Effets de la diversité des arbres sur le fonctionnement de l’écosystème dans deux plantations de forêts tempérées

Thèse

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Résumé

La grande majorité des scientifiques s'accordent depuis deux décennies sur le fait que les actions anthropiques sont responsables d'une grande perte de biodiversité à l'échelle terrestre via l'élimination de gènes, d'espèces et de traits biologiques à un rythme alarmant. Ce fait les a conduits à se questionner sur les impacts de la perte de la biodiversité sur le fonctionnement des écosystèmes. Bien qu'aujourd'hui les connaissances sur le lien entre biodiversité et fonctionnement des écosystèmes (BEF) commencent à être bien documentées, nous en savons encore très peu sur les mécanismes sous-jacents à la relation entre BEF, et en particulier concernant les mécanismes appuyant les processus souterrains des écosystèmes forestiers.

L'objectif de ce projet de doctorat était de développer les connaissances sur le lien entre la biodiversité des parties aériennes et le fonctionnement souterrain des écosystèmes artificiels (plantations d'arbres). Nous avons pour cela examiné l'implication de différents acteurs et paramètres du compartiment souterrain susceptibles d'intervenir dans le cycle du C - et de l'N - en lien avec la biodiversité (notamment via le recours à l'approche des traits fonctionnels). D'une part nous avons étudié la productivité des racines fines, leur chimie ainsi que le fonctionnement et la composition des communautés microbiennes du sol en lien avec des mesures de diversité (la richesse spécifique et la diversité fonctionnelle) dans une jeune plantation (4 ans). D'autre part, nous avons étudié la décomposition des racines fines en lien avec les communautés d'arbres et de végétation du sous-bois subséquentes à l'application de traitements dans une plantation plus âgée (27 ans). Dans tous les cas, nous avons étudié le lien entre ces différents paramètres et processus et les C et N du sol (totaux et dans les fractions). Les feuillus et les conifères diffèrent quant à la productivité des racines fines ainsi que par rapport à la métabolisation des sources de C. Les conifères étaient plus productifs que les feuillus (racines fines), et les feuillus métabolisaient un plus grand nombre de sources de C que les conifères. Par ailleurs, la richesse spécifique a influencé le fonctionnement des microbes mais pas leur composition ni la productivité des racines fines, tandis que l'identité des arbres (et de leurs traits fonctionnels) ont influencé tous ces paramètres et processus. La valeur moyenne des traits a plus influencé la productivité des racines fines, la respiration basale et la biomasse microbienne que la variance de ces traits. La diversité fonctionnelle (considérée en tant que gradient) n'a quant à elle pas eu d'effet sur aucun des paramètres et processus étudiés. Finalement, notre étude a révélé

que la végétation du sous-bois (couvert de type fonctionnel et certaines espèces) plus que les arbres, les propriétés du sol ou la chimie des racines fines influençait la décomposition de ces dernières.

De manière générale, cette thèse a permis de découvrir et de mettre en évidence des aspects jusqu'alors inconnus du lien entre BEF, notamment en ce qui concerne le lien entre la diversité des parties aériennes et le fonctionnement des parties souterraines. Nos résultats ont permis d'identifier avec précision les espèces d'arbres, de végétation du sous-bois ou encore les traits fonctionnels et les processus sur lesquels ils interviennent. Ceci pourrait permettre d'affiner les modèles de prédiction des cycles du C et de l'N ou encore de prodiguer des conseils avisés aux gestionnaires forestiers.

Abstract

In the last two decades, the vast majority of scientists have agreed that anthropogenic actions are responsible for an important and rapid loss of biodiversity at a global scale, through the elimination of genes, species and biological traits. This fact led to remarkable progress towards understanding how the loss of biodiversity affects the functioning of ecosystems. Although the link between biodiversity and ecosystem functioning (BEF) is now well documented, the mechanisms underlying this relationship are still poorly understood, especially with regards to belowground processes in treed ecosystems.

The objective of this Ph.D. project was to improve our understanding of the link between aboveground biodiversity and belowground functioning in two artificial ecosystems (tree plantations). For this purpose, we examined the implication of different actors and parameters of the belowground compartment that are likely to influence the C - and N - cycles, in relation to aboveground biodiversity (through the functional trait-based approach). On the one hand we studied the productivity of fine roots, their chemistry, the functioning and composition of soil microbial communities in relation to diversity measures (specific richness and functional diversity) in a young plantation (4 years). On the other hand, we studied the decomposition of fine roots in relation to over- and understory vegetation following the application of silvicultural treatments in an older plantation (27 years). In all cases, we studied the relationship between these parameters and processes, as well as soil C and N (total and in fractions).

Deciduous and conifer species differed in fine root productivity and in microbial community catabolic activity. Conifers were more productive than deciduous (fine roots), and soil microbial communities associated with deciduous trees used a greater number of carbon sources than those associated with conifers. Moreover, although tree specific richness influenced the functioning of microbes, it had no effect on their composition or the productivity of the fine roots, while tree identities (and their functional traits) influenced all these parameters and processes. The mean value of traits had a greater influence on fine root productivity, basal respiration and microbial biomass than the variance of these traits. The functional diversity (considered as a gradient) had no effect on any of the parameters and processes studied. Finally, our study revealed that the understory vegetation (cover of functional type and some species), more than overstory vegetation, soil properties or fine root chemistry influences the fine root decomposition.

In general, this thesis has uncovered and highlighted unknown aspects of the relationship between BEF, in particular with regard to the link between aboveground diversity and belowground functioning. Our results precisely identified tree species, understory vegetation and functional traits and the processes on which they intervene. This could help to refine predictive models of C and N cycles or provide advice to forest managers.

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Avant-propos

Insertion d’articles

La présente thèse est composée de trois chapitres rédigés en anglais sous forme d’articles scientifiques. Pour chaque chapitre, j’ai établi les objectifs de recherche et formulé les hypothèses, planifié les expériences sur le terrain et en laboratoire, et supervisé la collecte des données. J’ai également réalisé plusieurs analyses de laboratoires, les analyses statistiques, l’interprétation des résultats et la rédaction des articles scientifiques.

Chapitre I- Khlifa, R., Angers, D., and A.D. Munson. Do tree species richness and identity affect fine root production and soil C and N levels in young forest plantations? L’article sera soumis sous peu.

