for 48 h to determine the initial litter dry mass. Each mesocosm (PVC cylinder, 30 cm high, 10 cm diameter sealed at the base) was filled with 4.9 kg of soil in two steps. First, half of the soil was placed in the mesocosm and compacted to a bulk density of 1.3 g.cm^{-3} and a litterbag was placed corresponding to the 10 cm depth. Then the remaining soil was added, compacted to the same density and another litterbag was placed at the soil surface of the mesocosm. After 24 h, placed on a moist sponge in a plastic box for depuration, two adults of each epi-anecic earthworm species were weighed and placed in the appropriate mesocosm. They presented a marked gradient of initial fresh mass from 0.83 ± 0.02 g for LR to 4.59 ± 0.12 g for LT (Fig. 1). Mesocosms were closed with a thread of 1 mm to avoid earthworm escape during the experiment (Lubbers and van Groenigen, 2013). The mesocosms were placed in a climatic chamber at 12 °C, with a relative humidity of 85% and a 12 h: 12 h light: dark regime. Soil moisture was maintained by spraying deionized water at the soil surface twice per week.

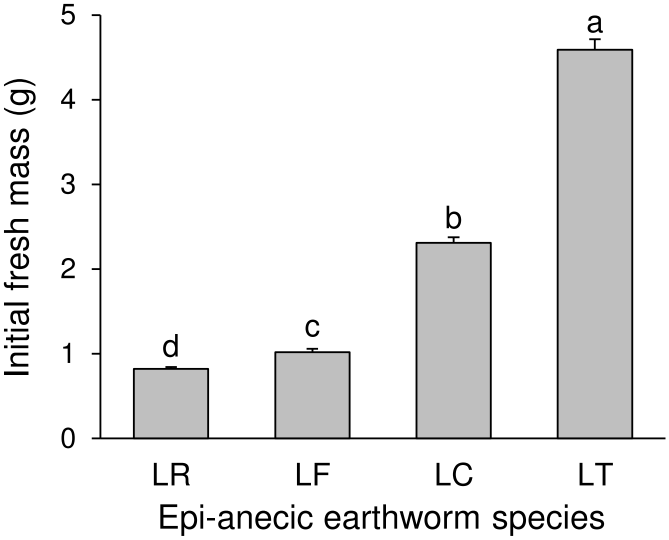

Fig. 1. Initial fresh mass of the four epi-anecic earthworms. Values are mean ± standard error; n = 48. Different letters denote significant differences among earthworm initial mass with a>b>c>d (post hoc Tukey test results). LR = *L. rubellus*; LF = *L. festivus*; LC = *L. centralis*; LT = *L. terrestris*.

After 10 or 20 days, the mesocosms were destructively sampled, and the litter remaining in the litterbags at both the soil surface and at 10 cm deep as well as the epi-anecic earthworm individuals were collected. The litter remaining in the litterbags was oven dried at 72 °C for 48 h, weighed in order to determine the litter dry mass, and burned at 550 °C for 6 h in order to determine the litter ash content. During the experiment, eleven epi-anecic earthworms died (6%), corresponding to 11 mesocosms: 6 LT, 2 LC, 2 LF and 1 LR. Despite the treatment, the surviving earthworms remained adult to the end of the experiment. All epi-anecic earthworms made burrows of a similar morphology, open at the surface, although the burrow diameter of LT and LC were higher than those of LF and LR (personal observations).

For both incubation times (10 and 20 days), the mass of the lightest earthworm at the end of the experiment was assigned to the mass of the lightest at the beginning of the experiment. Each earthworm individual mass was determined after 24 h on a moist sponge in a plastic box to empty its gut. Earthworm individuals' mass change was determined following the formula: (final fresh mass – initial fresh mass) / initial fresh mass × 100%.

Based on the ash-free dry mass (AFDM) of the plant litter, litter mass loss in each mesocosm was determined following the formula: initial litter AFDM – final litter AFDM. Epi-anecic earthworms' contribution to surface litter mass loss in each mesocosm was determined following the formula: (litter mass loss in presence of earthworms – mean litter mass loss in control mesocosms) / initial biomass of the two earthworms present in the mesocosm.

In mesocosms incubated for 20 days, the 2 mm of soil around burrow linings between 3 and 15 cm depth were harvested using a fin spatula while the bulk soil was sampled in control mesocosms. These soil samples were stored at -20 °C prior to microbial community analysis.

2.4. T-RFLP analysis of bacterial and fungal communities

Soil DNA was extracted from 0.5 g of epi-anecic burrow linings and control bulk soil following the protocol of (Griffiths et al., 2000) modified by Monard et al. (2013). DNA quality and quantity were checked on 1% agarose gel and using a NanoDrop spectrophotometer (NanoDrop Technologies). Bacterial 16S rRNA gene was amplified by polymerase chain reaction

(PCR) using the fluorescently labelled forward primer 63F (5'-[6FAM] CAGGCCTAACACATGCAAGTC-3') (Marchesi et al., 1998) and the reverse primer 1389R (5'-ACGGGCGGTGTGTACAAG-3') (Osborn et al., 2000). Two PCRs were run separately using 25 µL reaction volumes containing 2 µl of DNA template, 1X PCR buffer, 400 µM dNTP and 2.5 units of AmpliTaq polymerase (Applied Biosystems) and, 0.4 µM of each primer. The PCR amplifications were performed with an initial denaturing step of 4 min at 95 °C, followed by 25 cycles of 95 °C for 30 s, 58 °C for 1 min and 72 °C for 1 min and a final extension step of 72 °C for 10 min.

The fungal ITS region was amplified by PCR using the same reaction mixture as for the bacterial 16S rRNA gene but with the fluorescently labelled forward primer ITS1F (5'-[6FAM]-CTTGGTCATTTAGAGGAAGTAA-3') (Gardes and Bruns, 1993) and the reverse primer ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990). The following PCR program was used: an initial step at 95 °C for 4 min, 35 cycles of 95 °C for 30 s, 55 °C for 1min and 72 °C for 1 min and a final extension step of 72 °C for 10 min.

The replicated amplicons were pooled together, purified with the High Pure PCR Product Purification Kit (Roche Diagnostics GmbH, Manheim, Germany) according to the manufacturer's instructions and digested with 0.25 units of MspI (Promega Corporation, Madison, USA) for 3 h at 37 °C. The digested DNA was precipitated overnight and resuspended in 10 µL of sterile water. The T-RFs were separated and accurately sized on the Biogenouest platform using an ABI system analyzer (3730; Applied Biosystems). T-RFLP profiles were analyzed using Peakscanner version 1.0 (Software, Applied Biosystems), and peaks from 50 to 500 bp were retained. Data compilation, determination of true peaks and T-RFLP alignment were undertaken using the T-REX platform (Culman et al., 2009) and each T-RF was coded as a discrete variable (0 for its absence or 1 for its presence). Bacterial and fungal richness were estimated as the number of T-RFs detected. Bacterial and fungal diversities were estimated by using the Shannon diversity index following the formula: $H' = -\Sigma\, p_i \times \log_{10} p_i$, where p_i is the proportion of the total abundance arising from the i^{th} T-RF.

2.5. *Statistical analyses*

Replicates found to contain dead earthworms were removed from the data processing. Statistical analyses were performed with the R software 3.2.3 (R. Core Team, 2017). Significance was evaluated in all cases at $P < 0.05$. Data met the conditions of normality and homoscedasticity. Differences in initial litter characteristics between the three plant species as well as differences in initial mass between the four epi-anecic earthworms were assessed using separated one-way ANOVAs, followed by Tukey HSD tests for post hoc pairwise comparisons (“agricolae” package).

Regarding litter mass loss, we used first separated one-way ANOVAs, followed by Tukey HSD tests for post hoc pairwise comparisons, to assess the differences on litter mass loss between the presence of epi-anecic earthworms and control treatment (*i.e.* without epi-anecic earthworms) at both surface and 10 cm deep. Second, we used a linear mixed-effects model approach (“lme4” package), followed by Tukey HSD tests for post hoc pairwise comparisons, to test for the effects of initial biomass of epi-anecic earthworms, litter type, litter localization, sampling date, and their interactions on litter mass loss. To account for two litterbags per mesocosm, the random part of the model indicated that two litterbags were nested within a mesocosm with the following R syntax (“random = ~1|mesocosm/litterbag”). Third, we used separated one-way ANOVAs, followed by a Tukey HSD tests for post hoc pairwise comparisons, to test for the differences between epi-anecic earthworms’ contributions to surface litter mass loss for each litter type.

Regarding earthworm mass, we used first a linear model approach, followed by Tukey HSD tests for post hoc pairwise comparisons, to test for the effects of epi-anecic earthworm identity, litter type, sampling date, and their interactions on epi-anecic earthworm mass change. Second, we used separated one-sample Student's *t*-tests to test whether epi-anecic earthworms had significantly lost or gained mass during the experiment according to litter type or sampling date.

Regarding microbial communities, we used first separated one-way ANOVAs, followed by Tukey HSD tests for post hoc pairwise comparisons, to assess the differences on fungal and bacterial richness and diversity (i) between the three initial litters, (ii) between the three bulk soils (*i.e.* control treatment without earthworms) in which one of the three litter types was added, and (iii) between the initial soil, the bulk soil and the presence of a burrow (independently of the epi-anecic earthworm identity or litter type we used). Second, we used a two-way ANOVA, followed by Tukey HSD tests for post hoc pairwise comparisons, to test for the effects of epi-anecic earthworm identity, litter type, and their interactions on fungal and bacterial richness and diversity in earthworm burrows. Third, in order to compare the structure of bacterial and fungal communities (i) between the three initial litters, (ii) between the three bulk soils (*i.e.* control treatment without earthworms) in which one of the three litter types was added, (iii) between the bulk soil and the burrows of the four epi-anecic earthworms for each litter type, and (iv) between the three litter types for each epi-anecic earthworm species, a data matrix of pairwise comparisons among samples was then calculated using the Bray-Curtis distance index. Non-Metric multiDimensional Scaling (NMDS, “vegan” package) was used to find the best low-dimensional representation of the distance matrix. The null hypothesis of no difference in patterns of bacterial or fungal community structure was tested with a permutational multivariate analysis of variance (PERMANOVA, “vegan” package). PERMANOVAs were run on the Bray-Curtis distance with 1000 permutations per analysis.

3. Results

3.1. Litter mass loss

In the control treatment (*i.e.* without epi-anecic earthworms), litter mass loss was higher at 10 cm deep than at the soil surface for *L. perenne* and *H. lanatus* whereas there was no difference for *C. avellana* (Supplementary Fig. S1). In the presence of epi-anecic earthworms, litter mass loss at the soil surface was enhanced compared to the control treatment (0.81 ± 0.05 and 0.50 ± 0.04 g, respectively; $F = 9.42$, $P = 0.003$) and varied depending on the litter type (*L. perenne* > *H. lanatus* > *C. avellana*; significant litter × localisation interaction, $F = 33.11$, $P < 0.001$, Supplementary Table S1). Litter mass loss at the soil surface was always positively correlated to epi-anecic earthworms' initial biomass with the strongest relationship observed in the presence of *L. perenne* litter compared to *H. lanatus* and *C. avellana* (Fig. 2). At 10 cm deep, litter mass loss in the presence of epi-anecic earthworms was similar to that observed in the control treatment (0.68 ± 0.09 and 0.72 ± 0.04 g, respectively; $F = 0.01$, $P = 0.910$) but varied according to the litter type (*L. perenne* = *H. lanatus* > *C. avellana*; significant litter × localisation interaction, $F = 33.11$, $P < 0.001$, Supplementary Table S1).

Whatever its localization, litter mass loss increased with time (0.58 ± 0.04 after 10 days and 0.89 ± 0.04 g after 20 days; $F = 201.76$, $P < 0.001$, Supplementary Table S1) and was 2 times faster during the first 10 days than the following 10 days of the experiment.

Regarding the epi-anecic earthworms' contribution to the surface litter mass loss, LF exhibited a higher contribution than LR, LC or LT ($F = 4.02$, $P = 0.010$; Fig. 3).

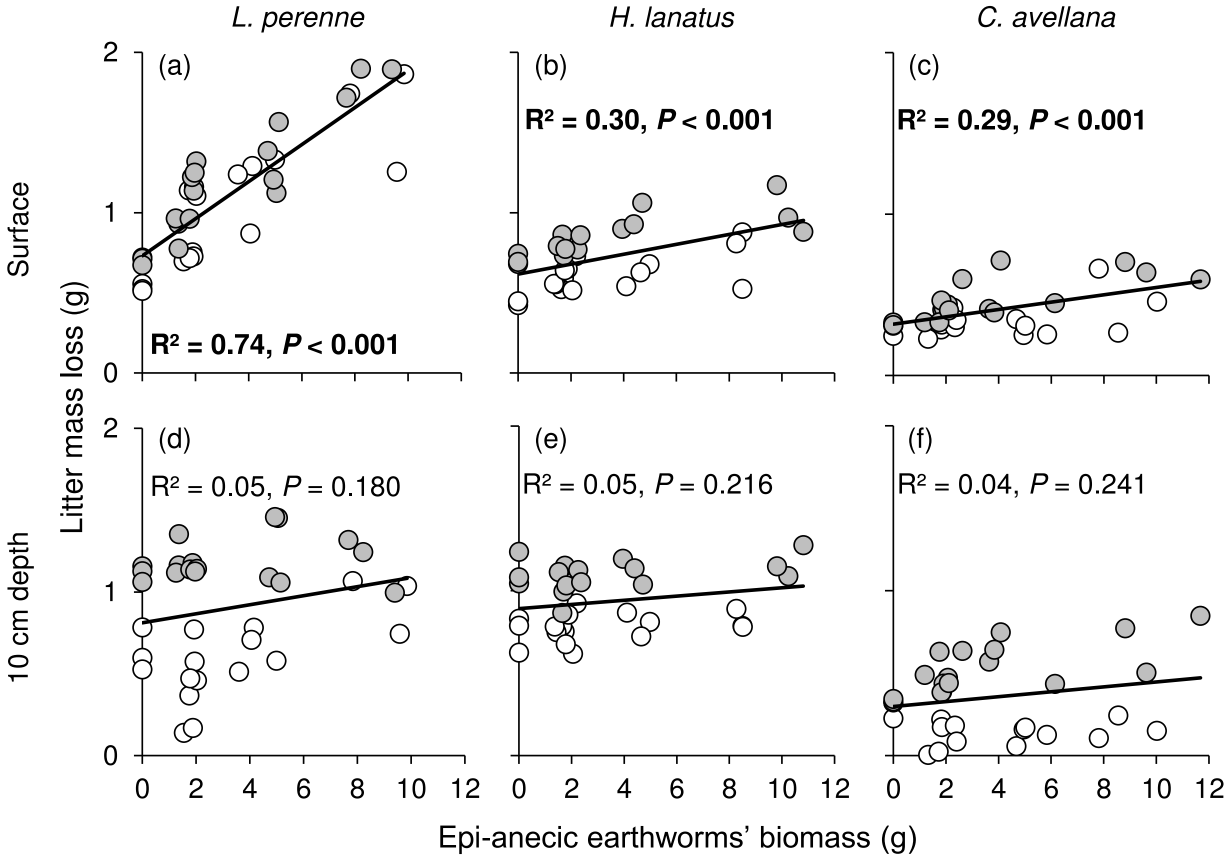

Fig. 2. Relationships between litter mass loss and epi-anecic earthworms' initial biomass according to litter type (*L. perenne* (a, d), *H. lanatus* (d, e) and *C. avellana* (c, f)) and litter localisation (soil surface (a, b, c) and at 10 cm deep (d, e, f)). Adjusted R² and associated *P*-values of the linear regression are indicated. Each symbol represents one observation after 10 (white dots) and 20 days (grey dots) of incubation.

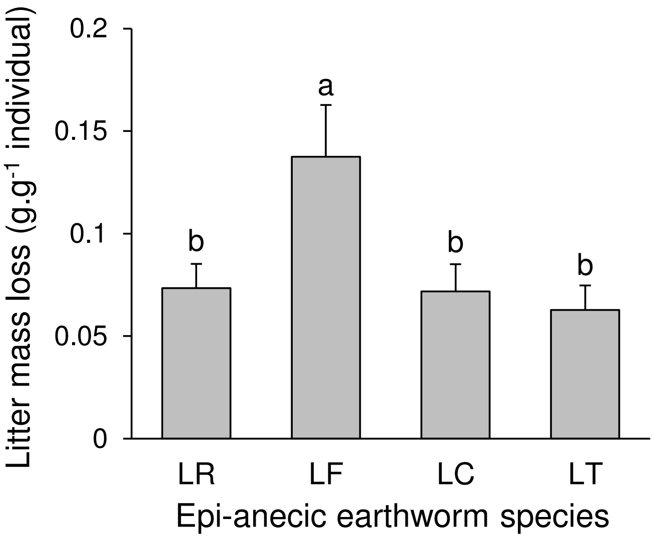

Fig. 3. Surface litter mass loss according to epi-anecic earthworms' initial biomass. Values are means ± SD; n = 21 to 23. Different letters denote significant differences between earthworm species with a > b (post hoc Tukey test results). LR = *L. rubellus*; LF = *L. festivus*; LC = *L. centralis*; LT = *L. terrestris*.

3.2. Epi-anecic earthworms mass change

Epi-anecic earthworms mass change differed significantly according to earthworm species identity ($F = 10.34$, $P < 0.001$, Supplementary Table S2, Fig. S2a) and litter type ($F = 47.91$, $P < 0.001$, Supplementary Table S2, Fig. S2b). In the presence of *L. perenne*, epi-anecic earthworms did not lose mass significantly (*t*-value = -1.78, $P = 0.081$) by contrast with the two other litter types (for *H. lanatus*: *t*-value = -10.90, $P < 0.001$ and for *C. avellana*: *t*-value = -11.97, $P < 0.001$). In the presence of *H. lanatus*, LR had a higher mass loss (-19.54 %) than LT, LC and LF (-5.53%, -10.65% and -11.33%, respectively) whereas with *C. avellana*, both LR and LF masses were more impacted (-23.12% and -17.45 %, respectively) compared with LC and LT (-7.73% and -10.65%, respectively). When fed with *H. lanatus*, the individual mass loss happened only during the first 10 days (Fig. S3), while in the presence of *C. avellana*, earthworms lost mass all throughout the experiment (significant litter × time interaction, $F = 5.34$, $P = 0.005$, Supplementary Table S2, Fig. S3).

3.3. Microbial community analyses

Initially the three litter types exhibited similar bacterial and fungal richness, diversity and community structure (F and pseudo-$F = 0.76$ to 1.83, $P > 0.05$). In the control treatment (*i.e.* without epi-anecic earthworms), both bacterial and fungal richness and diversity were similar in the bulk soil whatever the litter type added ($F = 0.08$ to 0.76, $P > 0.05$, Table 2). However, fungal community structure was different in the bulk soil according to the litter type added (pseudo-F = 8.16, $P = 0.004$), whereas no change in bacterial community structure was observed (pseudo-F = 1.18, $P = 0.284$).

Compared to the initial soil, bacterial richness and diversity in the bulk soil (*i.e.* without epi-anecic earthworms) after 20 days of incubation decreased whereas earthworm burrows maintained the initial bacterial richness and diversity ($F = 9.05$ and 27.90, $P < 0.001$, Table 2). In the meantime, fungal richness and diversity were, on the whole, similarly affected in the bulk soil and epi-anecic earthworms' burrows compared to the initial soil ($F = 3.49$ to 4.06, $P < 0.\ 05$; Table 2).

After 20 days of incubation, while no significant effect of the epi-anecic earthworm species or the litter type was observed on the richness and diversity of bacteria in the burrows (Table 2 and Supplementary Table S3), those of fungi varied according to the litter type and its interaction with epi-anecic earthworm species identity (Table 2 and Supplementary Table S3). LF fed with *L. perenne* maintained a fungal diversity as large as the initial soil, whereas with the two other litter types and/or epi-anecic earthworm presence the fungal diversity tended to be lower than in the initial soil (Table 2 and Supplementary Table S3).

Table 2 Fungal and bacterial richness (a) and diversity (b) within the initial soil, initial litter, bulk soil (*i.e.* without earthworms) and the burrows of the four epi-anecic earthworms. Microbial richness (a) is expressed as number of T-RFLP fragments; microbial diversity (b) is expressed as Shannon diversity index. Values are mean ± standard error; n = 3 or 4. LR = *L. rubellus*; LF = *L. festivus*; LC = *L. centralis*; LT = *L. terrestris*.