Chapitre II- Khlifa, R., Paquette, A., Messier, C., Reich, P.B., A.D. Munson. Do temperate tree species diversity and identity influence soil microbial community function and composition? L’article sera soumis sous peu.

Chapitre III- Khlifa, R., Angers, D., and A.D. Munson. Understory vegetation is the strongest driver of fine root decomposition in a temperate plantation. L’article sera soumis sous peu.

Mon travail a été encadré par ma directrice de thèse, Alison Munson, qui est donc co-auteure de tous les chapitres de cette thèse. Mon co-directeur, Denis Angers, a également participé à l’encadrement de mon travail notamment pour l’analyse des sols et l’interprétation des résultats des chapitres I et III. Christian Messier et Alain Paquette ont fourni le dispositif expérimental de

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Introduction générale

Au cours du siècle dernier, le réchauffement global a augmenté dû à la hausse des émissions anthropogéniques de gaz à effet de serre, tel que le dioxyde de carbone (CO_2). En 2015, la concentration moyenne journalière de CO_2 atmosphérique mesurée à l'observatoire de Mauna Loa, Hawaii a atteint un niveau record puisqu'elle dépassait les 400 ppm (parties par million) sur l'ensemble de la planète (NOAA/ESRL 2015) soit 120 ppm de plus qu'à l'ère préindustrielle. La température a également augmenté d'environ 0,85°C depuis la fin du $19^{ème}$ siècle et devrait continuer d'augmenter d'environ 2 à 4°C jusqu'à la fin du siècle (Collins et al. 2013, Hartmann et al. 2013). Dans ce contexte actuel de réchauffement climatique croissant, les communautés scientifiques se retrouvent confrontées à de nombreux défis concernant l'aménagement des écosystèmes terrestres. Il s'agira, par exemple, d'augmenter l'utilisation des écosystèmes comme sources de services écosystémiques de sorte qu'ils soient de plus en plus productifs pour répondre aux demandes croissantes. En même temps, il faudrait instaurer des modes de gestion plus efficaces et durables, permettant de continuer à avoir recours aux services des écosystèmes, tout en en assurant la pérennité.

L'une des pistes de gestion proposée pour atténuer les changements climatiques serait d'utiliser les sols comme des « puits » de carbone (C) (Janzen 2004). En effet, la vaste majorité du C terrestre est stockée dans les sols (Jobbágy and Jackson 2000), une augmentation du stockage du C dans les sols permettrait de contrer l'augmentation du CO_2 atmosphérique d'origine anthropique. De manière naturelle, les écosystèmes forestiers mondiaux jouent un rôle essentiel dans le cycle du C et sont capables à eux seuls de séquestrer 4.0 Pg C an^{-1} (Pan et al. 2011). En particulier, les sols des forêts boréales agissent depuis des milliers d'années comme d'importants puits de C, comptant parmi les plus importants stocks de C des sols mondiaux (Harden et al. 1997) aux côtés des tourbières qui contiennent à elles seules 1/3 de ces stocks (Bragazza et al. 2006). Avec le réchauffement climatique, la température de l'air augmente, ce qui a pour effet d'augmenter l'absorption du C atmosphérique au sein des écosystèmes forestiers via la photosynthèse en particulier au printemps durant la saison de croissance (Tanja et al. 2003, Krishnan et al. 2008). Cependant, l'augmentation de la température de l'air a également pour effet d'augmenter la respiration des microorganismes des sols et des plantes, ce qui se traduit au contraire par une perte de C des écosystèmes terrestres vers l'atmosphère, en particulier durant les mois d'automne (Piao et al. 2008, Vesala et al. 2010, Ueyama et al. 2014). Néanmoins, certaines études récentes montrent que des changements dans le fonctionnement des écosystèmes (e.g. variations saisonnières) auraient un impact plus important sur l'équilibre du C que le réchauffement climatique en soi (Wu et al. 2012a, Hadden and Grelle 2016). Les activités anthropiques pourraient également entraîner des changements dans les régimes de perturbations naturelles ou les perturbations directes plus ou moins grandes dans le fonctionnement des écosystèmes (e.g. aménagement forestier, traitements sylvicoles), et en conséquence, les sols forestiers pourraient donc passer potentiellement de « puits » à « sources » de C (Kurz et al. 2008), dans certaines situations. La différence entre ces deux flux opposés détermine l'équilibre net de carbone de l'écosystème. Cet équilibre est dynamique et varie en fonction du temps et de l'espace (Stoy et al. 2009, Yuan et al. 2009).

Le C dans les sols

Dans les sols, le C se retrouve sous différentes formes qui n'évoluent pas toutes à la même vitesse. Ces différentes formes de C du sol sont appelées « pools », et sont généralement qualifiées de rapides (10^0 an), lents (10^{1-2} ans) ou passifs (10^{3-4} ans et plus) (Amundson 2001), surtout à des fins de modélisation. De plus, la teneur en C organique du sol est influencée par des facteurs aussi divers que les facteurs climatiques, la productivité primaire nette de la végétation (Ingram and Fernandes 2001), les propriétés physiques et chimiques du sol, les communautés microbiennes du sol ainsi que le type de couvert forestier ou les espèces d'arbres (Jandl et al. 2007). En plus d'influencer les entrées de C dans les sols via la litière des feuilles et des racines dans les différents horizons des sols forestiers, les espèces d'arbres agissent également sur la capacité de rétention de l'N de ces sols (Swift et al. 1979). En effet, les différences de qualité des litières des arbres influencent le « turnover » du C et donc par la même occasion le ratio C/N du sol, ce qui aura un effet sur la rétention de l'N. Toute action anthropique susceptible d'influencer l'un de ces facteurs pourrait donc avoir un impact sur les teneurs en C organique du sol. Or, la prise en compte de certains de ces acteurs (e.g. les communautés microbiennes du sol, les impacts d'une perte de la biodiversité) dans les modèles actuels permettant de quantifier les flux de matière et d'énergie est encore insuffisante (Ingwersen et al. 2008). Il apparait donc important de mieux connaître et comprendre les interactions qui lient les différents acteurs et processus qui interviennent dans la séquestration du C dans les sols, afin d'être en mesure de fournir des modèles plus précis et réalistes. Ceci dans le double but de soutenir à la fois les pratiques mises en œuvre pour le calcul du budget national ou global du C, ainsi que pour mieux orienter les efforts futurs de mitigation du CO_2 face aux changements climatiques (Lal 2010).

Des méthodes ont été proposées pour étudier la dynamique et le stockage du C et de l'N dans les sols en rapport avec l'utilisation et la gestion des terres ou encore le couvert végétal. Par exemple celles décrites par Gregorich and Beare (2008) permettent d'isoler la matière organique (MO) « libre » (i.e. qui n'est pas encore liée aux particules minérales du sol). Le C organique de cette MO « libre » est considéré comme labile puisqu'il est plus facilement disponible pour les communautés microbiennes du sol (Khanna et al. 2001). La MO provient d'un mélange de plantes, d'animaux ou encore de parties de microorganismes à différents stades de décomposition et peut contenir différents composants tels que des pollens, des spores et des invertébrés (Spycher et al. 1983, Baisden et al. 2002). Elle peut être isolée soit en fonction de sa densité (via des liquides lourds) ou de sa taille (via des tamis). Dans le présent projet de doctorat nous avons eu recours à l'étude de la fraction de MO particulaire isolée en fonction de la taille. Tout au long de la thèse, cette fraction a été caractérisée selon qu'elle contenait i) des particules de sables et de larges particules de MO (fraction plus labile notée POM : taille > 53 µm) ou au contraire ii) des particules plus fines d'argiles et de limons (fraction présumée plus stable et notée S&C : taille < 53 µm).

La suite de cette introduction va détailler un à un quelques-uns des différents acteurs étudiés au sein de ce projet de doctorat et qui sont susceptibles d'influencer le cycle du C des sols.

Biodiversité et fonctionnement des écosystèmes

La grande majorité des scientifiques s'accordent depuis deux décennies sur le fait que les actions anthropiques sont responsables d'une grande perte de biodiversité à l'échelle terrestre via l'élimination de gènes, d'espèces et de traits biologiques à un rythme alarmant, ce qui pourrait avoir un impact négatif sur le fonctionnement des écosystèmes (Cardinale et al. 2012). Plusieurs études postulent un lien entre la diversité des espèces végétales ainsi que leurs traits fonctionnels (i.e. la richesse spécifique (SR) et la diversité fonctionnelle (FD)), et la séquestration du C dans les sols (De Deyn et al. 2008).Cependant, peu d'études ont réellement testé ce lien (Saha et al. 2009, Fornara and Tilman 2008, Bunker et al. 2005). La SR fait référence au nombre des différentes espèces présentes dans l'aire d'étude, et la FD est définie comme la diversité et l'abondance relative de différents traits fonctionnels. Les traits fonctionnels lient les espèces aux rôles qu'elles tiennent dans un écosystème et sont définis comme étant « toute fonction morphologique, physiologique ou phénologique mesurable au niveau individuel, et qui a un impact sur la fitness de l'individu via ses effets sur la croissance, la reproduction et la survie de ce dernier » (Violle et al. 2007). Bien que ces deux approches aient toutes deux grandement fait avancer la science de la biodiversité, de plus en plus d'études présentent le rôle de la FD comment pouvant être plus important que celui de la SR pour expliquer le fonctionnement des écosystèmes (Naeem and Wright 2003, Petchey and Gaston 2006). En effet, l'étude de la SR comme seul représentant de la biodiversité a rapidement montré des limites puisque cette mesure se base uniquement sur le nombre d'espèces différentes présentes dans une communauté sans tenir compte de l'effet fonctionnel de ces espèces. En ce sens, il serait plus important et pertinent de s'intéresser à la FD comme mesure de la diversité, puisqu'elle est une mesure qui présente l'avantage supplémentaire de tenir compte de l'aspect mécanique de la relation entre biodiversité et fonctionnement -BEF (Hooper et al. 2005). En effet, en plus de tenir compte du nombre d'espèces présentes dans la communauté, la FD tient également compte de la complémentarité des niches entre les espèces. Lorsque la diversité des traits est plus élevée, il y a une meilleure complémentarité des niches c'est-à-dire une meilleure exploitation des ressources par les différentes espèces présentes (Hooper 1998, Petchey 2003). De plus, la FD peut être décrite via deux aspects complémentaires de la structure des communautés tels que la moyenne et la dispersion des traits fonctionnels au sein d'un ensemble d'espèces donné (Ricotta and Moretti 2011). Dans ce projet de doctorat ces deux aspects ont été pris en compte via des mesures de la valeur moyenne de ces traits pondérée par la communauté (CWM) et le FDt (la dispersion - ou variance - de ces traits). L'intérêt de considérer la CWM lorsque l'on étudie la FD est basé sur l'hypothèse de « mass-ratio » (Grime 1998) selon laquelle l'effet de chaque espèce dans un processus écosystémique est proportionnel à son abondance relative dans la communauté. Lorsque l'on applique cela aux traits, l'importance de chaque trait est donc proportionnelle à la valeur moyenne de ce trait dans chaque espèce. Des études introduisant l'étude de la diversité de groupes fonctionnels seuls (Hooper and Vitousek 1998, Symstad and Tilman 2001) ou accompagnés de la SR (Hooper 2005, Hector et al. 1999, Díaz and Cabido 2001) ont donc émergés. Dans le présent projet de doctorat, la FD a été mesurée via un indice de FD (l'indice de dispersion FDis) proposé par Laliberté and Legendre (2010). Cette méthode présente de nombreux avantages qui faisaient défauts à d'autres indices proposés pour calculer la FD, tels que le « FDvar », le « FRO » ou encore le « Quadratic entropy » (Villéger et al. 2008). Le FDis présente notamment les avantages d'être très flexible (e.g. possibilité d'utiliser un ou plusieurs traits, possibilité d'utiliser plusieurs types de traits; quantitatif, semi-quantitatif, qualitatif ou encore possibilité d'effectuer le calcul malgré des données manquantes ainsi que de tenir compte de l'abondance des espèces en mélanges en pondérant chaque trait). Le FDis est la distance moyenne entre chaque espèce et le centroïde (centre de masse) de toutes les espèces dans un espace de trait multidimensionnel. L'abondance des espèces peut être utilisée pour pondérer les distances. Lors de l'établissement de la plantation qui a servi à cette étude (en 2009), l'état des connaissances stipulait qu'à la différence d'autres indices, la FD était supposée être mathématiquement indépendante de la SR pour les communautés de 2 espèces et plus (Tobner et al. 2014). Depuis, une étude (Dias et al. 2013) suggère que ce ne serait pas le cas, puisqu'en étudiant le relation entre la variance (mesure de la FD) et la moyenne des traits fonctionnels, les auteurs stipulent que ces deux mesures varient en fonction du nombre d'espèces présentes, de leur composition et de l'abondance relative de ces espèces dans les mélanges considérés. Contrairement à la SR, dont la mesure ne présente aucune difficulté, la FD est plus complexe à mesurer puisque le calcul fait à appel à de nombreux choix qui peuvent êtres arbitraires (e.g. identité et nombre des traits choisis). De plus, les traits choisis pour le calcul pourraient ne pas tous être étroitement reliés à la fonction étudiée et pourraient également provenir de la littérature et/ou d'espèces proches de celles étudiées et ne pas avoir été mesurés *in situ*. Paquette and Messier (2011) soulignent le fait que les résultats de leur étude auraient pu être plus robustes en utilisant des indices de FD avec une couverture plus complètes des traits des espèces étudiées et une meilleure connaissance de l'importance relative des différents traits impliqués.