(a)	Microbial OTU richness					
	Bacteria			Fungi		
	L. perenne	*H. lanatus*	*C. avellana*	*L. perenne*	*H. lanatus*	*C. avellana*
Initial soil	40.3 ± 9.1	40.3 ± 9.1	40.3 ± 9.1	62.3 ± 5.2	62.3 ± 5.2	62.3 ± 5.2
Initial litter	25.0 ± 8.1	23.67 ± 8.9	46.0 ± 5.3	39.3 ± 5.0	65.3 ± 6.8	70.3 ± 21.2
Control	7.0 ± 2.9	5.0 ± 1.4	13.5 ± 8.1	55.3 ± 2.2	45.3 ± 7.3	46.5 ± 3.2
LR	31.3 ± 4.7	37.7 ± 13.7	39.2 ±6.5	43.5 ± 1.6	43.0 ± 1.4	40.0 ± 4.0
LF	39.8 ± 6.2	24.5 ± 5.2	26.5 ± 7.4	66.0 ± 5.4	33.0 ± 8.6	32.8 ± 4.8
LC	29.5 ± 1.3	39.7 ± 7.3	44.3 ± 13.3	45.5 ± 2.6	41.0 ± 5.3	34.5 ± 1.6
LT	20.3 ± 5.2	35.3 ± 4.5	45.7 ± 6.8	33.7 ± 4.0	58.0 ± 6.7	36.0 ± 4.2

(b)	Microbial Shannon diversity index					
	Bacteria			Fungi		
	L. perenne	*H. lanatus*	*C. avellana*	*L. perenne*	*H. lanatus*	*C. avellana*
Initial soil	3.6 ± 0.3	3.6 ± 0.3	3.6 ± 0.3	4.1 ± 0.1	4.1 ± 0.1	4.1 ± 0.1
Initial litter	3.0 ± 0.4	2.6 ± 0.8	3.8 ± 0.1	3.7 ± 0.1	4.2 ± 0.1	4.1 ± 0.3
Control	1.7 ± 0.4	1.5 ± 0.3	2.0 ± 0.9	4.0 ± 0.0	3.8 ± 0.2	3.8 ± 0.1
LR	3.4 ± 0.2	3.4 ± 0.5	3.6 ± 0.2	3.8 ± 0.0	3.8 ± 0.0	3.7 ± 0.1
LF	3.6 ± 0.2	3.1 ± 0.2	3.1 ± 0.3	4.2 ± 0.1	3.4 ± 0.2	3.4 ± 0.2
LC	3.4 ± 0.0	3.6 ± 0.2	3.6 ± 0.3	3.8 ± 0.1	3.7 ± 0.1	3.5 ± 0.1
LT	2.9 ± 0.3	3.5 ± 0.1	3.8 ± 0.2	3.5 ± 0.1	4.0 ± 0.1	3.6 ± 0.1

The structure of fungal community was more impacted by the litter type and the epi-anecic earthworm species identity than that of bacteria. Indeed, a different bacterial community was only detected in the presence of LT fed with *L. perenne* compared with the other epi-anecic earthworms or litter types (data not shown). Fungal communities were structured differently depending on the interaction between epi-anecic earthworm species and litter type. With *H. lanatus*, whatever the epi-anecic earthworm species, the fungal communities in the burrows were similar (Fig. 4b). In the presence of *L. perenne* and *C. avellana*, the burrows of LR and LF contained similar fungal community structures that were different from those of LC and LT burrows (Fig. 4a and c). Additionally, with *L. perenne*, fungal community structures of LC and LT were different (Fig. 4a). According to the litter type, specific fungal communities were always observed in the burrows of LT and LC (Fig. 5a and b) by contrast to the smaller species, LR and LF, for which litter types had less impact on fungal community structures in their burrows (Fig. 5c and d).

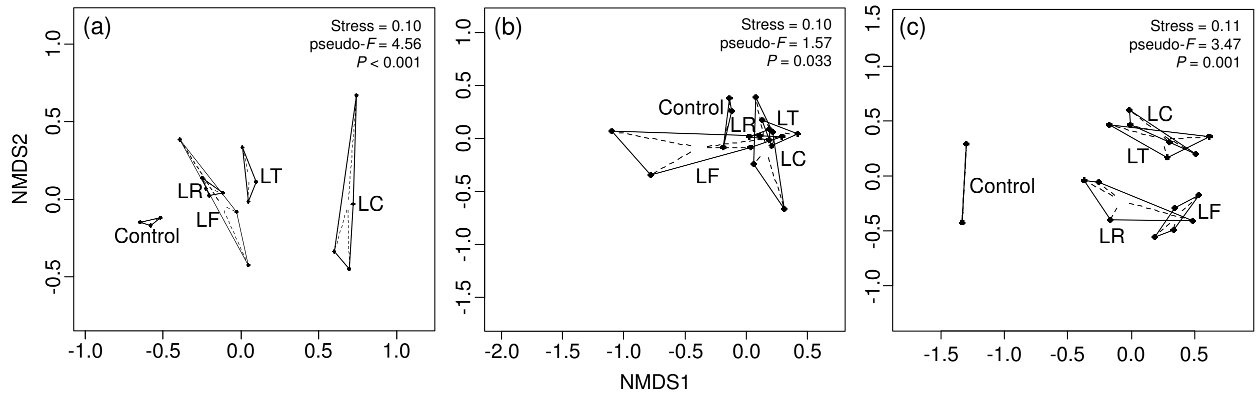

Fig. 4. Non-metric multidimensional scaling ordinations showing similarities, based on Bray-Curtis distance index, of fungal communities into the burrows of the four epi-anecic earthworm species fed with *L. perenne* (a), *H. lanatus* (b) and *C. avellana* (c). Significant dissimilarities between modalities were assessed by PERMANOVA. Pseudo-*F*-values and associated *P*-values are indicated. LR = *L. rubellus*; LF = *L. festivus*; LC = *L. centralis*; LT = *L. terrestris*.

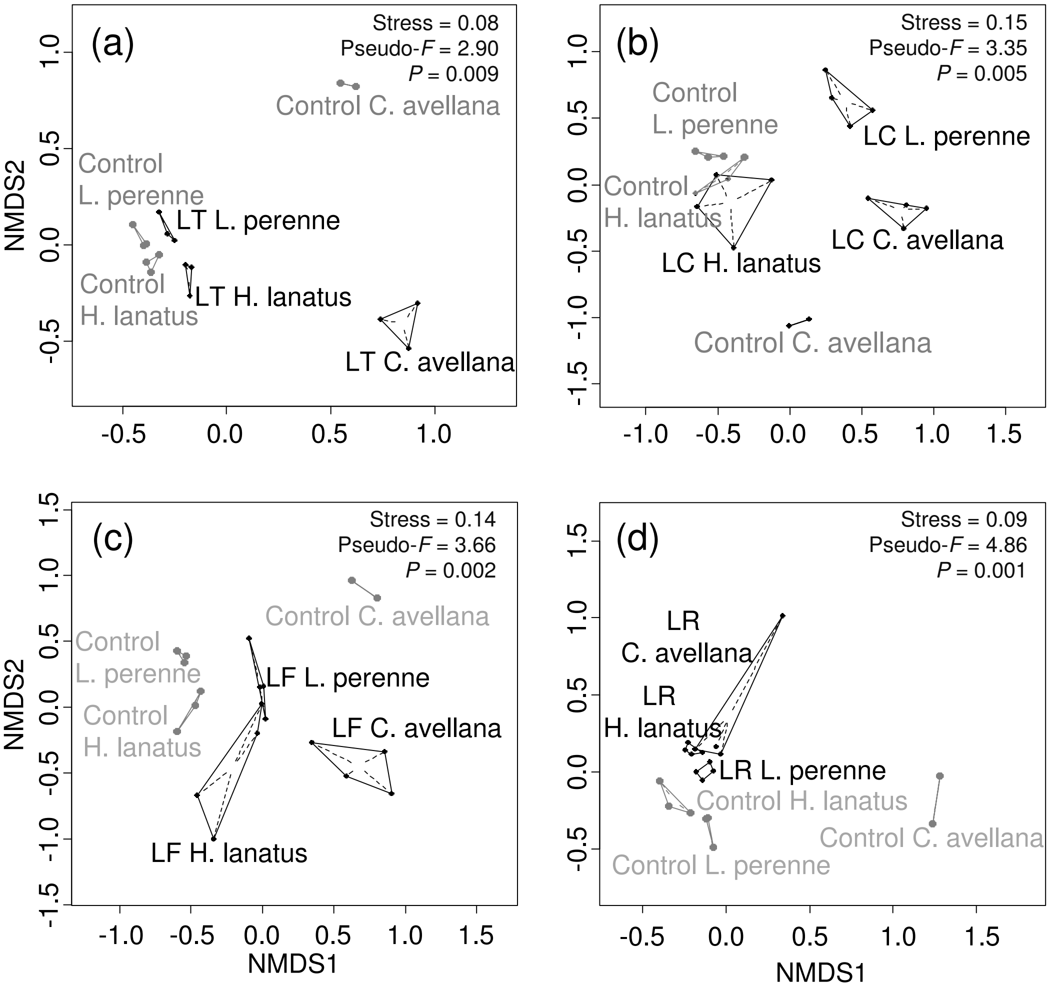

Fig. 5. Non-metric multidimensional scaling ordinations showing similarities, based on Bray-Curtis distance index, of fungal communities into the burrows of *L. terrestris* (a), *L. centralis* (b), *L. festivus* (c) and *L. rubellus* (d) fed with the three litter types (black symbols) compared to the bulk soil (grey symbols). Pseudo-*F*-values and associated *P*-values are indicated. LR = *L. rubellus*; LF = *L. festivus*; LC = *L. centralis*; LT = *L. terrestris*.

4. Discussion

4.1. *Surface feeding preference of epi-anecic earthworms*

Although it is well known that anecic earthworms feed at the soil surface, no studies have been done to test it specifically (Bouché, 1972, 1977; Syers and Springett, 1984; Sims and Gerard, 1999). In this study we clearly demonstrate that epi-anecic earthworms feed preferentially at the soil surface when they have the choice between buried and surface-deposited litters. *L. terrestris* was previously observed to gain more mass with surface applied than buried solid cattle manure after 24 weeks of experimentation (Lowe and Butt, 2002b). Nevertheless this manure was either placed at the soil surface or buried, so *L. terrestris* did not have any choice and manure mass loss was not measured. By demonstrating the surface litter feeding preference of epi-anecic earthworms, our study also provides answers on the impact of grassland ploughing to the decomposition function these earthworms sustain. It is well known that grassland ploughing has direct negative consequences on anecic earthworm communities by killing individuals (Chan, 2001) and, according to the present study, it can also have an indirect long-term impact by making organic matter less available (*i.e.* buried deeper) for epi-anecic earthworms. Overall, our study shows that epi-anecic earthworms did not interact with 10 cm deep organic matter which could affect their contribution to litter decomposition. Interestingly, at 10 cm deep, in the presence of the smaller epi-anecic earthworm species (*L. rubellus* or *L. festivus*), litter mass loss was even lower than observed without earthworms. This somewhat surprising result could be explained by an inhibition of microbial litter degradation at 10 cm deep due to direct consumption of microorganisms by these small epi-anecic earthworms or to a negative impact of their biotubartion activity on microbial activity at this depth (Edwards and Fletcher, 1988; Brown, 1995).

4.2. Surface litter mass loss in relation to epi-anecic earthworm biomass

Rate of surface mass loss of *L. perenne* in the presence of *L. terrestris* was on average of 9.5 mg g^{-1} day^{-1}, which is of similar magnitude to those found in previous studies. For example, Binet and Trehen (1992) observed a rate of 2.4 mg g^{-1} day^{-1} for *L. terrestris* when fed with *L. perenne* litter in a microcosm experiment, while Knollenberg et al. (1985) reported various rates ranging from 11 to 14 mg g^{-1} day^{-1} for *L. terrestris* when fed with fresh grass in a field experiment. At the soil surface, litter mass loss was highly correlated to the initial biomass of epi-anecic earthworms. This finding suggests that the surface litter decomposition performed by epi-anecic earthworms may in certain ecological contexts be predicted by their biomass in the field. Thus, up to an optimal density, the higher the epi-anecic earthworm biomass, the faster the surface litter decomposition is expected to be. However, this correlation is slightly modified by the presence of efficient species such as *L. festivus* that exhibited the highest rate of litter mass loss per unit of biomass. This epi-anecic earthworm species is described as widespread in Northern Europe and America (Bouché, 1972; Sims and Gerard, 1999) and could thus be considered as a keystone species for litter decomposition under these climates (Mills et al., 1993; Blondel and Aronson, 1995). Given this, more studies could usefully be undertaken on *L. festivus*' contribution to litter decomposition and possible consequences on carbon and nutrient cycling which are still poorly studied (Hendriksen, 1991c; Winding et al., 1997; Schmidt et al., 1999).

4.3. Litter quality effect on epi-anecic earthworms' contribution to litter mass loss

At the soil surface, this experiment demonstrated differences in litter mass loss depending on the initial litter quality. As previously observed (Satchell and Lowe, 1967; Wright, 1972; Edwards and Lofty, 1977), higher C:N ratio and phenolic compound concentration made *C. avellana* the least palatable litter for epi-anecic earthworms leading to the lowest litter mass loss.

Conversely, lower C:N ratio and phenolic concentration made *L. perenne* the most palatable litter leading to higher litter mass loss compared to *H. lanatus* and *C. avellana*. Interestingly, the four epi-anecic earthworms exhibited a similar sensitivity to leaf litter traits, constituting a homogeneous ecological category from this point of view. Epi-anecic earthworms did not lose mass when fed with *L. perenne*, which means that they were able to directly and quickly assimilate this food resource, by contrast to *H. lanatus* and *C. avellana* litters. Similar results were observed in a previous study in which *L. terrestris* individuals lost 30% of their mass when they were fed with *Quercus ilex* litter**,** whereas they gained 7.9% of mass in presence of *L. perenne* litter after 45 days of experimentation (Cortez and Hameed, 1988). Thus, according to Shipitalo et al. (1988), *L. perenne* can be considered as high-quality litter for epi-anecic earthworms. It seems that lower quality litters such as *H. lanatus* and *C. avellana,* were mainly left at the soil surface. A small part was buried and probably colonized by soil microorganisms that made them more palatable and/or digestible for epi-anecic earthworms (Brown, 1995; Brown et al., 2000). As the loss of mass by earthworms was absent with *H. lanautus* litter or limited with *C. avellana* litter during the last 10 days of the experiment, we can hypothesize that, when the experimental time increases, epi-anecic earthworms no longer lose energy by digging burrows and can feed on ‘microbial-incubated’ litter.

4.4. Responses of the drilospheric microbiota

By digging burrows and burying litter, epi-anecic earthworms create microhabitats for soil microorganisms that are enriched in organic carbon and inorganic nitrogen and phosphorus (Parkin and Berry, 1999; Tiunov and Scheu, 2000a; Jégou et al., 2001; Le Bayon and Binet, 2006) providing trophic resources for microbial development. It is well known that soil bioturbation by earthworms induce changes in microbial community structures (Tiunov and Dobrovolskaya, 2002; Furlong et al., 2002; Egert et al., 2004; Stromberger et al., 2012). However, this effect on soil microorganisms has never been compared between different earthworm species within a same ecological category and in presence of different litter types (Brown, 1995). In the present study, we demonstrated that whatever the litter type or the epi-anecic earthworm species considered, bacterial richness and diversity were stimulated in epi-anecic earthworm burrows compared to control bulk soil. Thus, bacteria are essentially influenced by the epi-anecic earthworm capacity to burrow, graze and disperse them into the soil profile (Brown, 1995). This is especially true in burrows formed by *L. terrestris* fed with *L. perenne,* that, linked to the highest litter mass loss observed, contained a specific bacterial community. Thus, interactions between *L. perenne* and *L. terrestris* may have recruited and stimulated specific bacteria through the supply of high content of soluble compounds easily available to the bacteria. In contrast, fungi are known to decompose more complex molecules and to be more specialized than bacteria towards soil organic matter degradation (Chapin et al., 2002). Thus, fungal community structures were shaped by both the litter type and the identity of the epi-anecic earthworm, however no common pattern was observed between epi-anecic earthworm species. Depending on the litter type, the structure of fungal communities were either similar or very different due to epi-anecic earthworm presence. Our results demonstrate that epi-anecic earthworms control the drilospheric microbiota diversity which can be partly explained by their different contributions to burying litter depending on the earthworm species considered and the litter quality.

5. Conclusion

Many studies have shown that earthworms contribute significantly to litter decomposition especially through microbial enhancement, but these studies did not take into account earthworm species diversity within a single ecological category. Here, we reported for the first time that epi-anecic earthworms fed preferentially on surface litter and exhibit a same sensitivity to litter quality which makes them a homogeneous ecological category based on these criteria. Litter mass loss was also highly correlated to initial epi-anecic earthworm biomass, and the intensity of this relationship was strongly dependent on litter quality. Nevertheless, we observed that *L. festivus* is quite efficient in surface litter mass loss which means that within the epi-anecic ecological category, some earthworm species may be more effective in the decomposition process than others. Epi-anecic earthworm presence enhanced bacterial richness and diversity. Interestingly, epi-anecic earthworms modified fungal community structure, in particular *L. festivus*, which enhanced fungal diversity, depending on litter quality. Thus, epi-anecic earthworms and litter type contribute to enhance drilosphere microbiota diversity. Consequently, during the decomposition process, epi-anecic earthworms and soil microbiota interact with each other, depending on the litter quality.

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SUPPORTING INFORMATION

Table S1 ANOVA results from linear mixed-effects model testing for the effects of earthworm initial biomass (EW biomass), litter type, sampling date (Time), litter position (Localisation), and their interactions on leaf litter mass loss. *F*-values and associated *P*-values are indicated. d.f.n. = degrees of freedom numerator; d.f.d. = degrees of freedom denominator.

	d.f.n	d.f.d	*F*-value	*P*-value
(Intercept)	1	104	5118.97	<0.001
EW biomass	1	99	88.72	<0.001
Litter type	2	99	350.79	<0.001
Localisation	1	104	0.32	0.576
Time	1	99	201.76	<0.001
EW biomass × Litter type	2	99	25.05	<0.001
EW biomass × Localisation	1	104	29.92	<0.001
EW biomass × Time	1	99	0.40	0.528
Litter type × Time	2	99	2.61	0.079
Litter type × Localisation	2	104	33.11	<0.001
Localisation × Time	1	104	32.87	<0.001

Table S2 ANOVA results of multiple linear models testing for the effects of earthworm species identity (EW species), litter type, sampling date (Time), and their interactions on epi-anecic earthworm mass change. *F*-values and associated *P*-values are indicated. d.f. = degrees of freedom.

	d.f.	*F*-value	*P*-value
EW species	3	10.34	<0.001
Litter type	2	47.91	<0.001
Time	1	0.32	0.570
EW species × Litter type	6	2.97	0.009
EW species × Time	3	0.09	0.966
Litter type × Time	2	5.34	0.005
Residuals	151		

Table S3. ANOVA results of multiple linear models testing for the effects of earthworm species identity (EW species), litter type, and their interactions on fungal and bacterial richness and diversity. *F*-values and associated *P*-values are indicated. d.f. = degrees of freedom.

		Fungi				Bacteria			
		Richness		Diversity		Richness		Diversity	
	d.f.	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
EW species	3	1.82	0.163	1.49	0.236	1.44	0.251	1.19	0.329
Litter type	2	4.90	0.014	3.63	0.038	2.97	0.068	2.70	0.082
EW species × Litter type	6	4.75	0.001	3.80	0.006	2.00	0.097	1.43	0.233
Residuals	33								

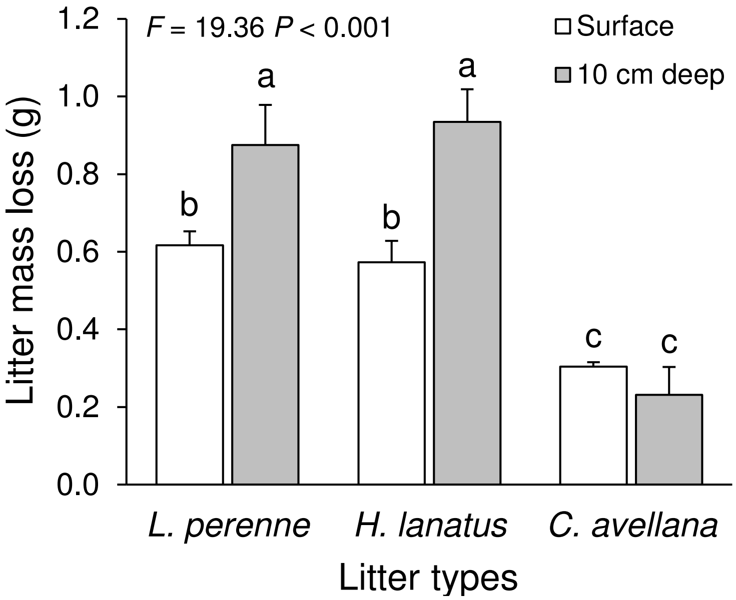

Fig. S1. Litter mass loss in control (without epi-anecic earthworms) according to litter species (*L. perenne*, *H. lanatus* and *C. avellana*) and localisation (Surface and 10 cm deep). Values are means ± SD; n = 4. Different letters denote significant differences between each modalities with a > b > c (post hoc Tukey test results).

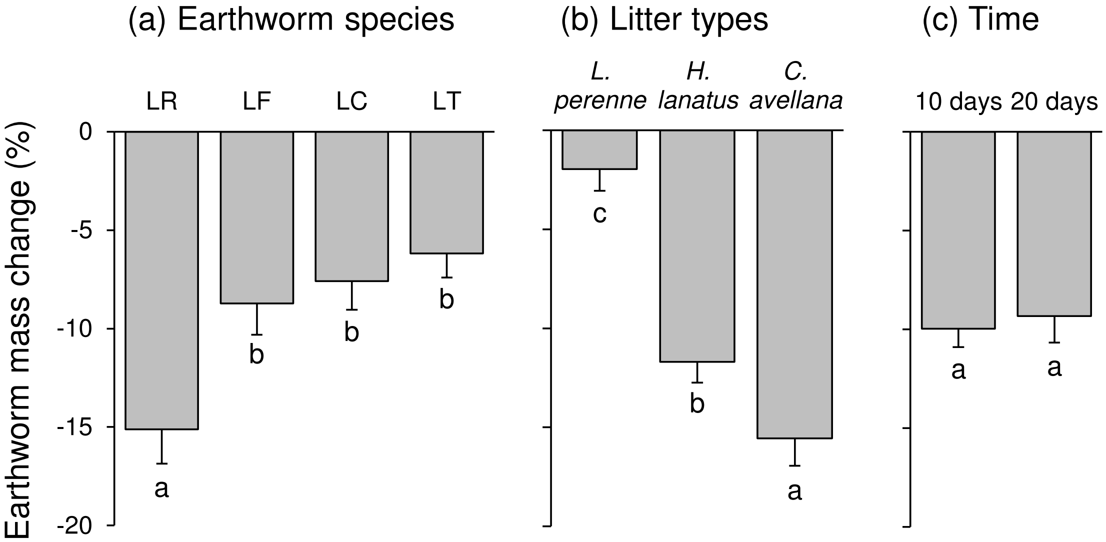

Fig. S2. Earthworm mass change according to earthworm species identity (a), litter type (b) and time of decomposition (c). Values are means ± SD; n = 21 to 86. Different letters denote significant differences among earthworm mass change with a>b>c (post hoc Tukey test results). LR = *L. rubellus*; LF = *L. festivus*; LC = *L. centralis*; LT = *L. terrestris*.

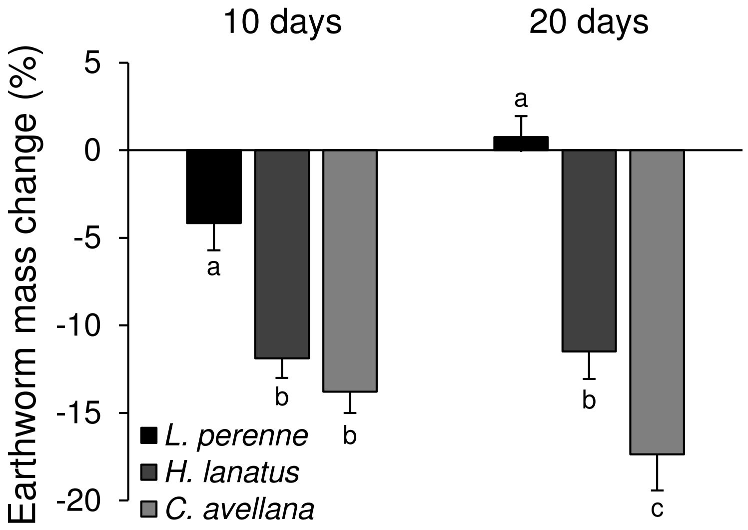

Fig. S3. Earthworm mass change according to litter type (*L. perenne*, *H. lanatus* and *C. avellana*) and time of decomposition (10 and 20 days). Values are means ± SD; n = 30 to 32. Different letters denote significant differences among earthworm mass change for each incubation time with a>b>c (post hoc Tukey test results).

CHAPITRE 4

Influence des lombriciens anéciques stricts et épi-anéciques et de leurs interactions sur les activités enzymatiques du sol

Le quatrième chapitre correspond à un article publié dans la revue internationale Soil Biology and Biochemistry 132 (2019) 93–100, DOI : 10.1016/j.soilbio.2019.02.001

Objectifs de l'étude

Les objectifs de cette expérimentation étaient de déterminer dans un premier temps si au sein des lombriciens anéciques stricts et épi-anéciques toutes les espèces (i) avaient le même rôle fonctionnel quant à la décomposition des litières et (ii) impactaient spécifiquement les activités enzymatiques des microorganismes des galeries et des cabanes. Dans un second temps, nous avons cherché à savoir si ces effets étaient modifiés par les interactions entre les différentes espèces anéciques ciblées.

Méthodologie

Nous avons mené une expérimentation en conditions contrôlées au laboratoire avec trois espèces lombriciennes anéciques stricts (*Aporrectodea caliginosa meridionalis*, *Aporrectodea nocturna* et *Aporrectodea giardi*) et trois espèces lombriciennes épi-anéciques (*Lumbricus rubellus*, *Lumbricus centralis* et *Lumbricus terrestris*) assemblées au sein de mésocosmes de sol selon toutes les combinaisons possibles de deux individus (6 paires mono- et 15 paires bispécifiques). Après 30 jours d'expérimentation, nous avons mesuré la perte de masse de la litière apportée en surface du sol et quantifié le potentiel de cinq activités enzymatiques (FDAse, β-D-glucosidase, cellobiohydrolase, leucine amino-peptidase et la phosphatase acide) dans les galeries et les cabanes produites par chaque assemblage de lombriciens anéciques.

Principaux résultats

Dans les assemblages mono-spécifiques, la perte de masse de la litière végétale et les activités enzymatiques étaient significativement plus élevées en présence de lombriciens épi-anéciques qu'en présence de lombriciens anéciques stricts, quelle que soit l'identité de l'espèce. Ces différences sont les plus importantes pour la β-D-glucosidase, la leucine amino-peptidase et la FDAse (+78%, +57% et +34%, respectivement). Nous avons également observé que l'activité de la FDAse était plus élevée dans les cabanes des lombriciens anéciques que dans leurs galeries alors que l'inverse était observé pour la phosphatase acide. Les interactions entre les espèces lombriciennes (intra- ou inter- sous-catégorie écologique) n'ont pas modifié la perte de masse de litière et les activités enzymatiques. Ces résultats indiquent que les deux sous-catégories écologiques des anéciques ont des impacts différents sur le processus de décomposition des litières.

Epi-anecic rather than strict-anecic earthworms enhance soil enzymatic activities

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Lumbricidae, *Aporrectodea*, *Lumbricus*, ecological category, nutrient cycling

Abstract

Earthworms in interaction with soil microorganisms play a key role in litter decomposition. Moreover, as soil engineers, earthworms modify microbial communities and their enzymatic activities. Most studies focusing on earthworms and soil enzymatic activities compare distinct ecological categories of earthworms whereas their contributions and interactions within a given ecological category remain largely unknown. In this context, the aims of the present study were to determine and compare the contribution of (1) three strict-anecic earthworm species, (2) three epi-anecic earthworm species and (3) the pairwise interactions between these different species on *Lolium perenne* leaf litter decomposition and soil microbial activity. After 30 days of incubation, the surface litter mass loss and five soil enzymatic activities (FDAse, β-D-glucosidase, cellobiohydrolase, leucine amino-peptidase and acid phosphatase) were measured in both earthworm burrows and middens. In mono-specific assemblages, leaf litter mass loss and enzymatic activities were significantly higher in the presence of epi-anecic compared to strict-anecic species, whatever the species identity. These differences were higher for the β-D-glucosidase, leucine amino-peptidase and FDAse (+78%, +57% and +34%, respectively). Earthworm species interactions at both intra- and inter-ecological sub-categories did not enhance either leaf litter mass loss or enzymatic activities. Interestingly, FDAse activity was higher in earthworm burrows whereas acid phosphatase activity was higher in earthworm middens. These results indicate that the two anecic ecological sub-categories have different impacts on soil functioning and each of them regroups earthworm species with similar behaviour. This functional distinction highlights the key role of epi-anecic earthworms in fresh surface litter burial and decomposition, featuring their importance on nutrient cycling in soil and for microbial activities stimulation through resource availability.

1. Introduction

Organic matter decomposition is a key ecosystem function due to its importance for carbon (C) and nutrient cyclings (Bardgett et al., 2005; Wall, 2012), soil structure (Oades, 1984; Soane, 1990), and water storage (Tisdall and Oades, 1982; Hudson, 1994). As roughly 90 % of the global terrestrial plant production enters the dead organic matter pool (Cebrian, 1999), the decomposition of plant material is one of the most crucial processes in terrestrial ecosystems (Tiessen et al., 1994). At the fine scale, soil organic matter (SOM) decomposition is performed by saprotrophic microorganisms that catalyze several biochemical reactions involved in the mineralization process (Coûteaux et al., 1995). Both soil fungi and bacteria secrete enzymes (Dick and Tabatabai, 1992; Dick et al., 2000; Tabatabai, 2003) acting on specific chemical bounds to hydrolyze complex molecules and polymers into low molecular weight compounds (Burns and Dick, 2002). Unlike many bacteria which tend to focus on one substrate at a time, fungi can secrete enzymes targeting several compounds simultaneously and are known to degrade more complex molecules than bacteria (Adl, 2003; Burns et al., 2013). Soil enzymatic activity measurements are used as soil quality indicators (Karaca et al., 2010; Schloter et al., 2018) since both enzyme production and enzymatic activity efficiencies are known to be highly sensitive to soil texture, pH, organic matter content, climate and microbial biomass changes (Bandick and Dick, 1999; Acosta-Martínez and Tabatabai, 2000; Tabatabai, 2003). Despite it is well established that microbial communities and the processes they drive depend strongly on their interactions with soil fauna (Lussenhop, 1992; Brown, 1995; Tiunov and Dobrovolskaya, 2002; Jouquet et al., 2005), our knowledge about the extent to which the soil microbial enzymes reacts to these interactions is still limited.

Earthworms are soil ecosystem engineers able to modify soil physical, chemical and biological properties (Lavelle, 1988; Jones et al., 1994; Blouin et al., 2013) with consecutive effects on soil microorganisms including changes in microbial biomass, respiration, or enzymatic activities

(Ross and Cairns, 1982; Brown, 1995; Binet et al., 1998; Tiunov and Scheu, 1999; Brown et al., 2000; Tiunov et al., 2001; Aira et al., 2009). According to their physiological, morphological and behavioral characteristics, earthworms are classified into three main ecological categories, epigeic, anecic and endogeic (Bouché, 1972, 1977). Among those, anecic earthworms play a key role in SOM degradation (Edwards, 2004; Curry and Schmidt, 2007) since they consume a mixture of organic matter with mineral soil and bury fresh leaf litter into their vertical burrows. Moreover, during their activity, anecic earthworms often create middens at the entrance of their burrows (Bouché, 1977; Brown et al., 2000) which are a surface structure made up of a mix of soil, casts, mucus and buried organic matter fragments (Nielsen and Hole, 1964). Within anecic earthworms, several studies have highlighted two ecological sub-categories according to earthworm morphological traits (Briones and Álvarez-Otero, 2018), burrowing (Jégou et al., 2000, 2001; Bastardie et al., 2005) and feeding behaviors (Ferrière, 1980; Eisenhauer et al., 2008; Andriuzzi et al., 2016; Larsen et al., 2016; Hoeffner et al., 2018). Thus, epi-anecic earthworms preferentially consume fresh leaf litter at the soil surface which is then buried into the soil profile whereas strict-anecic earthworms consume a mix of leaf litter and humified organic matter within the soil profile (Cortez et al., 1989; Jégou et al., 1998; Stromberger et al., 2012; Larsen et al., 2016; Hoeffner et al., 2018).

The average fresh individual biomass of each earthworm species within these two ecological sub-categories can vary greatly, from 0.5 g for *Lumbricus rubellus* to 15.0 g for *Lumbricus terrestris* within epi-anecic earthworms and from 0.3 g for *Aporrectodea caliginosa meridionalis* to 3.2 g for *Aporrectodea giardi* within strict-anecic earthworms (Bouché, 1972). This huge variability of biomasses between earthworm species of a same ecological sub-category suggests different metabolic needs and capacities to bury leaf litter in their burrows (Hoeffner et al., 2018). Thus, depending on both, their belonging to the epi- or strict-anecic sub-categories and their individual biomass, anecic earthworm species could influence soil microorganisms. For example, Jégou et al. (2001) reported a higher dehydrogenase activity in *L. terrestris* (epi-anecic earthworm) than in *A. giardi* (strict-anecic earthworm) burrow walls but no difference between these two species was observed on acid and alkaline phosphatase activities. Moreover, Hoeffner et al. (2018) reported that fungal communities were differentially impacted by four epi-anecic earthworm species. However, to our knowledge, no previous study compared the specific impact of these two ecological sub-categories on soil enzymatic activities involved in the main biochemical cycles such as those of C or N.

Several authors reported that anecic earthworm communities can be composed from one to six different species (Gerard, 1967; Poier and Richter, 1992; Boag et al., 1997; Margerie et al., 2001; Decaëns et al., 2008). Interactions between earthworm species within and between ecological categories can thus potentially modify their contributions to litter burial and their impacts on soil microbial communities and activities. For example, Xia et al. (2011) observed a synergistic effect on surface leaf litter mass loss between *L. rubellus* (epi-anecic earthworm) and *Octalasion lacteum* (endogeic earthworm). Within epi-anecic earthworms, Postma-Blaauw et al., (2006) reported that the interaction between *L. rubellus* and *L. terrestris* reduced mineral N concentrations derived from SOM and increased bacterial biomass probably due to an increase in litter incorporation. However, to our knowledge, no previous studies attempted to demonstrate if both strict- and epi-anecic earthworms could interact together in driving soil enzymatic activities.

The present study aims to determine the effects of strict- and epi-anecic earthworm species in addition to their interactions on leaf litter mass loss and soil microbial enzymatic activities. We used a block design experiment with three strict-anecic earthworm species (*Aporrectodea caliginosa meridionalis*, *Aporrectodea nocturna* and *Aporrectodea giardi*) and three epi-anecic earthworm species (*Lumbricus rubellus*, *Lumbricus centralis* and *Lumbricus terrestris*) in all possible pairwise combinations of two individuals (6 mono- and 15 bi-specific pairs). After 30 days of incubation, surface leaf litter mass loss was determined and five enzymatic activities were measured in earthworm middens and burrows. Since epi-anecic earthworms bury higher amount of leaf litter than strict-anecic earthworms, we first hypothesized greater leaf litter mass loss and enzymatic activities in the presence of epi- than strict-anecic earthworms. Secondly, we hypothesized a synergistic effect on leaf litter mass loss and enzymatic activities with bi-specific pairs, an effect that should be increased when combining one strict- and one epi-anecic species.

2. Materials and methods

2.1. Material collection

Soil (5-20 cm depth) was collected in a temporary grassland near Trans-La-Forêt, France (48°50’ N, -1°58’ W) in the Long Term Ecological Research (LTER) site ‘‘Zone Atelier Armorique’’. The climate of the region is oceanic with a mean annual temperature of 11.7 °C, a mean annual rainfall of 815.0 mm and a mean annual relative humidity of 80.9 % (mean values over the period 2010-2016, data from Météo France). The collected soil was hand-sieved at 4 mm and homogenized. The soil was identified as a loam Cambisol (IUSS Working Group, 2015) with 48% sand, 38% silt and 14% clay, characterized by 1.7% organic carbon, 0.2% total nitrogen, a C:N ratio of 9.5 and a pH of 6.4 (data from INRA SAS, Arras, France). The soil was pre-incubated for one week at 12 °C under a 12 h:12 h light: dark regime with a water content adjusted to 29% w/w by addition of deionized water prior to the experiment.

Fresh leaf litter of *Lolium perenne* L., a typical grassland species, was collected from a temporary grassland close to the soil sampling location. The leaf litter was air-dried and stored at room temperature until the beginning of the experiments. The leaf litter characteristics of *L. perenne* were determined according to the protocols described in Hoeffner et al. (2018). The leaf litter was characterized by 43.1% organic carbon, 3.1% total nitrogen, 3.4% phenolics, a C:N ratio of 13.9, a water holding capacity of 392.6% and a specific leaf area of 330.2 $cm^2 g^{-1}$.

Adult earthworms were collected in temporary grasslands around the soil sampling location. Three strict-anecic earthworm species, *A. caliginosa meridionalis* (Bouché, 1972; hereafter called AM), *A. nocturna* (Ude, 1885; hereafter called AN), *A. giardi* (Savigny, 1826; hereafter called AG); and three epi-anecic earthworm species, *L. rubellus rubellus* (Hoffmeister, 1843; hereafter called LR), *L. centralis* (Bouché, 1972; hereafter called LC), *L. terrestris,* (Linné, 1758; hereafter called LT) were studied. The six earthworm species were hand collected two weeks before the beginning of the experiment, grouped in mono-specific boxes containing the hand-sieved soil, and fed with air-dried leaves of *L. perenne* studied here.

2.2. Experimental setup

From the three strict-anecic and the three epi-anecic earthworm species selected, 21 pairwise combinations of two individuals were performed in five replicates following 5 earthworm assemblages (Supplementary Fig. S1): i) mono-specific assemblages of 2 individuals of strict-anecic earthworms (3 treatments), ii) mono-specific assemblages of 2 individuals of epi-anecic earthworms (3 treatments), iii) bi-specific assemblages of 2 individuals within strict-anecic earthworms (3 treatments), iv) bi-specific assemblages of 2 individuals within epi-anecic earthworms (3 treatments), and v) bi-specific assemblages of 2 individuals including one strict- and one epi-anecic earthworm (9 treatments). Control without earthworm accounting for the leaf litter mass loss due to microbial decomposition and leaching was performed in 5 replicates.

Each mesocosm (PVC cylinder, 30 cm high, 10 cm diameter sealed at the base) was filled with 4.9 kg of fresh soil. The soil was placed in the mesocosm and compacted to a bulk density of 1.3 $g.cm^{-3}$ in two steps. A plastic grille (mesh size 1.2 cm) was placed on top of the soil and 3.5 g of air-dried leaves of *L. perenne* cut into sections of approximately 7 cm length were deposited just before start of the experiment on the grille and re-humidified with deionized water. In parallel, 5 replicates of 3.5 g of leaves were dried at 72 °C during 48 h to determine the initial leaf litter dry weight. Earthworms were gut voided (36 h starving on a moist sponge in a plastic box) and weighed before being placed in the corresponding mesocosms. Individual earthworms presented an initial fresh biomass gradient varying from 0.62 g for LR to 4.72 g for LT (Supplementary Fig. S2). Mesocosms were closed with a mesh of 1 mm to avoid earthworms' escape during the experiment. The mesocosms were incubated in a climatic chamber at 12 °C, with a relative humidity of 85% and a 12 h: 12 h light: dark regime. Soil moisture was maintained by spraying deionized water at the soil surface twice per week. Given the large number of mesocosms, different sets of replicates were launched one day apart leading to 5 blocks of 22 mesocosms.

After 30 days, the mesocosms were destructively sampled: The leaf litter remaining on the grille at the soil surface and the earthworms were collected. In the mesocosms containing earthworms, two soil microsites were sampled and analyzed separately: the surface middens between +3 and -2 cm and a 2 mm soil layer from the inside (i.e. close to the burrow lumen) of the entire burrow network (regardless of the earthworm species) between -3 and -15 cm deep using a thin spatula. Strict-anecic earthworms do not form middens sensu stricto (Nielsen and Hole, 1964), however we sampled the entrance of the burrow constituted of casts and mucus. In the control mesocosms, the bulk soil was collected. These fresh soil samples were stored *at 4 °C* prior to enzymatic activity analyses performed within the following 14 days.

The remaining leaf litter was first dried at 72 °C for 48 h to determine leaf litter dry mass and then burned at 550 °C for 6 h to determine the leaf litter ash content. Based on the ash-free dry mass (AFDM) of the plant litter, leaf litter mass loss in each mesocosm was determined as the difference between initial and final AFDM.