À ce jour, peu d'études se ont porté sur la diversité fonctionnelle des arbres et le fonctionnement de l'écosystème. Le projet BIOTREE (Scherer-Lorenzen et al. 2007) est sans doute le premier à l'avoir fait. Par exemple, Potvin and Gotelli (2008, dans BIOTREE) se sont penchés sur le sujet pour une plantation d'arbres tropicaux et ont trouvé qu'après cinq ans de croissance, les mélanges mixtes présentaient 30 à 58 % plus de surface terrière d'arbres que les monocultures. Les auteurs suggèrent que ces différences seraient dues à un renforcement de la croissance individuelle des arbres lorsqu'ils sont mis en mélange. Loreau & Hector (2001) proposent que les relations positives entre la BEF (e.g. l'augmentation de la productivité des arbres lorsqu'ils sont en mélanges vs en monocultures) pourraient être causées par des mécanismes de complémentarité (répartition plus efficaces des ressources entre les espèces en raison du partitionnement des niches), ou de sélection (augmentation de la productivité en raison de la présence d'espèces hautement performantes). Le projet IDENT (Tobner et al. 2014) actuellement en cours à plusieurs sites, fait partie du projet TreeDivNet (voir Verheyen et al. (2016) pour plus de détails) en manipulant la SR, et la FD le long d'un gradient calculé à partir d'indices introduits par Laliberté and Legendre (2010).

Biodiversité et processus racinaires

De nos jours, la majorité des recherches portant sur l'étude du lien entre BEF ont surtout mis l'accent sur les parties aériennes et les études sur les parties souterraines sont encore peu nombreuses. De plus, les rares fois où ces effets ont été étudiés sur les racines, ces derniers concernaient surtout la comparaison entre des monocultures et principalement des mélanges de deux espèces (Leuschner and Hertel 2003). Toutefois, malgré la rareté des études effectuées sur les racines, il a été démontré que l'N racinaire est corrélé positivement au taux de décomposition des racines d'arbres (climat tempéré) tandis qu'inversement, le C et la lignine lui sont corrélés négativement (Aulen et al. 2012). Par ailleurs, Rasse et al. (2005) ont montré que les racines représentent une source non négligeable d'entrée de C dans les sols que ce soit par leurs exsudats ou par leur turnover élevé. De même, nous savons que dans la majorité des cas, la biomasse des racines fines est plus élevée dans les mélanges de deux espèces (Berish and Ewel 1988, Cuevas et al. 1991, Schmid and Kazda 2002) ou de quatre espèces (Lei et al. 2012b) que dans leurs monocultures respectives. Cette biomasse se retrouvait majoritairement (environ 63-77%) dans les 20 premiers centimètres de profondeur pour des arbres matures âgés entre 80 et 150 ans en forêt tempérée. Selon Schenk and Jackson (2002), 95% de la biomasse racinaire totale se retrouverait dans les deux premiers mètres de profondeur. Lei et al. (2012b) ont également rapporté une augmentation du « turnover » et de la productivité racinaire dans les communautés, attribuable aux effets complémentaires issus de la diversité fonctionnelle dans une plantation expérimentale d'arbres âgés de 6 ans. Un effet de l'identité d'espèces dominantes (*Picea abies* et *Pseudotsuga menziesii*) ainsi que l'ajout de fertilisant ont également été soulignés par ces auteurs afin d'expliquer ce gain de biomasse racinaire. Il a également été montré que les effets complémentaires de la diversité des espèces ont des effets sur les cycles biogéochimiques du sol, comme en témoigne l'étude de Fornara and Tilman (2009), qui ont trouvé un effet significativement positif sur le pourcentage d'N total du sol, ainsi que sur les taux de minéralisation de l'N du sol.

Les communautés microbiennes du sol

Au-delà des facteurs abiotiques comme la température, l'humidité, ou encore le pH, les microorganismes du sol jouent un rôle central dans les processus biogéochimiques du sol. En effet, ils représentent une composante essentielle, principalement caractérisée par les champignons (eucaryotes) et les bactéries (procaryotes). Une densité de 10^9 microorganismes par gramme de sol peut être atteinte, ainsi que de formidables niveaux de diversité : quelques dizaines à centaines de milliers de taxons ou groupes microbiens par gramme (Torsvik and Øvreås 2002). Chaque année, 90% de la biomasse végétale terrestre échappe aux herbivores et entre dans le « pool » de matière organique morte, alimentant ainsi les réseaux trophiques complexes à base de détritus qui déterminent l'équilibre essentiel entre la minéralisation et la séquestration du C dans les sols (Cebrian 1999). Les différentes espèces d'arbres qui varient énormément entre elles en terme de qualité et quantité de litière ainsi que d'exsudats racinaires (Mitchell et al. 2012, Vesterdal et al. 2012) vont grandement influencer la diversité des communautés microbiennes du sol. En effet, nous savons que les décomposeurs du sol n'ont pas tous les mêmes rôles ni les mêmes besoins et qu'ils n'ont pas la même importance dans les processus de dégradation. Par exemple, les bactéries du sol sont capables de répondre plus rapidement à des amendements organiques que les champignons, qui interviendraient à un stade plus tardif de la décomposition lorsque la matière organique est plus récalcitrante (Ingwersen et al. 2008, McMahon et al. 2005). Or, certains type de litières vont être riches en nutriments ou en C facilement utilisables (C labile) tandis que d'autres contiennent des concentrations plus élevées en composants organiques difficilement dégradables telle que la lignine (C récalcitrant). D'autres types de litières peuvent contenir des composants riches en N (facilement dégradable) mais contenir en plus plusieurs composants répulsifs pour les microbes tels que des tannins ou encore des alcaloïdes (Coq et al. 2010). Cette grande variabilité présente dans les mélanges de litières permet aux microbes d'optimiser leur acquisition des nutriments (i.e. utilisation complémentaire des ressources) par rapport aux monocultures (Gartner and Cardon 2004, Hättenschwiler et al.