During the experiment, 4 earthworms died (2% of the whole community): two LR, one LC and one AM. Whatever the treatment, the surviving earthworms were adults (with a turgid clitellum) and no juveniles were detected. All earthworms made burrows of a similar morphology, open at the soil surface, although the burrow diameters of LT, LC, AG were larger than those of LR, AC, AN (personal observations).

2.3. Enzymatic activity analyses

Five enzymatic activities commonly used as indicators for soil quality and involved in C, N and P cycles were studied (Bandick and Dick, 1999; Dodor and Tabatabai, 2003; Baldrian, 2009): fluorescein diacetate hydrolase (EC 3.1.1.x, FDAse for broad-spectrum indicator of soil activity), β-D-glucosidase (EC 3.2.1.21, C cycle), cellobiohydrolase (EC 3.2.1.91, C cycle), leucine-aminopeptidase (EC 3.4.11.1, N cycle) and acid phosphatase (EC 3.1.3.2, P cycle).

β-D-glucosidase, cellobiohydrolase, leucine-aminopeptidase and acid phosphatase assays were adapted from Marx et al. (2001) and FDAse from Green et al. (2006) using microplate assays. For each enzyme, the corresponding substrate and standard were obtained in crystalline form from Sigma-Aldrich (MI, USA). Fluorescence-based soil assays for FDAse, β-D-glucosidase, cellobiohydrolase, leucine aminopeptidase and acid phosphatase were based on protocols using the respective following substrates: Fluorescein diacetate, 4-methylumbelliferyl β-d-glucopyranoside, 4-methylumbelliferyl β-d-cellobioside, l-leucine 7-amido-4-methylcoumarin hydrochloride, and 4-methylumbelliferyl phosphate. Stock solutions of the substrates and calibration solutions of 4-methylumbelliferone (MUB), amino-4-methylcoumarin (AMC) and fluorescein were prepared in appropriate diluents (Marx et al., 2001; Green et al., 2006) and subsequently used. Substrate-saturating concentrations were assayed from 5 random soil samples, and substrate concentrations were adapted for the measurements.

Briefly, 0.5 g of fresh soil were suspended and mixed in 50 mL of appropriate buffer for 5 min using an orbital shaker and then sonicated for 120 s. Aliquots of 50 µL were dispensed into black 96-well microplates in 3 replicates (Greiner bio-one, Kremsmünster, Austria). The specific substrate solutions were added to reach a final volume of 200 µL, and the microplates were stored at 30 °C in the dark. Fluorescence was read from each well every 60 min during a 300 min period (spectrophotometer Safas Monaco Xenius) at excitation and emission wavelengths of 490 and 514 nm for FDAse, 360 and 450 nm for MUB-substrates and 380 and 440 nm for AMC-substrates, respectively. Enzymatic activities were expressed as FDA, MUB or AMC released g^{-1} soil h^{-1}.

2.4. Statistical analyses

The 4 soil mesocosms that contained dead earthworms were removed from the data processing. Statistical analyses were performed using the R software 3.2.3 (R. Core Team, 2017). Significance was evaluated at $P < 0.05$. Data met the conditions of normality and homoscedasticity.

The differences between the initial biomass of the six earthworm species were assessed by one-way ANOVA, followed by Tukey HSD tests for post hoc pairwise comparisons (“agricolae” package).

Litter mass loss was first analyzed by one-way ANOVA, followed by Tukey HSD tests for post hoc pairwise comparisons, to assess the differences between litter mass loss in the presence of mono-specific assemblages of strict- and epi-anecic earthworms and the control treatment (i.e. without earthworm). Second, a three-way ANOVA was applied, followed by Tukey HSD tests for post hoc pairwise comparisons, to test the effects of earthworm assemblages, earthworm pairs, and blocks on litter mass loss.

Enzymatic activities were first analyzed by one-way ANOVAs, followed by Tukey HSD tests for post hoc pairwise comparisons to assess the differences between mono-specific assemblages of strict- and epi-anecic earthworms and the control treatment (independently of the middens and the burrows). Second, 4-way ANOVAs, followed by Tukey HSD tests for post hoc pairwise comparisons were used to test the effects of earthworm assemblages, earthworm pairs, microsites, and block on each enzymatic activity.

3. Results

3.1. Litter mass loss

In mesocosms with mono-specific assemblages, litter mass loss was 57% higher in the presence of epi-anecic earthworms compared to the control, whereas no significant effect was observed in the presence of strict-anecic earthworms (1.65 ± 0.04, 2.59 ± 0.09 and 1.81 ± 0.03 g for control, epi- and strict-anecic earthworms, respectively, $F = 45.10$, $P < 0.001$). Within the mono-specific assemblages of epi-anecic earthworms, litter mass loss was 23% and 17% higher with LT compared to LR and LC, respectively, whereas no difference was observed between strict-anecic earthworm species ($F = 9.94$, $P < 0.001$, Supplementary Table S1, Fig. 1a).

In the mesocosms with bi-specific assemblages of either strict- or epi-anecic earthworms, the mean litter mass losses were similar to those observed with the corresponding mono-specific assemblages ($F = 97.55$, $P < 0.001$, Supplementary Table S1, Fig. 1b). The mean litter mass losses in the presence of bi-specific assemblages including one strict- and one epi-anecic earthworm were between those observed in the presence of the corresponding mono-specific assemblages ($F = 97.55$, $P < 0.001$, Supplementary Table S1, Fig. 1b). Additionally, litter mass loss in the presence of mono- and bi-specific assemblages of epi-anecic earthworms correlated to their initial biomass (monospecific assemblage: $R^2 = 0.81$, $P < 0.001$; bispecific assemblage: $R^2 = 0.73$, $P < 0.001$; Supplementary Fig. S3).

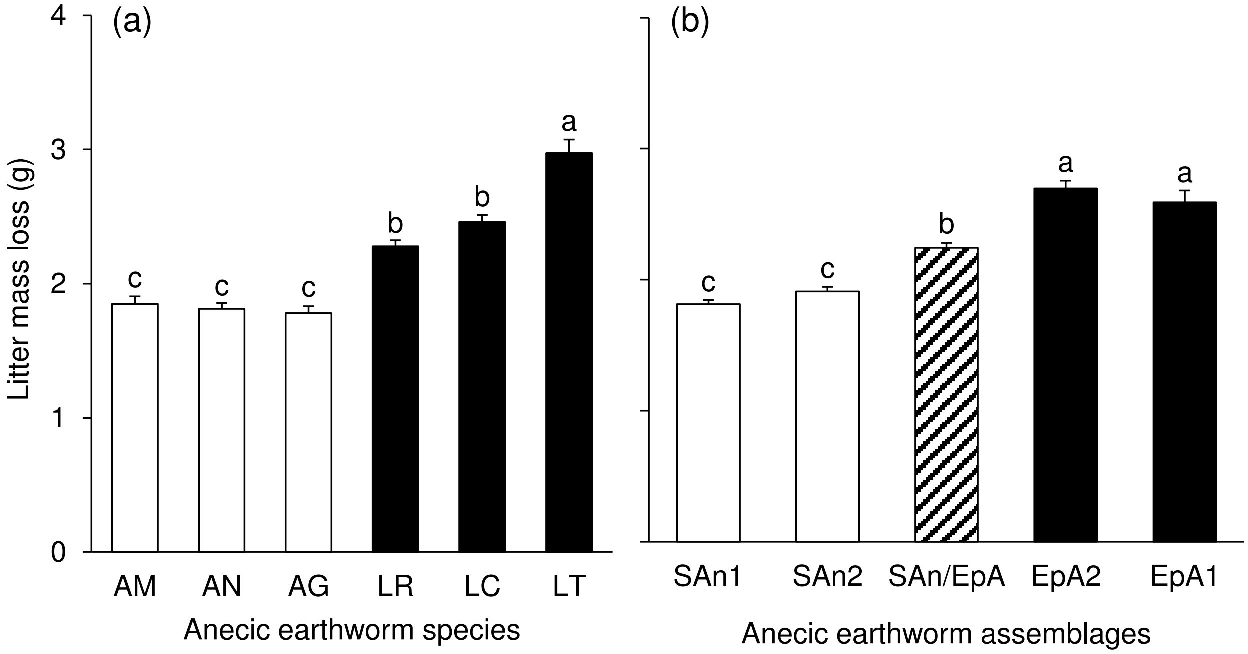


Fig. 1. Litter mass loss of *Lolium perenne* after 30 days of experiment according to 6 anecic earthworm species in mono-specific assemblages (a) and to the combinations of earthworm species within and between the two ecological sub-categories (b). Values are means ± SD; initial litter mass = 3.5 g. Different letters denote significant differences among earthworm pairs with a > b > c (Tukey HSD test results). AM = *A. caliginosa meridionalis*; AN = *A. nocturna*; AG = *A. giardi*; LR = *L. rubellus rubellus*; LF = *L. festivus*; LC = *L. centralis*; LT = *L. terrestris*. n = 5 for each anecic earthworm species in monospecific assemblages (panel a). SAn1 = Mono-specific assemblages of strict-anecic, n=14; SAn2 = Bi-specific assemblages of strict-anecic, n=15; SAn/EpA = Bi-specific assemblages with one strict- and one epi-anecic, n=44; EpA2 = Bi-specific assemblages of epi-anecic, n=13; and EpA1 = Mono-specific assemblages of epi-anecic, n=15.

3.2. Enzymatic activities

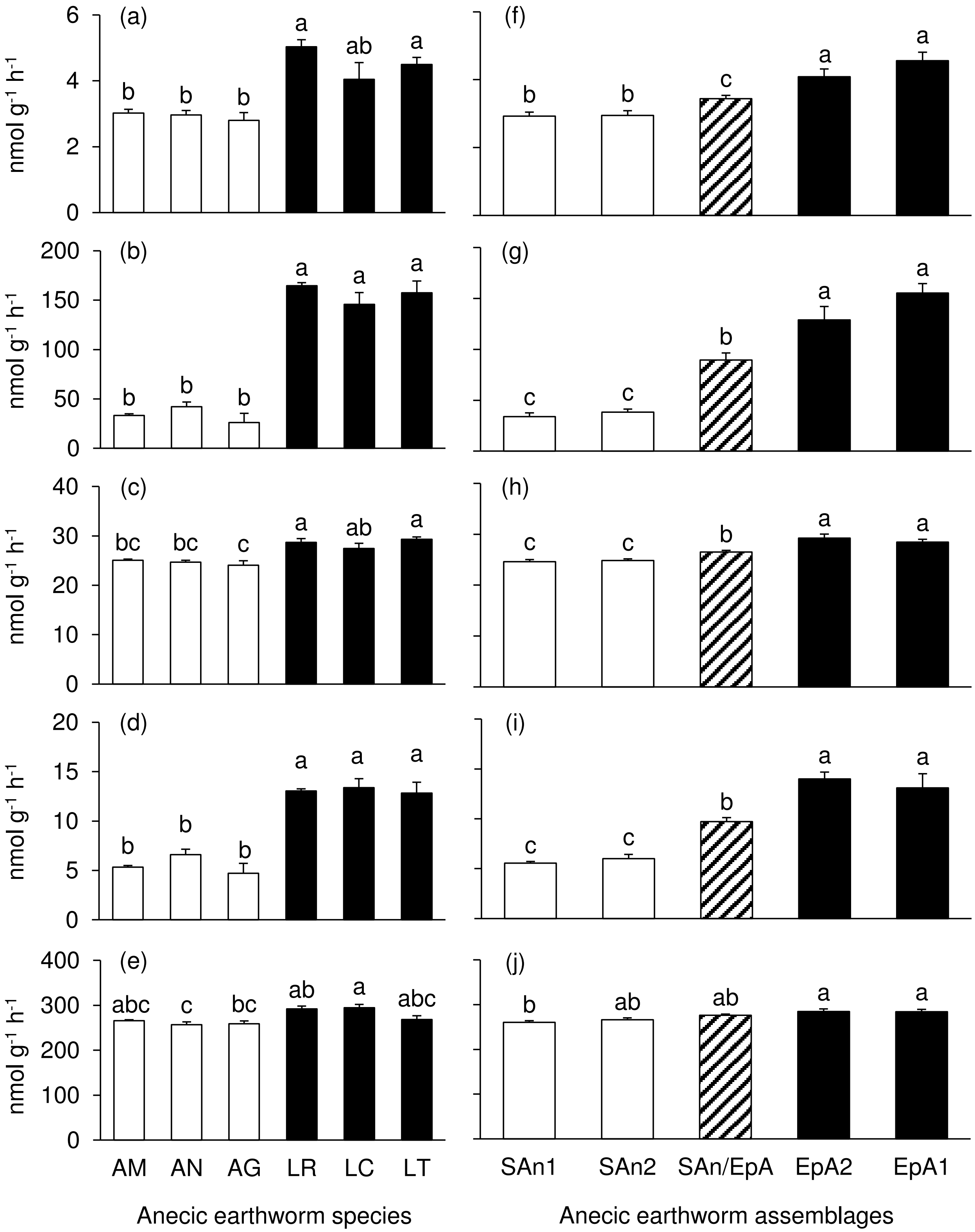
Compared to the bulk soil of the control, the five enzymatic activities were enhanced in soil microsites from mesocosms with mono-specific assemblages of epi-anecic earthworms ($F = 8.49$ to 93.46, $P < 0.001$, Supplementary Fig. S4). In soil microsites from mesocosms with mono-specific assemblages of strict-anecic earthworms however, only the β-D-glucosidase was enhanced by 50% compared to bulk soil ($F = 93.46$, $P < 0.001$, Supplementary Fig. S4b).

Except for the acid phosphatase, enzymatic activities were significantly higher in soil microsites from mesocosms with mono- and bi-specific assemblages of epi-anecic earthworms than in those of strict-anecic earthworms ($F = 19.02$ to 44.01, $P < 0.001$, Supplementary Table S2, Fig. 2f-j). These differences were higher for the β-D-glucosidase (+75%; Fig. 2g), leucine-aminopeptidase (+58%; Fig. 2i) and FDAse (+32%; Fig. 2f) than for the cellobiohydrolase activity (+14%; Fig. 2h).

Overall, the enzymatic activity levels in soil microsites from mesocosms with bi-specific assemblages of either strict- or epi-anecic earthworms were similar to those observed in the presence of their respective mono-specific assemblages (Supplementary Table S2, Fig. 2f-j). In the presence of mono-specific assemblages within each sub-category, the enzymatic activity levels were similar whatever the earthworm species considered (Fig. 2a-e). However, within these mono-specific assemblages, either strong differences or slight similarities were observed between strict- and epi-anecic pairs (Fig. 2a-e). For example, the β-D-glucosidase and leucine-aminopeptidase activities in the presence of LC were +77% and +59% higher than those observed in the presence of mono-specific assemblages of strict-anecic earthworms (Fig. 2b and d), respectively. Conversely, the FDAse and cellobiohydrolase activities in the presence of LC were similar to those observed in the presence of mono-specific assemblages of strict-anecic earthworms (Fig. 2a and c).

In the presence of bi-specific assemblages composed of one strict- and one epi-anecic earthworm, the enzymatic activity levels were generally intermediate between those observed in the respective mono-specific assemblages (Supplementary Table S2, Fig. 2f-j). Thus, the activities of FDAse, β-D-glucosidase, cellobiohydrolase and leucine-aminopeptidase in the presence of both strict- and epi-anecic earthworms were significantly higher than in the presence of strict-anecic and lower than in the presence of epi-anecic earthworms (Supplementary Table S2, Fig. 2f-j). In addition, some differences were observed between the earthworm pairs composed of one epi- and one strict-anecic earthworm. For example, the activities of the β-D-glucosidase, cellobiohydrolase and leucine-aminopeptidase in the presence of the AG/LT pair were significantly higher than those of the AG/LR pair ($F = 2.03$ to 3.09, $P < 0.02$, Supplementary Table S2).

Concerning the two soil microsites, the FDAse activity was significantly 16% higher in middens than in burrows ($F = 41.53$, $P < 0.001$, Supplementary Table S2, Fig. 3a), conversely to the acid phosphatase activity that was significantly 3% lower in middens than in burrows ($F = 5.19$, $P = 0.024$, Supplementary Table S2, Fig. 3e). The β-D-glucosidase and leucine-aminopeptidase activities were similar in the two soil microsites ($F = 0.38$ and 0.95, $P > 0.331$, Supplementary Table S2, Fig. 3b and d). With the exception of the cellobiohydrolase activity, most enzymatic activities did not differ between the microsites of mono- and bi-specific assemblages of either strict- or epi-anecic earthworms, (Fig. 3c). This enzymatic activity (cellobiohydrolase) was indeed higher in the middens obtained in the presence of the AG/LT pair than in both middens and burrows in the presence of the AG/LR and AG/LC pairs (significant EW pairs × Site interaction, $F = 1.79$, $P = 0.039$, Supplementary Table S2, Supplementary Fig. S5).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Anecic earthworm species
Anecic earthworm assemblages

Fig. 2. Enzymatic activities of FDAse (a, f), β-D-glucosidase (b, g), cellobiohydrolase (c, h), leucine-aminopeptidase (d, i) and acid phosphatase activities (e, j) after 30 days of experiment according to the 6 anecic earthworm species in mono-specific assemblages (a, b, c, d, e) and to the combinations of earthworm species within and between the two ecological sub-categories (f, g, h, i, j). Enzymatic activities were expressed in nmol of substrate consumed h^{-1} g^{-1} dry soil. Values are mean ± SD across burrows and middens. Different letters denote significant differences among earthworm assemblages with a > b > c (Tukey HSD test results). AM = *A. caliginosa meridionalis*; AN = *A. nocturna*; AG = *A. giardi*; LR = *L. rubellus rubellus*; LF = *L. festivus*; LC = *L. centralis*; LT = *L. terrestris*. n = 8 to 10 for each anecic earthworm species in monospecific assemblage (panels a, b, c, d and e). SAn1 = Mono-specific assemblages of strict-anecic, n=28; SAn2 = Bi-specific assemblages of strict-anecic, n=30; SAn/EpA = Bi-specific assemblages with one strict- and one epi-anecic, n=88; EpA2 = Bi-specific assemblages of epi-anecic, n=26; and EpA1 = Mono-specific assemblages of epi-anecic, n=30.

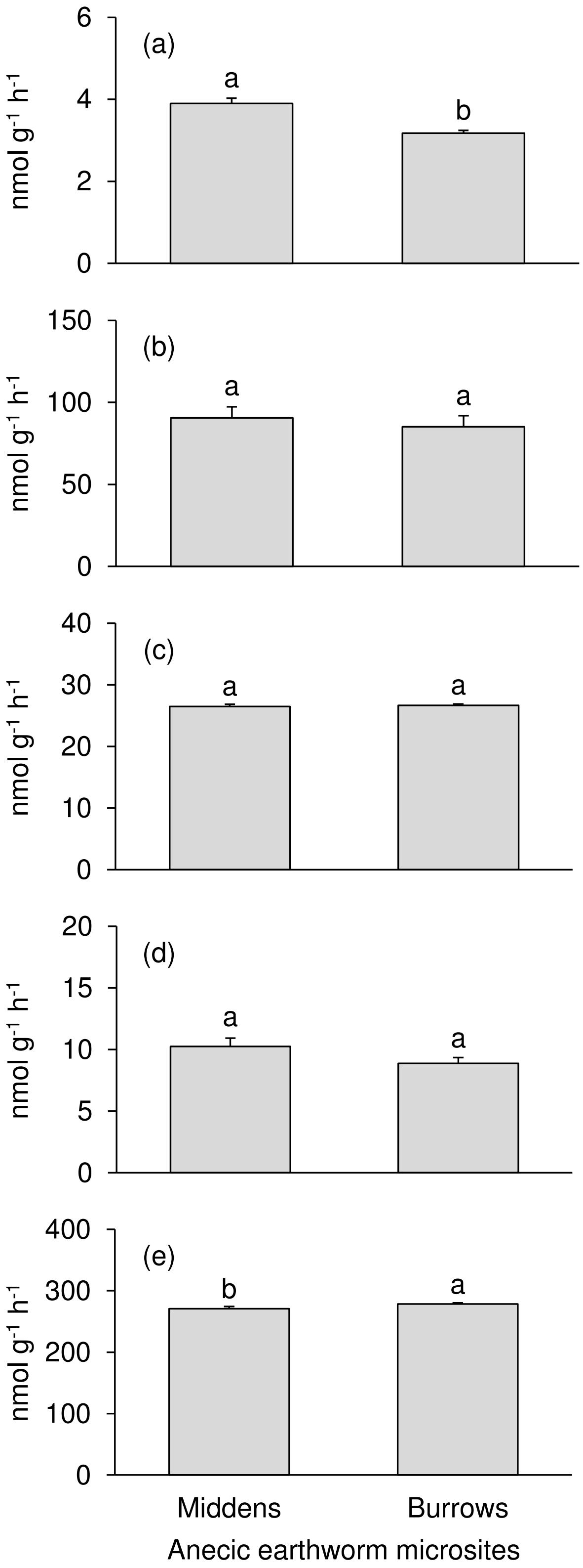

Fig. 3. Activities of (a) FDAse, (b) β-D-glucosidase, (c) cellobiohydrolase, (d) leucine-aminopeptidase and (e) acid phosphatase activities after 30 days of experiment in anecic microsites (middens and burrows). Enzymatic activities were expressed in nmol of substrate consumed h^{-1} g^{-1} dry soil. Values are mean ± SD; n = 101. Different letters denote significant differences among anecic microsites with a > b (Tukey HSD test results).

4. Discussion

4.1. Mono-specific assemblages

In this study, we observed that only epi-anecic earthworms contributed to surface litter mass loss of *L. perenne* and, as previously observed by Hoeffner et al. (2018), this contribution was strongly correlated to the initial earthworm biomass. The rate of surface litter mass loss in the presence of *L. terrestris* was 4.9 mg g^{-1} day^{-1} which is similar to previous studies that reported rates ranging from 2.4 (Binet and Trehen, 1992) to 10.4 mg g^{-1} day^{-1} (Curry and Bolger 1984). Moreover, in the present study, rate of litter mass loss with *L. centralis* was 5.3 mg g^{-1} day^{-1} confirming the first observation of Hoeffner et al. (2018). In the presence of *L. rubellus*, the rate of litter mass loss was 17.0 mg g^{-1} day^{-1}, which was slightly lower than the 23.1 mg g^{-1} day^{-1} observed by Xia et al. (2011) with *Liriodendron tulipifera* litter. Conversely, no significant contribution of the strict-anecic earthworms to the surface litter mass loss was observed after 30 days of experiment. Previous studies on the feeding behaviour of strict-anecic earthworms reported inconsistent results. Some of them highlighted rates of fresh litter mass loss varying from 7.1 mg g^{-1} day^{-1} with *A. giardi* fed *Triticum aestivum* (Cortez et al., 1989) to 16.3 mg g^{-1} day^{-1} with *A. caliginosa meridionalis* fed *Castanea sativa* (Cortez and Bouché, 2001). However, it is not clear if these studies took into account the litter mass loss resulting from microbial activity while their contribution can be substantial and should not be overlooked (Coûteaux et al., 1995). In the meantime and in line with our findings, Eisenhauer et al. (2008) observed that *Aporrectodea longa* (strict-anecic earthworm) did not contribute to surface litter mass loss and other studies, using isotopic markers, noticed that *A. longa* and *A. giardi* preferred to feed on humified SOM rather than on fresh surface litter (Cortez et al., 1989; Jégou et al., 1998, 2000; Andriuzzi et al., 2016; Larsen et al., 2016).

Such different behaviour between strict- and epi-anecic earthworms could induce differences in their interactions with soil microorganisms through a specific effect on microbial resources. Contrary to strict-anecic earthworms, epi-anecic earthworms, by contributing to surface litter mass loss, concentrate fresh leaf litter in their burrows (Jégou et al., 1998, 2000; Andriuzzi et al., 2016) and their gut contents were reported to be richer in litter than those of strict-anecic earthworms (Bouché and Kretzschmar, 1974; Piearce, 1978; Ferrière, 1980). Consequently, several studies reported enhanced contents of total C, N and P in the burrows (Parkin and Berry, 1999; Tiunov and Scheu, 2000; Hoang et al., 2017) and the middens of epi-anecic earthworms compared to the surrounding soil (Subler and Kirsch, 1998; Wilcox et al., 2002; Aira et al., 2009). The feeding activity of epi-anecic earthworms and the subsequent transfer of litter into the mineral soil is of key importance to enhance soil microbial activity (Binet et al., 1998; Subler and Kirsch, 1998; Zimmer et al., 2005; Hoang et al., 2016). Thus, in the present study, the five enzymatic activities measured were enhanced in the presence of epi-anecic earthworms. Several studies have also observed higher enzymatic activities in *L. terrestris* burrows or middens compared to the surrounding soil, including β-D-glucosidase (Don et al., 2008; Lipiec et al., 2016; Hoang et al., 2016; Athmann et al., 2017), leucine aminopeptidase (Athmann et al., 2017), acid and alkaline phosphatase (Jégou et al., 2001; Schrader and Seibel, 2001; Lipiec et al., 2016; Hoang et al., 2016) and cellobiohydrolase (Don et al., 2008; Hoang et al., 2016). However, for the first time, we report here that both *L. rubellus* and *L. centralis* also enhanced enzymatic activities in their burrows and middens. Interestingly, within the three epi-anecic earthworms studied, although the contribution of *L. terrestris* to litter mass loss was the biggest, corresponding to the heaviest species, it did not induce a higher increase of soil enzymatic activities compared to the smaller *L. rubellus* or *L. centralis*. This finding suggests that the stimulation of enzymatic activities by epi-anecic earthworms does not depend on earthworm individual biomass. In the present study, β-D-glucosidase (C cycle) and leucine-aminopeptidase (N cycle) were the most stimulated activities, which is in line with the presence of easily available compounds (cellulose or hemicellulose) in the *L. perenne* leaves and their low C:N ratio, respectively (Hoeffner et al., 2018).

Even if they did not contribute to surface litter mass loss, the strict-anecic earthworms stimulated the β-D-glucosidase activity. Jégou et al. (2001) also reported a specific enhancing effect of the presence of strict-anecic earthworms on the dehydrogenase activity in the burrows of *A. giardi* while the acid and alkaline phosphatase activities were not affected. Strict-anecic earthworms, by feeding mainly on SOM, might enrich their burrows and middens with their mucus, urine and cast deposits that should be concentrated in organic compounds as observed for *L. terrestris* (Needham, 1957; Laverack, 1963) and increase soil moisture. Thus, they might stimulate other enzymatic activities involved in the hydrolysis of more recalcitrant compounds, such as lignin or cutin which represent a significant part of plant litter input into the soil but were not measured in the present study (Gleixner et al., 2001). Moreover, the burrow network of strict-anecic earthworms is more expanded and broadened than that of epi-anecic earthworms which build only one or two main burrows (Jégou et al., 2000, 2001; Bastardie et al., 2005). This could dilute the organic matter inputs in strict-anecic earthworms burrows compared to those of epi-anecic earthworms, reducing the observed effects on soil microbial enzymatic activities.

4.2. Bi-specific assemblages

In contrast to our second hypothesis of synergistic effect between species, the litter mass loss in the presence of earthworms from different species within and between each anecic earthworm ecological sub-categories was simply additive. Similar results were observed for the enzymatic activities that were additive in pairwise interactions within each sub-categories while few exceptions were observed in some strict- and epi-anecic assemblages. For example, when *A. giardi* was paired with *L. terrestris*, the level of several enzymatic activities was higher than when it was paired with *L. rubellus*, suggesting that some specific earthworm combinations promote enzymatic activities. Such specific effects might be explained by the enhanced decomposition of relative recalcitrant organic compounds when easily decomposable organic matter is present due to mechanisms of facilitation or complementary resource used by diverse detritivores (Wardle et al., 1997; Hättenschwiler and Gasser, 2005).

According to the differences in the feeding behaviour observed between the two earthworm sub-categories, one could have expected an increase in surface litter mass loss induced by some commensal interactions. The epi-anecic earthworms, by actively burying fresh litter, should indeed provide SOM for the strict-anecic earthworms without being impaired. For example, Xia et al. (2011) previously observed a synergistic effect on surface litter mass loss in the presence of *L. rubellus* (epi-anecic earthworm) and *Octolasion lacteum* (endogeic earthworm), i.e. between two different earthworm ecological categories, with endogeic earthworms feeding on SOM. Based on the literature, synergistic effects on litter mass loss might be expected when organisms show high functional dissimilarity between their feeding behaviour (Heemsbergen et al., 2004; Zimmer et al., 2005; De Oliveira et al., 2010; Coulis et al., 2015). It seems that, in the present study, strict- and epi-anecic earthworms, even if they relied on different nutrient sources (fresh surface litter *vs.* SOM), should not be functionally dissimilar enough to synergistically interact. Thus, pairwise species interactions between the two sub-categories led mainly to additive effects on both surface litter mass loss and enzymatic activities. Moreover, as previously reported by Hoeffner et al. (2018), our study confirms that litter mass loss may in certain ecological contexts be predicted by epi-anecic earthworm biomass in the field.

4.3. Anecic earthworm microsites and enzymatic activities

Very few studies have analysed the variability of enzymatic activities within the drilosphere (Hoang et al., 2016; Athmann et al., 2017). Hoang et al. (2016) observed that within the network of burrows of *L. terrestris*, activities of the acid phosphatase, chitinase and β-D-glucosidase were more stimulated in hotspots and were more frequently stimulated than in the surrounding soil. However, to our knowledge, no studies compared these activities in the different soil microsites created by earthworms (i.e. middens and burrows) while they present differences in their physical, biological and chemical properties due to the way they are constructed (Nielsen and Hole, 1964). In this study, enzymatic activities were differently affected according to these two microsites created by earthworms. FDAse, which is an indicator of global soil activity, was significantly higher in anecic earthworm middens than in their burrows. Middens are a mixture of buried litter, casts, and mucus that together make them a hotspot of biological activity thus stimulated soil microorganisms (Subler and Kirsch, 1998; Schrader and Seibel, 2001).

The β-D-glucosidase and leucine-aminopeptidase activities that are involved in C and N cycles, respectively, showed a trend to higher values in the earthworm middens compared to the burrows, while the opposite was observed for the acid phosphatase activity. These results might reflect contrasting nutrient requirements for soil microorganisms in anecic earthworm burrows and middens. Enzymatic activities are indeed commonly considered as indicators of microbial nutrient demand and do not necessarily indicate a greater amount of available elements (Olander and Vitousek, 2000; Moorhead and Sinsabaugh, 2006; Sinsabaugh et al., 2008). Thus, by being composed of higher amount of cast compared to the burrow, middens should be characterized by lower C and N availabilities that enhance the enzymatic activities involved in C and N cycling. Moreover, earthworm casts are known to be enriched in P (Tiwari et al., 1989; Basker et al., 1993) derived from plant litter, that is released during the earthworm gut transit (Mansell et al., 1981) and should thus end up in middens, inhibiting the activity of enzymes involved in this element cycling. Scheu (1987) also observed that P was not limiting in casts from *A. caliginosa* (endogeic earthworm) while it was in burrow or in soil. This higher need of P for soil microorganisms in earthworm burrows should have enhanced the acid phosphatase activity in this soil microsite.

5. Conclusion

Many studies have observed that anecic earthworms contribute to litter burying and thus improve litter decomposition through microbial enhancement, but these studies did neither take into account earthworm species diversity within this anecic earthworm category nor their interactions. Here, we report that epi-anecic earthworms (*Lumbricus* sp.) contributed to surface litter mass loss and stimulated five soil enzymatic activities measured in both burrows and middens conversely to strict-anecic earthworms (*Aporrectodea* sp.). These results reflected differences in the behaviour of these two earthworm sub-categories, epi-anecic earthworms consuming essentially fresh plant litter and strict-anecic earthworms feeding mainly on humified SOM. Moreover, we report for the first time that pairwise species interactions within and between strict- and epi-anecic earthworms did not enhance surface litter mass loss and enzymatic activities. Independently of earthworm species, enzymatic activities were differently affected in earthworm middens and burrows, probably reflecting some different nutrient requirements. This study highlights that within anecic earthworms, strict- and epi-anecic earthworms have different impact on litter decomposition and confirms the functional differentiation of these ecological sub-categories.

Acknowledgements

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SUPPORTING INFORMATION

Table S1. Effects of earthworm assemblages, earthworm pairs and blocks on litter mass loss. *F*-values and associated *P*-values are indicated.

	d.f	*F*-value	*P*-value
EC	4	97.55	<0.001
EW species	16	9.94	<0.001
Rep	3	0.79	0.503
Residuals	77		

Table S2. Effects of earthworm assemblages (EA), earthworm pairs (EP pairs), microsites (Site) and blocks on FDAse (FDA), β-D-glucosidase (GLU), cellobiohydrolase (CEL), leucine-aminopeptidase (LAP) and acid phosphatase (PHO) activities. *F*-values and associated *P*-values are indicated.

		FDA		GLU		CEL		LAP		PHO	
	d.f	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
EA	4	26.99	<0.001	44.01	<0.001	19.02	<0.001	22.24	<0.001	4.90	<0.001
EP	16	1.35	0.173	2.15	0.009	2.03	0.016	3.09	<0.001	0.91	0.554
Site	1	41.53	<0.001	0.95	0.331	0.01	0.928	0.38	0.541	5.19	0.024
Block	3	23.84	<0.001	2.55	0.057	23.36	<0.001	5.91	<0.001	5.96	<0.001
EA x Site	4	1.11	0.660	0.64	0.633	2.31	0.061	1.07	0.373	0.53	0.712
EP x Site	16	1.24	0.542	1.68	0.055	1.79	0.039	1.02	0.438	0.39	0.983
Residuals	161										

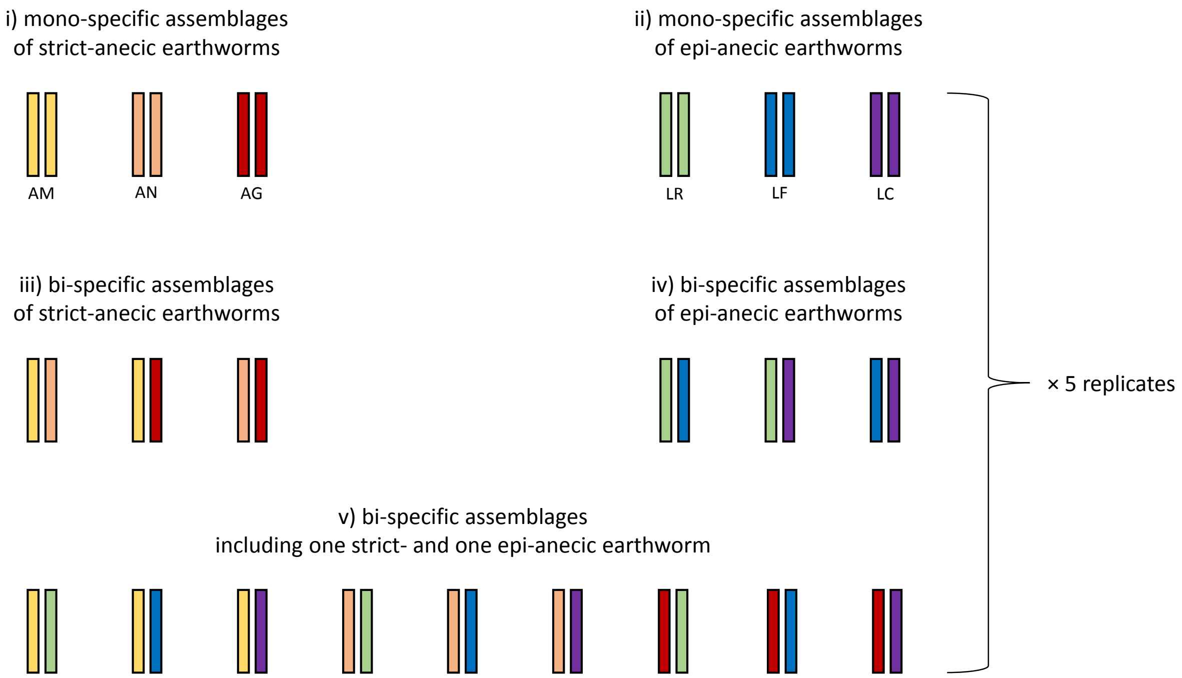

Fig. S1. Graphical illustration of the 21 pairwise combinations of two earthworm individuals within and between the two anecic ecological sub-categories. AM = *Aporrectodea caliginosa meridionalis*; AN = *Aporrectodea nocturna*; AG = *Aporrectodea giardi*; LR = *L. rubellus*; LC = *L. centralis*; LT = *L. terrestris*.

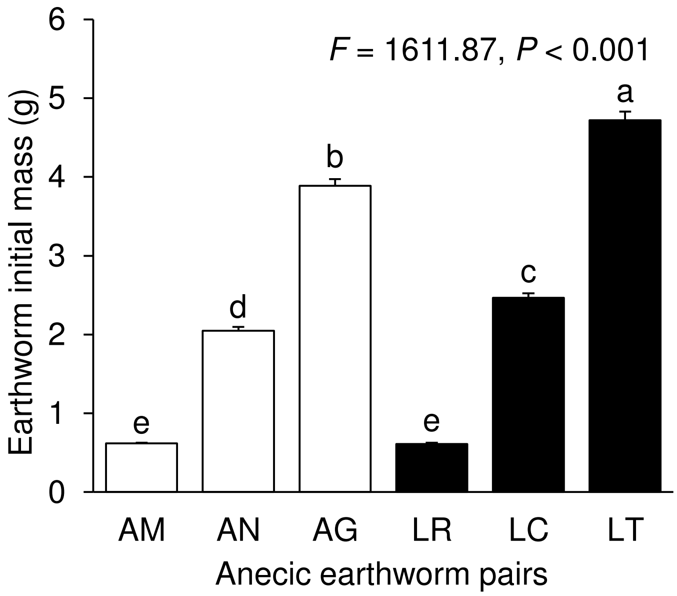

Fig. S2. Initial fresh mass of the six anecic earthworm species. Values are mean ± SD; n = 35. Different letters denote significant differences among earthworm species with a > b > c > d > e (post hoc Tukey test results). AM = *Aporrectodea caliginosa meridionalis*; AN = *Aporrectodea nocturna*; AG = *Aporrectodea giardi*; LR = *L. rubellus*; LC = *L. centralis*; LT = *L. terrestris*.

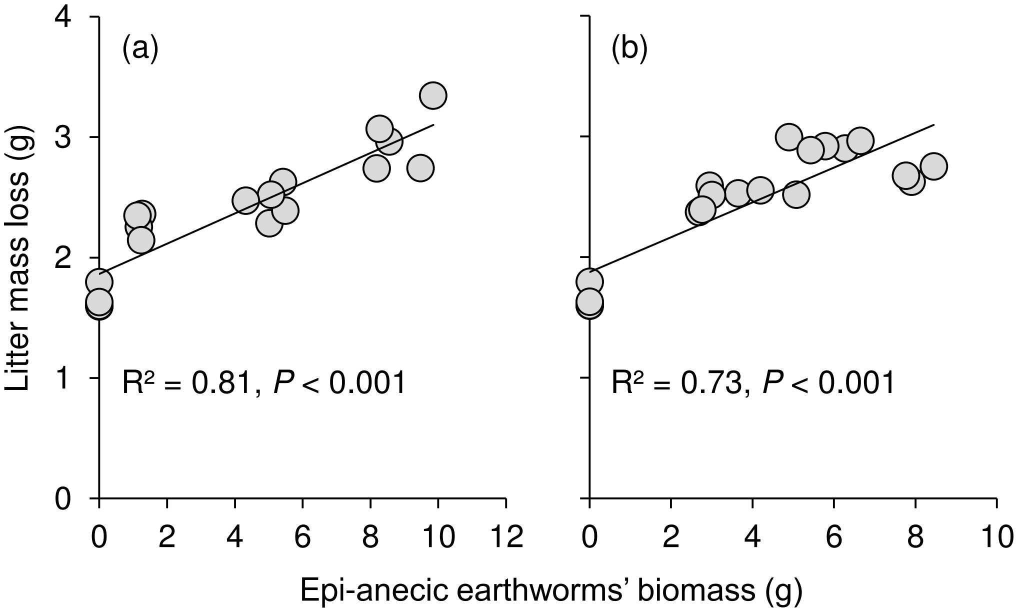

Fig. S3 Relationships between litter mass loss and epi-anecic earthworms' initial biomass according to (a) mono specific assemblages and (b) bi-specific assemblages. Adjusted R² and associated *P*-values of the linear regressions are indicated.