2005). En 2005, Hättenschwiler and Gasser ont mis en évidence un effet positif de la diversité de la litière sur la décomposition de la litière de mauvaise qualité; la décomposition de cette dernière étant accélérée par les détritivores dans les mélanges mixtes. Malgré leur importance, les microbes du sol sont encore mal connus et seulement 7000 procaryotes ont été décrits à l'heure actuelle (Alain and Querellou 2009). Pourtant, plusieurs approches sont possibles lorsque l'on veut étudier et comprendre le rôle et l'importance de cette composante microbienne tellurique dans les fonctions délivrées par le sol. En voici trois exemples:

Méthode de la **biomasse microbienne** qui consistera à déterminer la biomasse microbienne (initialement proposée par Jenkinson (1966)) afin de permettre de quantifier le pool microbien du sol. Cette méthode a souvent été utilisée afin d'évaluer les impacts de différentes perturbations (pratiques agricoles, pollution, etc.) dans les agrosystèmes (Chaussod 1996, Kandeler et al. 1999, Marschner et al. 2003, Lejon et al. 2005, Ranjard et al. 2006). Or, bien que la biomasse microbienne soit toujours considérée comme étant un bon bio-indicateur de la qualité des sols (Marschner et al. 2003), cette dernière manque de précision puisqu'elle prend en compte l'ensemble des microorganismes présents (vivants ou non) et ne fait pas de différence entre les domaines des microorganismes (archaea-champignons-bactéries). Elle nécessite donc d'être complétée par des méthodes plus informatives sur les processus étudiés telles que la méthode de la mesure des **capacités métaboliques et cataboliques** qui consistera à apporter une mesure fonctionnelle des communautés microbiennes du sol en nous renseignant sur leur activité. La méthode de ce type qui sera utilisée lors de ce présent projet de doctorat est la méthode de micro-respirométrie (MicroResp™) proposée par Campbell et al. (2003). Cette dernière permettra d'utiliser les aptitudes des communautés microbiennes à utiliser certains substrats organiques précisément choisis tels que des sucres, des acides aminés ou des acides carboxyliques. La technologie BioLog est une méthode similaire connue mais présentant moins d'avantages que MicroResp™. En effet, MicroResp™ est plus rapide (4 à 6 h) et permet d'analyser des sols entiers (pas d'extraction ni de culture d'organismes) par rapport à la technologie BioLog. De nombreuses études réalisées jusqu'à présent ont eu recours aux mesures de densité (biomasse microbienne) et d'activité (Lundquist et al. 1999, Griffiths et al. 2001, Gaillard et al. 2003, Zelenev et al. 2005), mais ces dernières ont leurs limites. En effet, elles ne permettent pas de refléter la diversité des communautés microbiennes responsables de la dégradation de la matière organique du sol (MOS). En effet pour connaître la composition des microorganismes du sol il faudra avoir recours à des méthodes d'analyse de la **structure des communautés microbiennes** à travers la technique d'extraction des acides gras phospholipidiques (PhosphoLipid Fatty Acid) « PLFA ». Les phospholipides sont des composants essentiels des membranes cellulaires vivantes et sont donc utilisés comme indicateurs de la microflore du sol (Zelles 1999, Nannipieri et al. 2003). La force de cette méthode réside dans sa capacité à fournir différentes informations à partir d'un même échantillon. En effet, l'analyse des PLFA peut fournir (i) des mesures de biomasses bactérienne et fongique; (ii) des informations taxonomiques sur les communautés microbiennes (e.g.champignons, bactéries, Gram +, Gram -); (iii) des profils représentant la structure des communautés microbiennes dans l'échantillon.

Traitements sylvicoles et fonctionnement des écosystèmes

L'afforestation (i.e. plantation d'arbres sur une surface longtemps restée dépourvue d'arbre) est considérée comme un outil important pour l'atténuation des émissions de gaz à effets de serre ainsi que la mitigation du CO_2 atmosphérique d'origine anthropique (Paul et al. 2002, Arevalo et al. 2009). Or, l'établissement de plantations implique nécessairement plusieurs traitements sylvicoles qui vont avoir un impact plus ou moins grand sur les propriétés du sol et sur les successions végétales. En effet, certaines études ont montré que les traitements sylvicoles appliqués peuvent influencer la succession végétale des sous-bois (Jones et al. 2009) ainsi que les propriétés physiques du sol telles que la densité (Scott et al. 2007) ou le pH (Poirier et al. 2016) qui à leur tour, peuvent influer sur la dynamique et l'accumulation du C et de l'N dans les sols. Plusieurs études ont exploré l'effet des espèces d'arbres ou du type de couvert forestier (Paré et al. 2006, Fissore et al. 2009) sur différentes fractions de C des sols. La fraction labile de C (i.e. le C de la matière organique non encore stabilisé dans la phase minérale et qui peut être facilement disponible) devrait répondre le plus rapidement aux propriétés physiques, chimiques, et aux changements microclimatiques du sol (Khanna et al. 2001). De plus, dans leur étude de 2007, Hobbie et al. confirment que les espèces d'arbres contribuent aux variations de la dynamique du C, de l'N et de la matière organique des sols (MOS) par différents mécanismes et différents degrés que ce soit dans les horizons organiques ou minéraux. Il apparait que la dynamique du C serait largement influencée par l'impact des espèces sur sa stabilité chimique alors que celle de l'N serait en grande partie influencée par des variations dans la chimie des détritus, les taux de minéralisation et la nitrification de l'N étant influencés par la chimie et la biomasse racinaire. Dans leur étude effectuée dans la forêt expérimentale de Petawawa (alors âgée de 20 ans), Maillard et al. (2010) ont étudié l'effet de différentes pratiques de gestion (scarifiage, fertilisation, contrôle de la végétation via application de phytocide) et des espèces d'arbres sur les stocks et la fraction labile de C dans ces sols. Leurs résultats montrent, 20 ans après application des traitements, une diminution mineure (9 %) du contenu en C dans le sol minéral de surface (0-15 cm) en condition de contrôle de la végétation par un phytocide. Bien que mineure, ce type de perte de C dans les plantations pourraient avoir des conséquences importantes pour les budgets de C dans ces écosystèmes. Le scarifiage est le traitement qui aura eu le plus d'effet sur les stocks de C organique du sol et sur leur stabilité. En effet, les auteurs ont pu montrer que la teneur en C était réduite de 54,2 % dans l'horizon F/H (équivalent des horizons Oi,Oe/Oa définies selon la Soil Survey Staff. (1993)) des parcelles scarifiées par rapport à celles des parcelles non scarifiées. Ces effets sur les horizons minéraux du sol sont moins évidents et en partie atténués par l'ajout d'engrais (traitement de fertilisation). Le scarifiage a également affecté la qualité générale du C dans l'horizon F/H en augmentant de 21.9 % la fraction de C labile (mesuré par incubation) sur C total. Néanmoins, dans les parcelles scarifiées le contenu absolu en C labile était 51,9 % inférieur que dans les autres traitements. De plus, une concentration en C totale plus élevée (18,6 %) dans les horizons F/H sous l'épinette blanche que sous le pin blanc a été également observée (traitement de contrôle de la végétation).