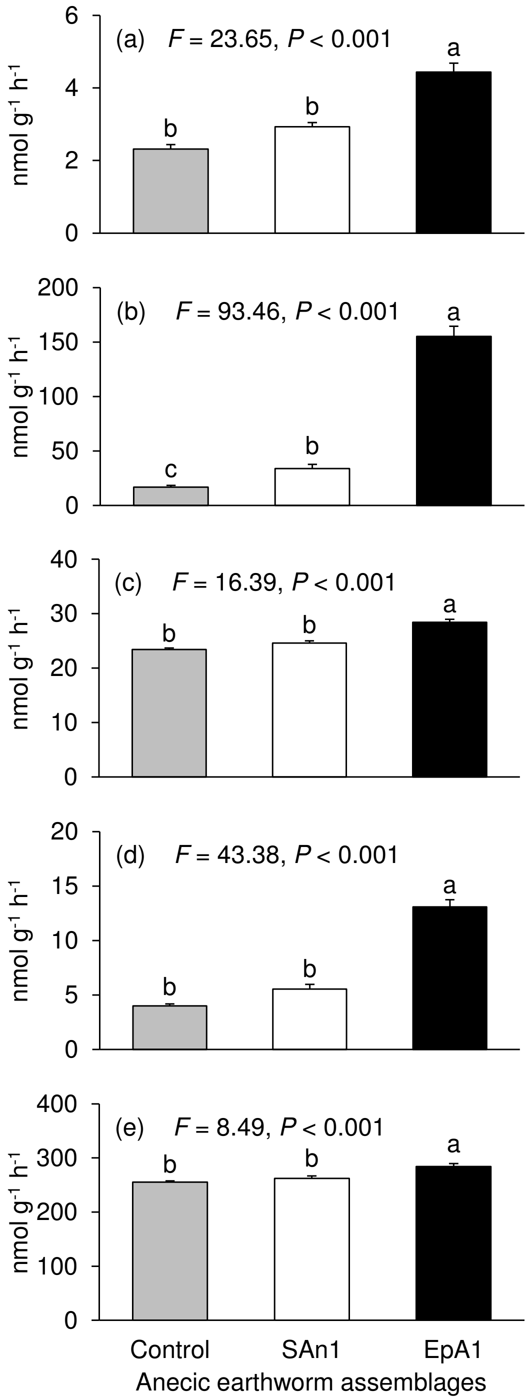

Fig. S4. Effect of mono-specific earthworm assemblages on enzymatic activities: FDAse (a), β-D-glucosidase (b), cellobiohydrolase (c), leucine-aminopeptidase (d) and acid phosphatase (e). Enzymatic activities were expressed in nmol of substrate consumed h^{-1} g^{-1} dry soil. Values are mean ± standard error; n = 5 to 30. Different letters denote significant differences among earthworm categories with a > b (Tukey HSD test). Control = without earthworm; SAn1 = Mono-specific assemblages of strict-anecic; EpA1 = Mono-specific assemblages of epi-anecic.

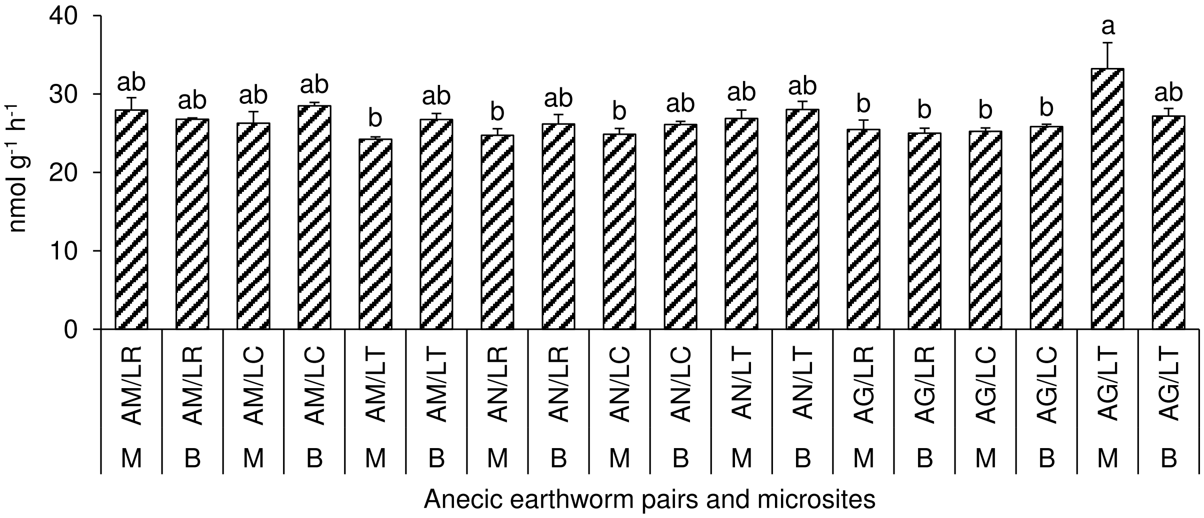

Fig. S5. Effects of bi-specific earthworm assemblages including one strict- and one epi-anecic earthworm on cellobiohydrolase activity in both middens and burrows. Values are mean ± standard error; n = 4 to 5. Different letters denote significant differences among earthworm pairs and microsites with a > b (Tukey HSD test results). AM = *A. caliginosa meridionalis*; AN = *A. nocturna*; AG = *A. giardi*; LR = *L. rubellus rubellus*; LF = *L. festivus*; LC = *L. centralis*; LT = *L. terrestris* and M = Middens, B = Burrows.

CHAPITRE 5

Réponse de la biomasse individuelle des lombriciens anéciques à l'interaction entre deux espèces

Le cinquième chapitre correspond à une courte communication en révision dans la revue internationale Applied Soil Ecology.

Objectifs de l'étude

Le but de cette expérimentation était d'évaluer l'impact des interactions entre des espèces lombriciennes anéciques occupant des niches écologiques similaires ou différentes sur le changement de leur biomasse individuelle. La biomasse étant un proxy de l'état de santé générale, impactant la reproduction des lombriciens.

Méthodologie

Nous avons mené une expérimentation en conditions contrôlées au laboratoire et déterminé les effets d'interactions entre espèce en utilisant trois espèces lombriciennes anéciques (*Aporrectodea caliginosa meridionalis*, *Aporrectodea nocturna* et *Aporrectodea giardi*) et trois espèces épi-anéciques (*Lumbricus rubellus rubellus*, *Lumbricus centralis* et *Lumbricus terrestris*) assemblées au sein de mésocosmes de sol selon toutes les combinaisons possibles de deux individus (6 paires mono- et 15 paires bispécifiques). Après 30 jours d'expérimentation, nous avons mesuré le changement de biomasse de chaque individu.

Principaux résultats

Nos résultats indiquent qu'indépendamment des interactions entre espèces, les lombriciens anéciques stricts maintiennent leur biomasse initiale pendant les 30 jours d'incubation. En revanche, les lombriciens épi-anéciques gagnent du poids avec une augmentation moyenne de 12 % et de 24 % de leur biomasse individuelle dans les assemblages monospécifiques et bispécifiques respectivement. Ainsi, pour la première fois, notre étude met en évidence un effet synergique des interactions entre espèces lombriciennes épi-anéciques sur leur changement de masse.

CHAPITRE 5

Response of anecic earthworm individual biomass to species interactions

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Courte communication en révision dans la revue internationale Applied Soil Ecology

Keywords:

Temperate grasslands, Lumbricidae, ecological category, *Aporrectodea*, *Lumbricus*

Abstract

Because earthworms contribute to a wide range of ecosystem services provided by the soil, it seems necessary to understand the assembly rules of earthworms, nevertheless synecology of these organisms is still not properly elucidated especially in terms of species interactions. The aim of the present study was to determine the effects of anecic earthworm species interactions on individual biomass. These effects were measured using three strict-anecic and three epi-anecic earthworm species in all possible pairwise combinations. Treatments were maintained in mesocosms for 30 days and individual change in biomass measured. Monospecific or bispecific assemblages of strict-anecic earthworms maintained their initial biomass. In contrast, epi-anecic earthworms exhibited a 12 and 24% increase in biomass in monospecific and bispecific assemblages, respectively. Bispecific assemblages combining one strict- and one epi-anecic earthworm showed a 7% increase of biomass that was solely due to the biomass gained by the epi-anecic earthworms. Surprisingly, the biomass' changes were not homogenous within the two sub-categories as the six earthworm species exhibited species-specific responses. Generally, *Aporrectodea giardi* (strict-anecic species) mainly lost biomass while, *Lumbricus terrestris* and *Lumbricus rubellus* (epi-anecic species) mainly gained biomass in monospecific assemblages or in combination with other species. Finally, in contrast with our expectations, the greatest increases in individual biomass were recorded in the bi-specific epi-anecic assemblages. Then, this study provides further evidence for the distinction between the two anecic sub-categories, as findings indicate that species interactions can be positive only for epi-anecic earthworm biomass.

1. Introduction

Earthworms are widespread organisms and constitute the most important animal biomass in soil under temperate climates (Hole, 1981; Lavelle, 1983; Curry, 1994; Bar-On et al., 2018). According to their physiology, morphology and behaviour, earthworms are classified into three ecological categories: epigeic, endogeic and anecic species (Bouché, 1972, 1977). Nevertheless, several studies highlighted that within anecic earthworms two ecological sub-categories can be identified based mainly on their feeding (Ferrière, 1980; Andriuzzi et al., 2016; Larsen et al., 2016) and burrowing (Jégou et al., 1998, 2001; Bastardie et al., 2003, 2005) behaviours. Briefly, epi-anecic earthworms feed preferentially on surface organic matter, mainly composed of leaf litter, which they can bury into their main permanent burrows forming middens at the entrance to their burrows (Nielsen and Hole, 1964). In contrast, strict-anecic earthworms feed on humified organic matter already incorporated into the soil with a slight proportion of leaf litter and they establish a denser burrow network than epi-anecic earthworms. Consequently, ecosystem services provided by earthworms belonging to these two anecic sub-categories are expected to be different.

In this context, improving our understanding of the assembly rules of anecic earthworm communities is important since (i) from one to 6 different anecic earthworm species can coexist in the same soil (Poier and Richter, 1992; Boag et al., 1997) and (ii) inoculations of anecic earthworms are frequently realised in order to enhance soil functions (Vimmerstedt and Finney, 1973; Hoogerkamp et al., 1983; Brun et al., 1987; Butt, 2008; Forey et al., 2018). The development of anecic earthworms is known to be sensitive to different environmental factors including climate (Butt, 1991; Daniel and Anderson, 1992; Butt, 1993), soil characteristics (Bouché, 1972; Lee, 1985) and the identity of the plant litter (Michon, 1954; Shipitalo et al., 1988; Daniel, 1991). Previous studies also reported that interactions within anecic earthworms could either delay or increase their growth rate depending on the degree of niche overlap between species (Butt, 1998;

Lowe and Butt, 1999, 2002a, 2002b; Uvarov, 2009). For example, after 24 weeks of experiment, the growth rate of *Lumbricus terrestris* (epi-anecic earthworm) was negatively impacted in the presence of *Lumbricus rubellus* (epi-anecic earthworm) whereas the growth rate of *L. rubellus* was either not impacted or enhanced in presence of *L. terrestris* (Butt, 1998; Lowe and Butt, 2002a, 2002b). However, our knowledge about these interactions within and between epi- and strict-anecic earthworms is still limited.

The present study aimed to determine the effects of anecic earthworm interactions on individual biomass as a specific proxy of earthworm fitness (Michon, 1954; Butt, 1991; Butt et al., 1994). Three strict-anecic earthworm species and three epi-anecic earthworm species were used in all possible pairwise combinations (6 monospecific and 15 bispecific pairs). We measured individual biomass change after 30 days of experimentation with leaf litter placed on the soil surface in order to mimic natural leaf litter deposition. First, we hypothesized an antagonism effect on biomass change in species mixture within each ecological sub-category due to resource competition. Second, we hypothesized no effect on biomass change when combining one epi- and one strict-anecic species due to reduced niche overlap.

2. Materials and methods

Soil (5-20 cm depth), fresh leaf litter of *Lolium perenne* (a typical grassland species), and the six anecic earthworm species were collected from temporary grasslands in the Long Term Ecological Research (LTER) site ‘‘Zone Atelier Armorique’’ (48°50’ N, -1°58’ W), Brittany, France. Soil and leaf litter characteristics were determined according to the protocols described in Hoeffner et al. (2018). The soil was identified as a brown soil with 48.2% sand, 37.5% silt and 14.3% clay, characterized by 2.9 % organic matter, a C:N ratio of 9.7 and a pH of 6.4. The leaf litter was characterized by a C:N ratio of 13.9, a phenolic concentration of 3.4%, a water holding capacity of 392.6% and a specific leaf area of 330.2 $cm^2.g^{-1}$. Three strict-anecic earthworm species, *Aporrectodea caliginosa meridionalis* (Bouché, 1972; hereafter called AM), *Aporrectodea nocturna* (Evans, 1946; hereafter called AN), *Aporrectodea giardi* (Savigny, 1826; hereafter called AG) and three epi-anecic earthworm species, *Lumbricus rubellus rubellus* (Hoffmeister, 1843; hereafter called LR), *Lumbricus centralis* (Bouché, 1972; hereafter called LC), *Lumbricus terrestris,* (Linné, 1758; hereafter called LT) were studied. Two weeks before the beginning of the experiment, adult earthworms from the six species were selected, grouped in monospecific boxes containing the soil hand-sieved at 4-mm and fed with air-dried leaves of *L. perenne*.

Thirty-six hours before the start of the experiment, each earthworm was placed on a moist sponge in a plastic box to void its gut content (depuration). From the six anecic earthworm species, 21 pairs of individuals were established in five replicates following 5 assemblages (Fig. S1): monospecific pairwise assemblages of strict- and epi-anecic earthworms (2 × 3 treatments), bispecific pairwise assemblages within strict- and within epi-anecic earthworms (2 × 3 treatments) and bispecific pairwise assemblages with one strict- and one epi-anecic earthworm (3 × 3 treatments).

Mesocosms (PVC cylinder, 30 cm high, 10 cm diameter) were filled with 4.9 kg of fresh sieved soil and two adults (i.e. presence of a turgid clitellum, to ensure taxonomic identity) of each pair of earthworm individuals had biomass determined and placed in the corresponding mesocosm. The six earthworm species presented an initial mean biomass gradient varying from 0.62 g for LR to 4.72 g for LT (Fig. S2). Then, 3.5 g of air-dried leaves of *L. perenne* were placed on the soil surface in order to mimic the natural leaf litter deposition and re-humidified with deionized water. The mesocosms were placed in a climatic chamber at 12 °C, with a relative humidity of 85% and a 12h: 12h light: dark regime. Soil moisture was maintained by spraying deionized water on the soil surface twice per week. Given the large number of mesocosms, individual replicates of each treatment were established one day apart leading to 5 blocks of 22 mesocosms.

During the experiment, only 4 earthworms died of the 105 used. Whatever the treatment, the surviving earthworms remained adults (with a turgid clitellum) until the end of the experiment. After 30 days, leaf litter was still available at the soil surface and remaining earthworm individuals were collected by destructively sampling mesocosms. Each individual earthworm biomass was determined after depuration, as previously described. Earthworm individuals' biomass change was determined following the formula: (final fresh biomass – initial fresh biomass) / initial fresh biomass × 100%. For monospecific assemblages, the biomass of the lightest earthworm at the end of the experiment was assigned to the biomass of the lightest at the beginning of the experiment.

Statistical analyses were performed with the R software 3.2.3 (R. Core Team, 2017). Significance was evaluated in all cases at $P < 0.05$. Data met the conditions of normality and homoscedasticity. Soil mesocosms that contained dead earthworms were excluded from the data analysis. First, we used separated two-way ANOVAs, followed by Tukey HSD tests for post hoc pairwise comparisons, to assess differences in (i) initial biomass between the six earthworm species and (ii) changes in earthworm biomass between the different pairwise assemblages. Second, we used separated one-sample Student's *t*-tests to test whether (i) earthworm species and (ii) earthworm assemblages significantly lost or gained biomass during the experiment.

3. Results

Overall, strict anecic earthworms in mono- and bispecific pairwise assemblages within their sub-category maintained their initial biomass during the experiment (Fig. 1). In contrast, the biomass of epi-anecic earthworms in mono- and bispecific pairwise assemblages within their sub-category increased (Fig. 1), and this increase was two times higher in bispecific compared to monospecific assemblages (+23.7% *vs.* +12.4%, respectively, Fig. 1). Finally, the biomass of earthworms in bispecific assemblages combining one epi- and one strict-anecic was 6.9% higher than at the beginning of the experiment (Fig. 1), but this increase was solely due to the biomass gained by epi-anecic earthworms (+17.0%, Fig. S3).

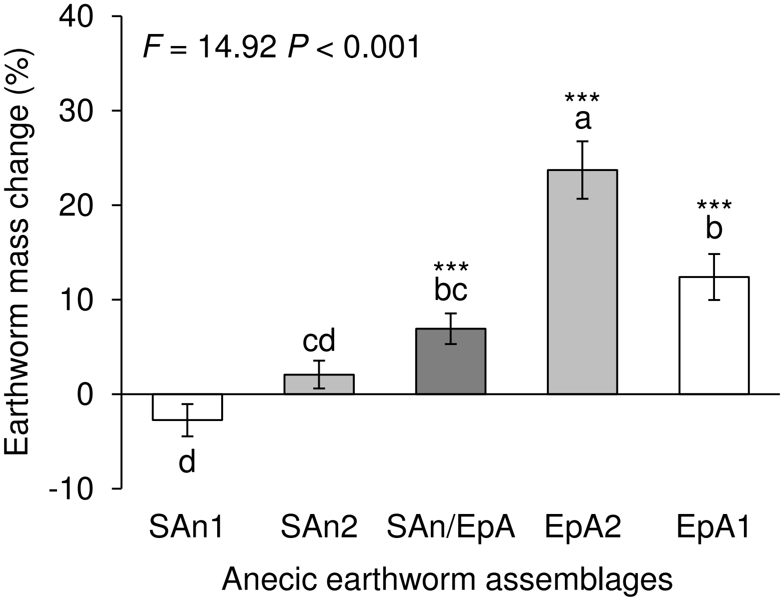

Fig. 1. Earthworm biomass changes according to anecic earthworm assemblages. Values are means ± SD. Different letters denote significant differences among earthworm assemblages with a>b>c>d (post hoc Tukey test results). Biomass changes significantly different from 0 are indicated with the respective symbols *** for $P < 0.001$. SAn1 = Monospecific assemblages of strict-anecic, n= 30; SAn2 = Bispecific assemblages of strict-anecic, n= 28; SAn/EpA = Bispecific assemblages with one strict- and one epi-anecic, n= 86; EpA1 = Monospecific assemblages of epi-anecic, n= 30; and EpA2 = Bispecific assemblages of epi-anecic, n= 28.

The biomass' changes were not homogenous within the two anecic sub-categories as the six earthworm species exhibited species-specific responses (Fig. 2). Contrary to the two other strict-anecic species, AG lost biomass in monospecific assemblage (-9.2%) as well as in bispecific assemblages with AN (-7.3%), LR (-7.5%) and LT (-13.6%, Fig. 2c). While the biomass of AM and AN did not vary significantly in both mono- and bispecific assemblages with an epi-anecic species, these two strict-anecic species gained biomass in presence of AG (+6.8% and +9.0% for AM and AN, respectively, Fig. 2a and b). Within the epi-anecic species, the biomass of LC only increased in presence of AM (Fig. 2e), while LT gained biomass whatever the pairwise assemblage considered (Fig. 2f). Finally, the biomass of LR increased in monospecific assemblage (+12.0%) as well as in bispecific assemblages with LC (+29.7), AN (+21.2%) and AM (+12.3%, Fig. 2d).