Plan de thèse

L'objectif général de ce projet de doctorat aura été de mieux caractériser le lien entre la biodiversité et le fonctionnement des écosystèmes (BEF), et de mieux comprendre les mécanismes sous-jacents à ce lien. Comme mesures de biodiversité, nous avons eu recours à des gradients artificiels (de SR et de FD) ou des gradients induits par des traitements sylvicoles. Les fonctions de l'écosystème étudiées ici étaient particulièrement en lien avec le cycle du C et de l'N dans les sols à savoir : diversité et fonctionnement des communautés microbiennes du sol, productivité, chimie et décomposition des racines fines du sol. De plus, puisque nos dispositifs expérimentaux nous le permettaient, nous avons également porté notre attention sur l'aspect identitaire des communautés d'arbres et d'espèces du sous-bois étudiées que cela soit par rapport à l'identité de l'arbre en soi, à son groupe fonctionnel (feuillus vs conifères) ou encore à l'identité des traits fonctionnels de ces arbres.

Cette thèse de doctorat se présentera sous forme de trois chapitres, chacun traitant de l'un des aspects listés dans l'objectif général ci-dessus.

Dans le premier chapitre nous nous sommes intéressés à examiner l'effet de la diversité des arbres ainsi que l'influence des espèces d'arbres et de leur groupe fonctionnel (feuillus vs conifères) sur la productivité des racines fines dans une jeune plantation d'arbres de forêt tempérée (dispositif IDENT de Sainte-Anne-de-Bellevue au sud de Montréal). De plus nous avons examiné les effets de l'identité et de la diversité de ces arbres sur la chimie des racines fines ainsi que sur les C et N totaux du sol et dans les fractions (fraction grossière et fraction fine).

Le second chapitre a fait appel au même dispositif expérimental que pour l'étude du chapitre 1, et a eu pour but de mieux caractériser le lien entre la diversité des arbres (gradients artificiels de SR et de FD) ainsi que leur identité (identité des arbres en soi et identité de leurs traits fonctionnels), et les communautés microbiennes du sol - en termes de structure (via PLFA) et de fonctionnement (via MicroResp™). Ce second chapitre a également eu pour but de mieux comprendre les mécanismes sous-jacents à ces liens.

Finalement, le troisième chapitre s'est concentré sur l'étude de l'effet de la diversité des espèces d'arbres et de la végétation du sous-bois, induite par des traitements sylvicoles appliqués il y a

27 ans, sur la décomposition des racines fines ainsi que sur les C et N totaux du sol et dans les fractions (fraction grossière et fraction fine). Cette étude a été effectuée sur une plantation de la forêt expérimentale de recherche de Petawawa, Ontario.

Chapitre I

Do tree species richness and identity affect fine root production and soil C and N levels in young forest plantations?[1]

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[1] Article soumis prochainement: Khlifa, R., Angers, D., and A.D. Munson. Do tree species richness and identity affect fine root production and soil C and N levels in young forest plantations?

Abstract

An increasing body of literature provides evidence of a positive relationship between biodiversity and ecosystem functioning (BEF), however, there are far fewer studies of this relationship belowground. On experimental gradients of tree species richness (SR) (1, 2 and 4 species) and functional diversity (FD), we studied annual fine root productivity (FRP), fine root chemistry, mineral soil organic carbon (SOC) and nitrogen (N) levels. The gradients were established in young forest plantations of 12 North American temperate tree species (*Acer saccharum, Acer rubrum, Betula alleghaniensis*, *Betula papyrifera, Quercus rubra*, *Abies balsamea*, *Larix laricina*, *Pinus strobus*, *Pinus resinosa*, *Picea glauca*, *Picea rubens* and *Thuja occidentalis*). Annual fine root productivity was assessed using the ingrowth core method and SOC and N in the mineral soil were determined for both total elements and two size fractions (particulate organic matter – POM; silt and clay size fraction – S&C). FRP was not higher with either SR or FD. Only fine root C and Ca concentrations were significantly higher in SR mixtures of four species compared to monocultures. However, the individual trait variance (FDt) and the community-weighted mean trait value (CWM) of several functional traits affected (both positively or negatively) FRP; in general, CWM of traits had stronger effects than did FDt. We also observed differences in FRP among tree species, with the most marked effect observed according to leaf habit; conifer tree species had significantly higher FRP than deciduous tree species, while the opposite was observed for aboveground productivity. In the mineral soil, the mixtures of two species were characterized by lower total SOC compared to mixtures of four species, and lower N concentration in total and fine fractions compared to monocultures and four species mixtures. We observed no differences among tree species in C and N concentrations in the two size fractions. The fact that tree species identity rather than tree SR influence FRP, and that tree SR rather than tree species identity influence mineral soil SOC and N confirms the importance of considering both SR and the identity of tree species when studying the belowground BEF relationship.