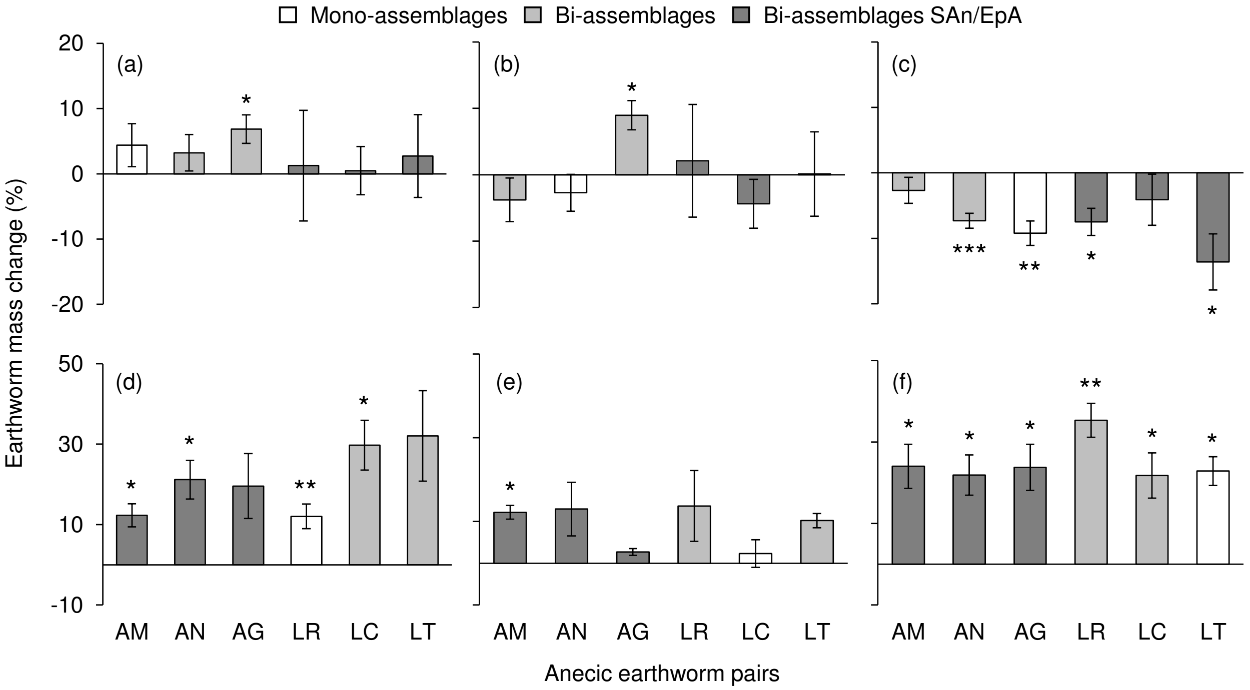

Fig. 2. Biomass changes of earthworms in mono- and bi-specific pairs of (a) AM, (b) AN, (c) AG, (d) LR, (e) LC and (f) LT. Values are means ± SD, n = 4 to 10. Biomass changes significantly different from 0 are indicated with the respective symbols * for $P < 0.05$, ** for $P < 0.01$, *** for $P < 0.001$. AM = *A. caliginosa meridionalis*; AN = *A. nocturna*; AG = *A. giardi*; LR = *L. rubellus rubellus*; LC = *L. centralis*; LT = *L. terrestris*.

4. Discussion

In contrast i) to our first hypothesis of an antagonist effect of species interaction on biomass change in pairwise assemblages within each anecic ecological sub-category and ii) to previous laboratory observations (Butt, 1998; Lowe and Butt, 1999, 2002b; Frazão et al., 2018), we observed no effect in bispecific assemblages of strict-anecic earthworm and a synergistic effect in those of epi-anecic earthworms. However, the organic resources and the experimental time used in previous studies differed to those applied in the present study (i.e. *Lolium perenne* and 30 days), as Lowe and Butt (1999) used cattle manure (separated cattle solids) during 84 days and Frazão et al. (2018) a mixture of *Triticum aestivum* and *Raphanus sativus* during 61 days. Postma-Blaauw et al. (2006) observed that the assemblage of *L. rubellus* with *L. terrestris* fed with *Solanum tuberosum* enhanced the bacterial biomass in their burrows. In addition, Hoeffner et al. (2018) observed specific fungal communities within *L. rubellus*, *L. centralis* and *L. terrestris* burrows fed with *L. perenne*. These previous observations of positive effects of epi-anecic species on microbial communities could lead to enhanced leaf litter decomposition and then to higher organic matter assimilation by epi-anecic earthworms.

Overall, the effect of the interactions between one strict- and one epi-anecic earthworm was mainly additive confirming our second hypothesis of reduced niche overlap. Nevertheless, we observed some synergistic or antagonistic effects depending on the earthworm assemblages. It may be assumed that other mechanisms could explain this different biomass change requiring additional experiments.

Within strict-anecic earthworms, *A. caliginosa meridionalis* and *A. nocturna* in monospecific assemblages maintained their initial biomass while *A. giardi* lost biomass. These findings are in agreement with previous studies that also reported no change in *A. caliginosa meridionalis* biomass after 31 days when fed with *Castanea sativa* (Cortez and Bouché, 2001) and a 38% loss of biomass of *A. giardi* after 31 days when fed with *Triticum aestivum* (Cortez et al., 1989). Overall, epi-anecic earthworms in monospecific assemblages gain biomass (Shipitalo et al., 1988; Binet and Trehen, 1992; Hoeffner et al., 2018). For example, Shipitalo et al. (1988) reported respectively a 100% and a 35% increase in biomass of *L. rubellus* and *L. terrestris* when fed with *Medicago sativa* litter after 32 and 36 days of experimentation, respectively. Thus, the present study confirms the two distinct feeding behaviours of strict- and epi-anecic earthworms and provides support to this sub-category distinction. In fact, feeding on leaf litter at the surface by epi-anecic earthworms (Andriuzzi et al., 2016; Larsen et al., 2016; Hoeffner et al., 2018) allowed them to increase their biomass with our experimental conditions. In contrast, strict-anecic earthworms by feeding mainly on soil organic matter had restricted access to organic matter (2.9% of soil content). Additionally, we cannot also exclude that the 1571 cm^{-3} soil volume in the experimental mesocosm restricted their soil organic matter consumption.

5. Conclusion

Because of their impact on soil functioning, great consideration is given to understand the assembly rules of earthworms, which are commonly explained by soil characteristics, land use and management. In addition to these abiotic factors, biotic parameters such as species interactions are also known to significantly govern the assembly rules of earthworms. In temperate grasslands' context where leaf litter fall naturally on the soil surface, we observed a synergistic effect of the epi-anecic earthworm species interactions on their biomass change leading to a gain of biomass. In addition, this study contributes to highlight the distinction between the two anecic sub-categories, as our findings indicate that species interactions can be very positive only for epi-anecic earthworm fitness.

Acknowledgements

We thank the Institut National de la Recherche Agronomique (Arras, France) for mineral soil analyses. We also thank Clément De Graaf, Clément Bertran, Albin Fertil, Alan Schneider and Anaïs Rancon for their technical assistance in the laboratory. Kevin Hoeffner was supported by a PhD grant from the French Ministère de la Recherche.

SUPPORTING INFORMATION

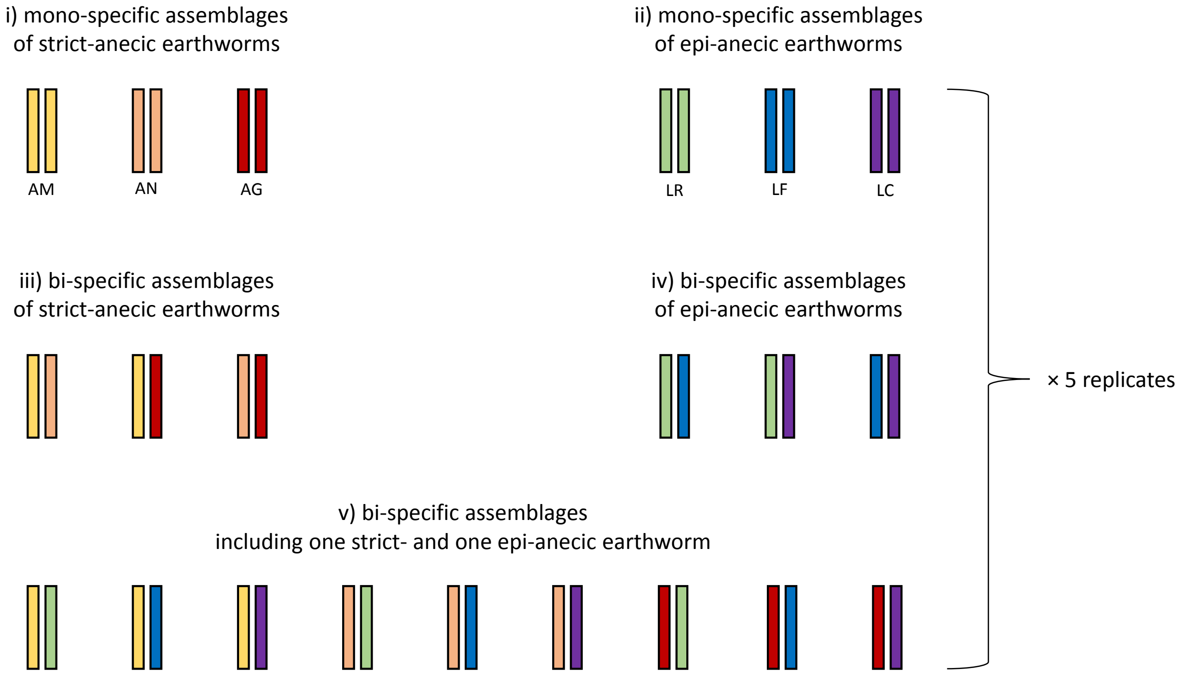

Fig. S1. Graphical illustration of the 21 pairwise combinations of two earthworm individuals within and between the two anecic ecological sub-categories. AM = *Aporrectodea caliginosa meridionalis*; AN = *Aporrectodea nocturna*; AG = *Aporrectodea giardi*; LR = *L. rubellus*; LC = *L. centralis*; LT = *L. terrestris*.

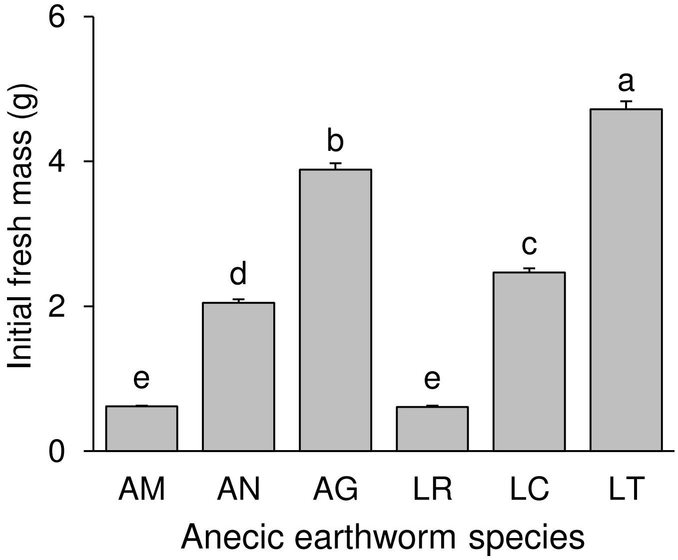

Fig. S2. Initial fresh biomass of the six anecic earthworm species at the beginning of the experiment. Values are means ± SD. Different letters denote significant differences among earthworm initial biomass with a>b>c>d>e (post hoc Tukey test results). AM = *A. caliginosa meridionalis*; AN = *A. nocturna*; AG = *A. giardi*; LR = *L. rubellus rubellus*; LC = *L. centralis*; LT = *L. terrestris*.

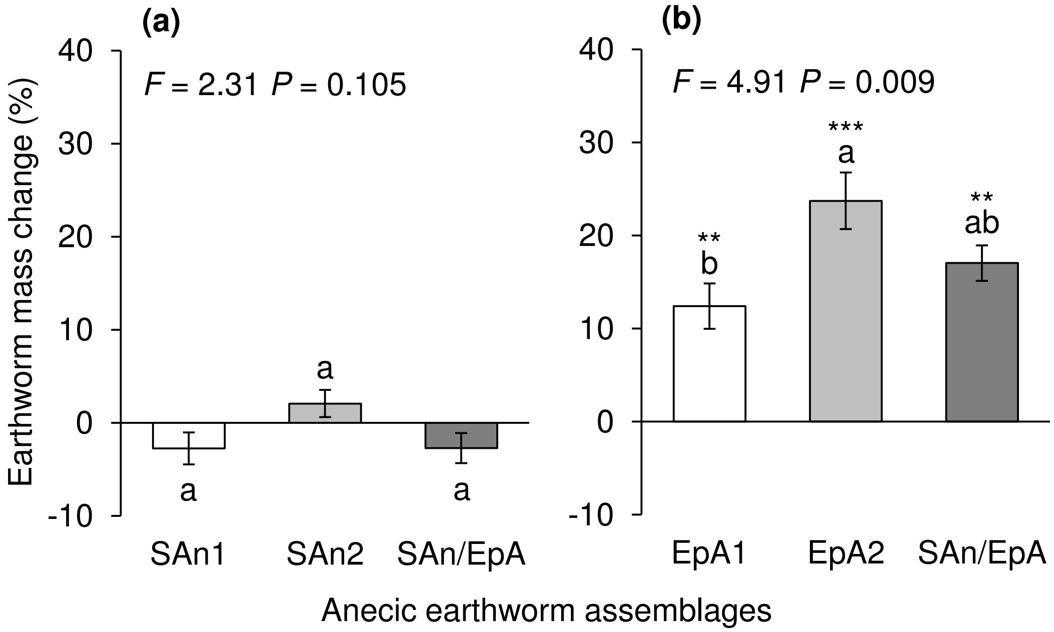

Fig. S3. Earthworm biomass changes according to anecic earthworm assemblages of (a) strict- and (b) epi-anecic earthworm. Values are means ± SD. Different letters denote significant differences among earthworm assemblages with a>b (post hoc Tukey test results). Biomass changes significantly different from 0 are indicated with the respective symbols ** for $P < 0.01$, and *** for $P < 0.001$. SAn1 = Monospecific assemblages of strict-anecic, n= 30; SAn2 = Bispecific assemblages of strict-anecic, n= 28; SAn/EpA = Bispecific assemblages with one strict- and one epi-anecic, n= 43; EpA1 = Monospecific assemblages of epi-anecic, n= 30; and EpA2 = Bispecific assemblages of epi-anecic, n= 28.

DISCUSSION ET PERSPECTIVES

A. Synthèse des résultats

Dans le premier chapitre de ce manuscrit de thèse, nous avons montré que l'assemblage des communautés lombriciennes en sols prairiaux était régi par les propriétés du sol (MO, pH et texture), les pratiques de gestion des prairies (age des prairies, chargement en bétail et fertilisation) et la diversité du paysage environnant. Nous avons également mis en évidence que l'effet de ces filtres environnementaux dépendait des catégories écologiques lombriciennes. Par exemple, contrairement aux anéciques stricts, l'abondance des lombriciens épi-anéciques était diminuée avec l'augmentation du chargement en bétail et leur richesse spécifique était stimulée avec une augmentation de la diversité du paysage environnant. La synthèse bibliographique présentée dans le chapitre 2 a confirmé qu'au sein des lombriciens anéciques, des différences de comportements existaient, notamment liées à leur alimentation avec des conséquences significatives sur les dynamiques du carbone et de l'azote dans les sols. Les épi-anéciques consomment principalement des litières fraichement tombées au sol et les anéciques stricts consomment principalement la matière organique humifiée du sol. Dans le troisième chapitre, nous avons démontré que les communautés fongiques des galeries étaient différemment impactées selon le type de litière présente et l'identité des espèces lombriciennes épi-anéciques. Les communautés bactériennes, quant à elles, étaient toujours stimulées quelles que soient le type de litière et les espèces épi-anéciques. Le taux de décomposition des litières par les espèces lombriciennes augmentait avec le poids initial des lombriciens épi-anéciques. Dans le quatrième chapitre, nous avons observé que, parmi les lombriciens anéciques, seules les espèces lombriciennes épi-anéciques participaient à la décomposition de la litière en surface du sol, notamment au travers de la stimulation des activités enzymatiques microbiennes du sol. De plus, nous avons montré que la décomposition des litières et la stimulation des activités enzymatiques n'étaient pas affectées par l'interaction entre les espèces lombriciennes au sein ou entre épi-anéciques et anéciques stricts (effet additif). Enfin, nous avons démontré dans les troisième et cinquième chapitres, que dans la durée de nos expérimentations, l'assimilation des litières et donc le gain de biomasse étaient limités aux lombriciens épi-anéciques. Cette assimilation était plus importante en présence de litière de bonne qualité (*i.e.* faible C/N et pauvre en composés phénoliques) et stimulée par la présence d'une autre espèce épi-anécique (commensalisme voir mutualisme).

B. Importance des sous-catégories écologiques lombriciennes

Au cours de nos observations sur le terrain, de la synthèse bibliographique et des différentes expérimentations menées, nous avons confirmé la distinction entre les deux sous-catégories écologiques au sein des anéciques selon leurs règles d'assemblage en sols prairiaux et leurs comportements alimentaires (chapitres 1, 2, 3, 4 et 5). En parallèle, une autre expérimentation réalisée en collaboration sur les mêmes prairies que celle du chapitre 1 avait pour objectif d'étudier les facteurs déclenchant le Home-Field Advantage[1] lors de la décomposition des litières (litterbags). Cette expérimentation a permis de mettre en évidence, que l'augmentation de la biomasse des lombriciens épi-anéciques augmentait la perte de masse des litières de prairies contrairement aux autres catégories écologiques lombriciennes (Barbe et al., 2018, *en révision dans Ecosystems*).

Ces résultats viennent préciser les travaux de Bouché (1972, 1977) sur les catégories écologiques lombriciennes basés sur des critères morphologiques, physiologiques et comportementaux. Cette distinction entre lombriciens anéciques stricts et épi-anéciques est également soutenue par d'autres études à travers des différences de comportements fouisseurs, de

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[1] Adaptation des décomposeurs à des litières particulières au cours de l'assemblage se traduisant par une décomposition plus rapide (Veen et al., 2015)

traits morphologiques et de sensibilité au travail du sol. Jégou et al. (1998a, 2000, 2001) et Bastardie et al. (2003, 2005) ont par exemple observé que le réseau de galeries des anéciques stricts était plus dense (en terme de nombre de galeries et de connexions entre elles) que celui des lombriciens épi-anéciques. Concernant les différences de traits morphologiques, Briones and Álvarez-Otero (2018) ont observé que l'épaisseur de l'épiderme et de la cuticule de *Aporrectodea longa*, une espèce anécique strict, était supérieure à celle de *Lumbricus rubellus* et de *Lumbricus terrestris*, deux épi-anéciques. Ces différences de traits morphologiques peuvent suggérer une adaptation des lombriciens anéciques stricts à leur comportement fouisseur, une cuticule et un épiderme épais leur conférant une meilleure protection contre l'abrasion du sol. Par ailleurs, dans une méta-analyse Briones et Schmidt (2017) ont observé que suite à une réduction du travail du sol, l'abondance des communautés lombriciennes était stimulée et que cet effet était plus marqué pour les populations de *Lumbricus rubellus* et *Lumbricus terrestris* (épi-anéciques) que pour celles d'*Aporrectodea longa* (anécique strict). Les auteurs ont suggéré que le caractère permanent des galeries de *Lumbricus terrestris* (potentiellement héritées de descendants ; Butt et al., 2003; Grigoropoulou et al., 2008) pouvait expliquer le plus grand effet positif de la réduction du travail du sol.

Bien que la distinction entre lombriciens épi-anéciques et anéciques stricts ne soit pas toujours acceptée et controversée au sein de la communauté scientifique internationale, l'ensemble de ces travaux menés de manière indépendante alimente un faisceau de preuves convergeant dans ce sens. De plus, cette distinction est souvent basée sur une à deux espèces modèles (*Aporrectodea longa* et *Aporrectodea giardi vs Lumbricus rubellus* et *Lumbricus terrestris*) et nécessite d'être confirmée avec d'autres espèces tout aussi répandues (*Apporectodea nocturna*, *Lumbricus festivus* et *Lumbricus centralis*). Ainsi ce travail de thèse enrichit les connaissances actuelles sur les anéciques en apportant des éléments sur un plus grand nombre d'espèces.

C. Evaluation du rôle des espèces lombriciennes épi-anéciques dans la décomposition des litières

Nous avons observé en conditions contrôlées que la perte de masse des litières était liée à la biomasse initiale des lombriciens épi-anéciques (chapitres 3 et 4). Compte tenu de la forte variabilité morphologique des espèces lombriciennes épi-anéciques, leur contribution à la perte de masse des litières est alors très dépendante de l’espèce considérée. *In natura,* cette relation peut être compensée par le nombre d’individu des espèces considérées, en effet dans certaines prairies échantillonnées dans le chapitre 1, la biomasse totale des individus de *Lumbricus festivus* ou de *Lumbricus centralis* (biomasse individuelle faible et moyenne respectivement) surpassait celle des individus de *Lumbricus terrestris* (biomasse individuelle élevée).

Nous avons également observé que l’identité des espèces lombriciennes épi-anéciques régissait, en partie, la structure des communautés fongiques au sein de leurs galeries (chapitre 3). Ces différentes communautés fongiques observées peuvent être liées au fait que les communautés de microorganismes se succèdent très rapidement au cours du processus de décomposition des litières (Tang et al., 2005; Voříšková and Baldrian, 2013). Ainsi les espèces épi-anéciques en contribuant différemment à la perte de masse des litières peuvent structurer les communautés fongiques par l’apport de litières plus ou moins rapidement et donc plus ou moins fraîches. De plus, il est possible que le mucus sécrété par les anéciques et déposé à chacun de leur passage dans les galeries, de par sa composition, sélectionne des communautés fongiques différentes selon les espèces. Cependant ces différences de composition des mucus selon les espèces d’épi-anéciques restent largement méconnues. Les caractéristiques des réseaux de galeries (*e.g.* diamètre, nombre de connexions…) sont très différentes selon les catégories écologiques lombriciennes (Jégou et al., 1998b; Bastardie et al., 2005; Capowiez et al., 2015). Compte tenu de la variabilité morphologique des espèces lombriciennes épi-anéciques (Bouché, 1972) il est également possible que les conditions microclimatiques au sein des galeries (*e.g.* oxygène, humidité…) soient également très différentes selon l'identité des espèces considérées et par conséquent qu'elles régissent les communautés fongiques.