Key words

Biodiversity, ecosystem functioning, tree species identity, tree species diversity, root productivity, root chemistry, soil organic carbon, IDENT, TreeDivNet

Résumé

Un nombre croissant d'études fait état d'une relation positive entre la biodiversité et le fonctionnement des écosystèmes (BEF). Cependant, il reste encore peu d'études axées sur les écosystèmes forestiers et les processus souterrains qui les caractérisent. Dans la présente étude, la productivité des racines fines (FRP) et leur chimie, le stockage du carbone (C) et de l'azote (N), ainsi que leurs fractions dans le sol, ont été mesurés le long de deux gradients artificiels de richesse spécifique (SR) d'arbres (1, 2 ou 4 espèces) et de diversité fonctionnelle (FD). L'échantillonnage a eu lieu dans une jeune plantation forestière de 12 espèces d'arbres tempérés d'Amérique du nord (*Acer saccharum, Acer rubrum, Betula alleghaniensis, Betula papyrifera, Quercus rubra, Abies balsamea, Larix laricina, Pinus strobus, Pinus resinosa, Picea glauca, Picea rubens et Thuja occidentalis*). La productivité des racines fines a été mesurée via la technique « ingrowth-cores », et le C organique du sol (SOC) et l'N du sol minéral superficiel ont été caractérisés à la fois en terme de concentrations totales et de concentrations retrouvées dans la matière organique particulaire (fraction grossière; POM et fraction fine; S&C). La FRP n'a pas été augmentée par l'augmentation de la SR ni de la FD. Seuls le C des racines fines et les concentrations de Ca étaient significativement plus élevés dans les mélanges de quatre espèces par rapport aux monocultures. Cependant, la variance individuelle (FDt) et la valeur moyenne pondérée sur la communauté (CWM) de plusieurs traits fonctionnels d'arbres ont eu un effet (que ce soit positivement ou négativement) sur la FRP; en général, la CWM des traits a eu des effets plus forts que ceux de la FDt. Nous avons également observé des différences de FRP entre les espèces d'arbres, avec un effet plus marqué observé selon le type de feuillage; les conifères avaient une FRP significativement plus élevée que les feuillus, alors que l'inverse a été observé pour la productivité des parties aériennes. Les sols des mélanges composés de 2 espèces présentaient moins de C total que les sols des mélanges de 4 espèces, et moins d'N (total et fraction fine) que dans les monocultures et les mélanges de 4 espèces. Nous n'avons observé aucune différence de concentration en C et N entre les espèces d'arbres dans les deux fractions (grossière et fine). Le fait que l'identité des espèces d'arbres plutôt que la SR influence la FRP, et que la SR plutôt que l'identité des espèces d'arbres influence le SOC et l'N du sol minéral confirme l'importance de considérer à la fois la SR et l'identité des espèces d'arbres lors de l'étude de la relation de BEF des parties souterraines.

Mots clés

Biodiversité; fonctionnement des écosystèmes; identité des espèces d'arbres; diversité des espèces d'arbres; productivité racinaire; chimie des racines; carbone organique du sol; IDENT; TreeDivNet

Introduction

Biodiversity and ecosystem function (BEF) studies have frequently noted a positive relationship between plant species diversity - plant species richness (SR) – and productivity, in a number of different ecological contexts (Cardinale et al. 2011). This positive effect may be the result of species complementarity, through facilitation or niche differentiation among species (Hooper et al. 2005, Cardinale et al. 2011), leading to overyielding (Cardinale et al. 2007), or the result of selection effects: the presence of more dominant species with particular traits (Loreau and Hector 2001). The diversity-function relationship has been less studied in treed ecosystems, and the link between diversity and belowground productivity (for example, fine root productivity: FRP) and processes is less well-documented than the aboveground compartment.

In grassland ecosystems, plant functional composition was shown to strongly influence root biomass (Tilman 2001, Fornara et al. 2009), affecting both carbon (C) inputs and nitrogen (N) cycling, and root chemistry (e.g. root N concentration of C4 grasses was lower than root N of C3 grasses and legumes; see Dijkstra et al. 2006; Vivanco and Austin 2006). Fornara et al. (2009) highlighted that the presence and number of different plant functional groups (in mixtures) with different effects on root mass loss, root N release and soil N mineralization rates, may be crucial for sustaining multiple ecosystem services such as productivity and soil C and N sequestration in N-limited grassland systems. Positive effects (Amoroso and Turnblom 2006, Pretzsch and Schütze 2009, Tobner et al. 2016) insignificant (Cavard et al. 2010) or negative effects (Edgar and Burk 2001) of SR on aboveground tree productivity were demonstrated, while both positive (Brassard et al. 2011) and no effects (Jacob et al. 2014, Domisch et al. 2015) were observed for fine root productivity with increasing SR.

Although we know that roots are a principal input to soil C and N (Rasse et al. 2005) few studies have reported the influence of species diversity on these parameters. In grasslands, a positive effect of plant species diversity on soil C and nitrogen N stocks was demonstrated (Steinbeiss et al. 2008, Fornara and Tilman 2008). Guckland et al. (2009) reported a negative effect of tree species diversity (monospecies, 3 and 5 species mixtures in beech-dominated stands) on forest floor C in mixed broadleaf forests in Germany, while Laganière et al. (2015) observed no effect of tree species mixtures (in natural boreal stands) versus monospecific stands, on soil carbon stocks.

Gurmesa et al. (2013) studied the effects of four tree species on soil C and N stocks in a common garden design replicated at eight sites in Denmark. After four decades, mineral soil C stocks were significantly higher in Norway spruce stands than in stands of oak and beech, while soil N stocks did not differ among stands. In a literature review, Augusto et al. (2015) noted that while stocks of soil C were comparable between deciduous and evergreen species, nutrient cycling was generally higher in deciduous-dominated versus evergreen-dominated ecosystems. Vesterdal et al. (2013) synthesized current knowledge of tree species effects on SOC stocks in temperate and boreal forests to demonstrate consistent tree species effects on SOC stocks. These latter authors suggested that some tree species may be better engineers for sequestration of C in stable form in the mineral soil, but it was unclear whether the key mechanism is root litter input or macrofauna activity.