Compte tenu du rôle des champignons dans la dégradation des matières organiques récalcitrantes (humifiées), la sélection de différentes communautés fongiques pourrait impacter la dégradation de la matière organique humifiée ou récalcitrante du sol. Si tel est le cas d'importantes conséquences pourraient avoir lieu sur la formation du complexe argilo-humique et plus globalement sur le stockage ou le déstockage du carbone des sols. Cependant, les mesures d'activités enzymatiques (chapitre 4) n'ont pas révélé de différences selon l'identité des espèces lombriciennes épi-anéciques. Il pourrait être intéressant de travailler sur d'autres enzymes plus spécifiques aux champignons notamment la lignin peroxidase (EC 1.11.1.14) et la manganese peroxidase (EC 1.11.1.13 ; Burns et al., 2013). D'autre part, l'utilisation de plaques Biolog ^{TM} permettrait d'analyser le métabolisme des micro-organismes en présence d'une variété de sources de carbone (Smalla et al., 1998; Widmer et al., 2001). Cependant, l'utilisation de ces techniques (activités enzymatiques et plaques Biolog), engendre un biais de mesure puisqu'elles représentent un potentiel d'activité en conditions optimales ce qui ne reflète pas les micro-conditions créées par chacune des espèces lombriciennes épi-anéciques dans leurs galeries. Les avancées technologiques en métagénomique et en métatranscriptomiques permettraient d'avoir une information plus précise sur l'ensemble des gènes fonctionnels et exprimés dans ces galeries (Bailly et al., 2007; Fierer et al., 2012; Bouchez et al., 2016). Enfin, l'analyse de la composition des MOS à l'échelle moléculaire en utilisant la thermochimiolyse (THM) GC-MS permettrait de différencier les apports microbiens des litières et d'étudier la composition de ces apports en se basant sur la distribution de différentes molécules organiques (Vidal et al., 2016).

Les récentes études ont tendance à observer que les lombriciens stimulent la décomposition des litières et de la MOS en libérant plus de gaz à effet de serre (*e.g.* CO_2, N_20…) et donc ne favorisent pas le stockage de carbone dans les sols (Lubbers et al., 2013, 2017). Nous avons également observé la stimulation de la richesse et de la diversité bactérienne et l'augmentation du potentiel d'activités enzymatiques dans les galeries lombriciennes (chapitres 3 et 4) susceptibles de déclencher le « priming effect » sur la matière organique des sols (Jenkinson et al., 1985; Lavelle et al., 1995; Kuzyakov et al., 2000; Hoang et al., 2017). Cela étant, au travers de leur impact sur la décomposition des litières et de la matière organiques du sol les lombriciens contribuent, au travers de cette stimulation de l'activité des micro-organismes, à la fertilité des sols et donc à la production végétale (Brown et al., 1999; Scheu, 2003; Groenigen et al., 2014).

Alors qu'il existe des relations entre *biodiversité et fonctionnement* du sol (Girvan et al., 2005; Balvanera et al., 2006; Cardinale et al., 2006; Philippot et al., 2013; Maron and Lemanceau, 2014), l'importance de la diversité est remise en question par le concept de redondance fonctionnelle : puisque différentes espèces peuvent contribuer à la même fonction dans les écosystèmes, la redondance fonctionnelle prédit que la perte ou le changement d'espèces n'altèrerait pas nécessairement le fonctionnement des écosystèmes en raison de leur substitution par d'autres espèces qui contribuent au maintien des processus (Loreau, 2004). Dans les écosystèmes terrestres où la diversité microbienne est très importante, la redondance fonctionnelle est potentiellement très importante (Nannipieri et al., 2003; Wertz et al., 2006; Rousk et al., 2009; Schimel and Schaeffer, 2012). Par contre, la comparaison des taux de décomposition de la litière dans des microcosmes de sol inoculés avec différentes communautés microbiennes, a montré que des différences dans la composition des communautés entraînent des dissemblances fonctionnelles (Strickland et al., 2009). La redondance fonctionnelle est aussi remise en question par la présence de microorganismes rares et non redondants dans les sols mais pouvant jouer un rôle déterminant dans le fonctionnement des écosystèmes en améliorant par exemple la défense des plantes contre les herbivores (Hol et al., 2010). Ces études mettent en évidence aussi notre connaissance limitée de l'étendue de la redondance fonctionnelle dans les communautés microbiennes, tout en déterminant que son rôle dans le fonctionnement des écosystèmes peut être essentiel.

D. Dynamique et suivi de la biodiversité lombricienne

De nombreuses études témoignent de l'érosion de la biodiversité (Bardgett, 2002; Butchart et al., 2010; Barnosky et al., 2011). Concernant la biodiversité lombricienne, très peu d'études permettent d'apprécier son évolution dans le temps. En Europe, des inventaires de la biodiversité lombricienne ont été réalisés à des échelles régionales ou nationales (Bouché, 1972; Boag et al., 1997; Rutgers et al., 2009; Cluzeau et al., 2012; Pulleman et al., 2012) et constituent des premières bases pour apprécier cette biodiversité. Malgré ces efforts d'échantillonnage conséquents, les bases de données sont souvent constituées de prélèvements réalisés à de faibles échelles spatiales (Cameron et al., 2016). Ainsi, les connaissances actuelles sur la biodiversité lombricienne restent encore largement méconnues et des zones d'ombres persistent. En se basant sur la théorie des niches, un premier modèle prédictif sur l'abondance et la diversité lombricienne en Europe a été créé par Rutgers et al. (2016). Ce modèle prédictif se base sur les caractéristiques du sol, le climat et l'occupation du sol. Cependant, cette approche ne permet pas de suivre la dynamique de la biodiversité lombricienne dans le temps. Pour compenser le manque d'observation à long terme et sur un vaste territoire, la France, depuis 2012, avec le plan Ecophyto (I et II) prévoit, entre autre, de suivre l'apparition éventuelle d'effets non intentionnels des pratiques agricoles sur l'environnement (Article L251-1 du code rural et de la pêche maritime). Ce plan a permis de constituer un réseau national de 500 parcelles réparties sur le territoire (Fried et al., 2018) et au sein desquelles les lombriciens sont échantillonnés et inventoriés chaque année. Ce premier réseau d'envergure nationale permettra à terme d'évaluer la dynamique de la biodiversité lombricienne. En parallèle, l'échantillonnage des lombriciens est relativement simple et à portée de tous, ce qui permet d'envisager le déploiement des sciences participatives comme un des leviers permettant de compenser le manque d'observations (Bone et al., 2012; Kaartinen et al., 2013; Rossiter et al., 2015). Plusieurs programmes de sciences participatives existent sur les vers de terre, en Angleterre (OPAL), France (OPVT), Finlande (Worm Tracker) ou encore au Canada et aux Etats-Unis (Worm Watch). L'identification des individus à l'échelle spécifique et l'acquisition des données explicatives (propriétés du sol) restent cependant encore limitées aux spécialistes.

E. Méta-communautés lombriciennes et prédateurs

Nous avons observé que les communautés lombriciennes des sols prairiaux étaient régulées par les propriétés du sol, ses pratiques de gestion et le paysage environnant. Seulement, selon les indicateurs sélectionnés entre 30 et 80 % de variance était non expliquée. Outre d'autres variables supplémentaires concernant les propriétés du sol (*e.g.* profondeur, tassement…), de gestion (*e.g.* pesticides, fauche…) et du paysage (*e.g.* taille des parcelles, qualification du paysage…), d'autres facteurs intervenant dans les règles d'assemblages des communautés lombriciennes pourraient être pris en compte : la pression de prédation et la dispersion.

La prédation est très importante chez les lombriciens puisqu'ils constituent un maillon très important de la chaîne trophique pour de nombreux animaux, invertébrés, mammifères, oiseaux… (Macdonald, 1983; Granval and Aliaga, 1988). Certaines études ont également observé un lien entre la densité de lombriciens et la densité de divers prédateurs (Kruuk and Parish, 1981; Granval et al., 2000; Goszczyński et al., 2000; Kowalczyk et al., 2004; Maerz et al., 2005; Duriez et al., 2005). Ainsi, la pression de prédation a sans doute un impact conséquent sur l'assemblage des communautés lombriciennes ; par exemple, *Lumbricus rubellus rubellus* et *Lumbricus terrestris* sont deux espèces souvent prédatées (Macdonald, 1980; Cuendet, 1983; Fiore et al., 2004; Brown et al., 2012). D'autres au contraire comme *Eisenia foetida* éviterait plus facilement la prédation d'un plathelminthe (*Bipalium adventitium* ; Fiore et al., 2004). Très peu d'études se sont intéressée pour l'instant à quantifier et qualifier ces pressions de prédation sur les communautés lombriciennes. Cuendet, (1983) a par exemple estimé qu'une mouette rieuse (*Larus ridibundus*) pouvait consommer jusqu'à 4.3 g de lombriciens par minute dans des champs cultivés et que selon l'occupation du sol (*i.e.* prairie et culture) les catégories écologiques lombriciennes étaient consommées différemment. Dans ce présent travail, nous avons identifié une relation négative entre la diversité du paysage et l'abondance des lombriciens. La diversité du paysage est souvent corrélée positivement à la présence de prédateur de lombriciens (Marshall and Moonen, 2002; Vickery et al., 2002; Maudsley et al., 2002; O'Brien et al., 2016), ce qui augmenterait ces pressions de prédation et pourrait en partie expliquer la plus faible abondance lombricienne observée à proximité de paysages très diversifiés. De plus, les lombriciens sont la proie principale (> 50% d'occurrence) ou régulière (entre 10% et 50% d'occurrence) de certains animaux (i) d'intérêt économique comme la bécasse des bois (*Scolopax rusticola*), le faisan (*Phasianus colchicus*) ou le sanglier (*Sus scrofa*) mais également (ii) patrimoniale comme le hérisson d'Europe (*Erinaceus europaeus*) ou la musaraigne aquatique (*Neomys fodiens* ; Granval and Aliaga, 1988). Ainsi, le maintien ou le développement des communautés lombriciennes est d'un intérêt majeur pour ces espèces. Dans ce présent travail, nous avons pu identifier une relation positive avec la diversité du paysage autour des prairies avec la diversité des épi-anéciques. Ces espèces épi-anéciques vont potentiellement occuper des niches écologiques légèrement différentes et augmenter la diversité de ressources pour divers prédateurs.

La dispersion est un trait d'histoire de vie qui affecte les processus de formation et de maintien des communautés, les flux géniques mais également la colonisation vers un patch ou une niche vide (Clobert et al., 2004; Hanski et al., 2004; Clobert et al., 2012). L'ajout d'une perspective spatiale à l'assemblage des communautés lombriciennes permet de les considérer comme potentiellement organisées en méta-communautés (Hanski et al., 2004). Le terme « méta-communauté » décrit un ensemble de communautés locales qui sont liées par la dispersion de multiples espèces potentiellement en interaction (Wilson, 1992) de sorte que les interactions locales et les processus régionaux influencent l'assemblée communautaire locale. Cette théorie fournit de nouvelles perspectives sur la façon dont les communautés sont structurées à de multiples échelles spatiales. La dispersion peut se décomposer en trois étapes : l'émigration, le transfert et l'immigration (Ims and Yoccoz, 1997; Mathieu et al., 2018). Chez les lombriciens, la grande difficulté pour observer la dispersion est liée au fait qu'une part de la dispersion peut se faire sous terre et est donc non visible. Plusieurs moyens peuvent être mis en place afin d'apprécier la dispersion chez les lombriciens : les observations (direct, localisation, capture-marquage-recapture, expérimentation en conditions contrôlées), la génétique et les modèles mathématiques (Nathan, 2001; Mathieu et al., 2018). Chez les lombriciens, les synthèses bibliographiques d'observation diachroniques de Eijsackers (2010, 2011) font état d'une dispersion allant de 1.5 à 14 m. an^{-1}. Les études sur les facteurs déclenchant l'immigration ont permis de mettre en évidence séparément le rôle de la qualité de l'habitat, de la densité lombricienne et de la recherche de partenaire (Nuutinen and Butt, 1997; Michiels et al., 2001; Mathieu et al., 2010; Caro et al., 2013; Chatelain and Mathieu, 2017). Ainsi, la capacité d'émigration a pu être reliée en partie aux catégories écologiques lombriciennes (épigés > anéciques = endogés) avec une certaine variabilité intra-catégories écologiques (Caro et al., 2013; Chatelain and Mathieu, 2017). Au cours de ce travail de thèse, nous avons pu identifier deux sous-catégories écologiques au sein des anéciques susceptibles de réagir différemment aux facteurs déclenchant l'émigration et ainsi expliquer en partie la variabilité intra-catégorie écologique observée. Très peu d'études ont été réalisées sur ce qui pouvait influencer le transfert et l'immigration des lombriciens. Récemment, Dupont et al. (2017) avec des techniques moléculaires, ont pu relier la dispersion de *Allolobophora chlorotica chlorotica* et *Aporrectodea icterica* (deux lombriciens endogés) à différents éléments du paysage facilitant (prairies) ou entravant (route) la dispersion. Les techniques moléculaires restent onéreuses à utiliser cependant elles offrent de bonnes perspectives pour étudier l'effet du paysage sur les communautés lombriciennes (Mathieu et al., 2018). La théorie des méta-communautés ouvre de nouvelles perspectives, en particulier sur la relation entre la richesse des espèces et le fonctionnement des écosystèmes

De plus, la destruction et la fragmentation des habitats sont depuis longtemps considérées comme des causes majeures d'érosion de la biodiversité (Wilson et al., 2016; Tilman et al., 2017) pouvant expliquer jusqu'à 75% de celle-ci (Haddad et al., 2015). Ainsi, l'analyse du paysage est très importante en écologie. La perte d'habitats et la fragmentation sont des processus distincts cependant il est complexe de séparer leurs influences relatives sur les variables mesurées (Ewers and Didham 2006). Les conséquences néfastes de la fragmentation et de la perte d'habitat sur la biodiversité sont multiples et présentes à différents niveaux d'organisation du vivant (Fahrig 2003; Haddad et al. 2015). Il apparaît important de maintenir des espaces vitaux pour les lombriciens dans le paysage, l'occupation et la gestion du sol sont deux facteurs pouvant être pris en compte par les gestionnaires. Face à ce constat, les stratégies de conservation par les institutions et les politiques sont la sauvegarde des habitats (permettant la conservation de grandes populations) et le maintien des flux entre les populations par la prise en compte de la connectivité entre les habitats.

La connectivité est définie comme le degré auquel un paysage facilite ou limite les mouvements des individus entre patchs de ressources (Taylor et al. 1993). Cette définition est la plus commune et souligne l'importance des effets du paysage (par sa composition et sa configuration) sur le mouvement des organismes qui, par extension, influencent les dynamiques de populations et la structure des communautés (Taylor, Fahrig, and With 2006). En Europe, les continuités écologiques font l'objet d'une prise en compte croissante et sont reconnues comme une stratégie de conservation de la biodiversité (Garmendia et al., 2016). En France, cette stratégie de conservation se décline au travers de deux articles de loi visant à enrayer la perte de biodiversité en participant à la préservation, à la gestion et à la remise en bon état des milieux nécessaires aux continuités écologiques, les Trames Verte et Bleu (Article L371-1 du code de l'environnement). Les continuités écologiques constituant la trame verte et bleue comprennent des réservoirs de biodiversité et des corridors écologiques (Article R371-19 du code de l'environnement). La Trame Verte et Bleu est un outil d'aménagement du territoire qui vise à (re)constituer un réseau écologique cohérent et fonctionnel. Cependant ces stratégies de continuités écologiques ne prennent pas du tout en compte la biodiversité du sol.

Compte tenu de la difficulté d'observer la dynamique de la biodiversité lombricienne, il est alors envisageable par anticipation de favoriser les facteurs bénéfiques aux communautés lombriciennes ce que cette thèse a pu mettre en avant.

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UNIVERSITE / ECOLOGIE
BRETAGNE \ GEOSCIENCES
LOIRE / AGRONOMIE ALIMENTATION

UNIVERSITÉ DE RENNES 1

Titre : Évaluation de la contribution fonctionnelle des espèces lombriciennes anéciques à la décomposition des litières prairiales

Mots clés : microorganismes, activités enzymatiques, communautés, interaction intra- et inter-spécifique, paysage

Résumé : La décomposition des litières est un processus clé du fonctionnement du sol contribuant à de nombreux services écosystémiques. En climat tempéré, les lombriciens en interaction avec les micro-organismes du sol, contribuent significativement à ce processus. Cependant, les connaissances sur les lombriciens ciblent le plus souvent les trois catégories écologiques selon lesquelles ils sont définis : les épigés, les endogés et les anéciques. Les anéciques sont très répandus dans les sols tempérés, constituent la majeur partie de la biomasse lombricienne et interviennent dans la décomposition des litières. Plusieurs études ont observé des traits comportementaux, morphologiques et physiologiques distinguant deux sous-catégories au sein des anéciques : les épi-anéciques et les anéciques stricts. Le premier objectif de ce travail de thèse était de vérifier si cette distinction avait une réalité dans le cadre du processus de décomposition des litières. En conditions contrôlées, nous avons évalué (i) le rôle des principales espèces anéciques dans le processus de décomposition, (ii) l'impact de ces espèces sur les communautés de microorganismes et (iii) les activités enzymatiques des microorganismes du sol et (vi) l'impact des interactions entre espèces anéciques sur le processus de décomposition.

A partir d'observations sur le terrain, le second objectif de ce travail de thèse était de définir les règles d'assemblages des communautés lombriciennes en prairie compte-tenu de leurs rôles majeurs dans divers processus du sol. Les résultats obtenus ont confirmé la distinction entre lombriciens anéciques stricts et épi-anéciques : les épi-anéciques étant les seuls à contribuer au processus de décomposition des litières et celui-ci étant corrélé à la biomasse individuelle moyenne de chaque espèce. Cette contribution passe par une plus forte stimulation des activités enzymatiques du sol, indépendamment des espèces considérées. En revanche, contrairement aux bactéries, les communautés fongiques du sol dépendent de l'espèce épi-anécique avec laquelle ils interagissent. Ce travail met également en évidence que l'abondance, la biomasse et la diversité des communautés lombriciennes des sols prairiaux sont régulées par différents filtres environnementaux dont la diversité du paysage. Cette thèse met en évidence que les deux sous-catégories écologiques au sein des anéciques ont des rôles différents sur le processus de décomposition des litières et qu'elles contribuent donc à des services écosystémiques fournis par le sol de manière différenciée.

Title: Assessment of the functional contribution of anecic earthworm species to the decomposition of grassland litters

Keywords: microorganisms, enzymatic activities, communities, intra- and interspecific interaction, landscape

Abstract : Litter decomposition is a key process in soil functioning that contributes to many ecosystem services. In temperate climates, earthworms interacting with soil microorganisms contribute significantly to this process. However, knowledge about earthworms most often targets the three ecological categories in which they are defined: epigeics, endogeics and anecics. Anecics are very common in temperate soils, constitute the major part of the earthworm biomass and are involved in the litter decomposition. Several studies have observed behavioral, morphological and physiological traits that distinguish two subcategories within anecics: epi-anecics and strict-anecics. The first objective of this thesis was to verify if this distinction had a reality in the context of the litter decomposition process. Under controlled conditions, we evaluated (i) the role of the main anecic earthworm species in the decomposition process, (ii) the impact of these species on microorganism communities and (iii) soil enzymatic activities and (vi) the impact of interactions between anecic species on the decomposition process.

Taking into account their major roles in various soil processes, based on field observations, the second objective of this thesis was to define the assembly of earthworm communities in grassland, The results obtained confirmed the distinction between epi and strict-anecic earthworms: only epic-anecics contribute to the litter decomposition process, which is correlated to the average individual biomass of each species. This contribution involved a greater stimulation of soil enzymatic activities, regardless of the species considered. In contrast to bacteria, soil fungal communities depend on the epi-anecic earthworm species with which they interact. This work also highlights that the abundance, biomass and diversity of earthworm communities in grassland soils are regulated by different environmental filters, including landscape diversity. This thesis highlights that the two ecological subcategories within the anecic category have different roles in the litter decomposition process and therefore contribute to ecosystem services provided by the soil in a differentiated way.