Several studies explored the effects of tree species or forest cover type on different C fractions (Paré et al. 2006, He et al. 2008, Yang et al. 2009). The labile fraction is generally a more sensitive indicator to physical, chemical, microclimatic changes in the soil environment (Khanna et al. 2001) and to management impacts (He et al. 2008) than the other fractions. In addition to reporting an effect of tree species identity on SOC stocks (higher SOC under black spruce in surface mineral soil (0–15 cm) than under mixedwood or aspen), Laganière et al. (2011) measured C in different soil aggregate size classes and soil physical fractions; they observed that black spruce soils accumulated more uncomplexed organic matter (the less protected fraction) compared to aspen. In addition to total C and N levels in surface mineral soil, the present study is the first to investigate the potential effect of SR on size fractions of C and N (POM and fine fraction) stored in soil.

In a study of fine root decomposition in a common garden among 11 temperate tree species, Hobbie et al. (2010) showed that species effects on C and N dynamics of fine roots were unrelated to those of leaf litter, highlighting that studies focusing solely on the aboveground compartment may obscure important mechanisms by which species influence ecosystem processes. The present study aimed to evaluate the influence of tree diversity (artificial SR and functional diversity (FD) (only on FRP) gradients of temperate species) and identity (of the trees and functional traits) on FRP, fine root chemistry and soil C and N concentration (including total and size fractions). To do so, we used a young, controlled experimental plantation of monocultures and mixed-species stands in southern Quebec, Canada (Tobner et al. 2014). Monocultures consisted of twelve deciduous or conifer North American species, while mixed species stands were represented by an SR gradient of two and four species (14 two-species mixtures and 10 four-species mixtures). The FD gradient was developed *a priori* (Tobner et al. 2014) by choosing species combinations at SR levels of 2 and 4 which varied in FD, enabling independent tests of FD while holding SR constant. The FD metrics were based on functional dispersion (FDis, Laliberté and Legendre 2010). We also tested the effect of the average values of traits (CWM: functional mean) and their variance (FDt) (Ricotta and Moretti 2011).

We hypothesized that:

Fine root productivity will increase with increasing tree SR and FD and that;

1) Fine root macronutrient concentrations (N, P, K, Ca, Mg) as well as soil organic C and N (in bulk soil and in the labile fraction: POM) will be higher in mixed compared with single-species stands due to higher complementarity (and lower competition) among tree species in mixtures, and greater input of litter under mixtures. Fine root C will be lower under mixtures compared to monocultures (higher quality of litter).
2) Tree species identity and functional group identity (conifer vs. deciduous species) will differentially affect below-ground functioning; we expect that some deciduous tree species will show elevated fine root productivity (as noted for aboveground; Tobner et al. (2016)), higher fine root nutrient concentrations and higher soil N (in bulk soil and in different fractions), but lower root C and soil C, compared with coniferous tree species. This difference is related to higher nutrient levels in above- and belowground litter and higher decomposition rates of deciduous compared to coniferous litter.

Methods

Study site

The study site is located at Ste-Anne-de-Bellevue, near Montreal, Québec, Canada (45°28N, 73°45W, 36 m asl). Mean annual temperature is 6.1 °C and mean annual precipitation is 973 mm (Dorval climate station, Environment Canada, 2014). The young experiment is part of « International Diversity Experiment Network with Trees » (IDENT), that includes seven sites in

North America and Europe (Tobner et al. 2014); it was established on a former agricultural field in spring 2009, with seedlings of 1 (deciduous) or 2 (conifer) years of age. The 12 North American temperate tree species included early and late successional species: *Acer saccharum* Marsh (Sugar Maple); *Acer rubrum* L. (Red Maple); *Betula alleghaniensis* Britton (Yellow Birch); *Betula papyrifera* Marsh (Paper Birch) and *Quercus rubra* L. (Northern Red Oak); *Abies balsamea* (L.) Mill. (Balsam Fir); *Larix laricina* (Du Roi) K. Koch. (Tamarack); *Pinus strobus* L. (Eastern White Pine); *Pinus resinosa* Aiton (Red Pine); *Picea glauca* Voss (White Spruce); *Picea rubens* Sarg. (Red Spruce) and *Thuja occidentalis* L. (Eastern White Cedar). Trees were planted in a square plot of eight by eight individuals, at a 50 cm planting distance (so that diversity effects on ecosystem processes are visible more rapidly) to create 36 different tree communities; each was replicated in 4 blocks. Each block consists of 12 monocultures (SR1), 14 combinations of two-species mixtures (SR2) and 10 combinations of four-species mixtures (SR4). Within each block, plots were randomly distributed and separated by 1.25 m corridors to allow circulation within the site and to minimize disturbance. Tree communities were weeded manually to eliminate understory plants, and corridors between plots were trenched at 3 and 4 years (2011 and 2012) to prevent the roots from growing into adjacent plots. An enclosing fence was installed to protect trees from herbivory.

Root productivity

We measured fine root productivity (≤ 2mm diameter) over one year using a modified ingrowth core method (Lund et al. 1970). In each plot in early June 2012, two cores (8 cm diameter, 15 cm depth; based on preliminary samples and data from Archambault et al. in preparation) were sampled randomly in a central point between four trees, to maximise species interactions in tree neighbourhoods, and were refilled with sieved (4 mm), root-free soil. The exact position of ingrowth core boundaries was marked with flags to allow sampling the same placement when retrieving the cores. In early June, 2013, the ingrowth cores were retrieved and all live and dead roots were manually removed from the soil samples and washed then dried in a forced-air oven at 65°C to constant weight. Total fine root production (≤ 2mm: FRP) was estimated as the sum of live and dead roots present in the ingrowth core in June 2013.

Data from a study on the same site and species (Tobner et al. 2016) allowed us to compare our FRP data to aboveground productivity data.

Fine root chemistry

Fine roots (≤ 2mm diameter) were rinsed, dried in a forced-air oven at 65°C to constant weight, then finely ground using a ball mill apparatus (Retsch mixer mill, MM200, Retsch GmbH, Haan, Germany). For root chemistry, C was determined by dry combustion (Trumac CNS, LECO Corp. MI, USA) and the concentrations of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) were determined by digestion (Parkinson and Allen 1975) followed by atomic absorption spectroscopy (Optima 4300 DV ICP-OES, Perkin-Elmer Inc., Waltham, MA, USA).

Soil sampling and analyses

A second series of soil cores was sampled at the end of the growing season in October 2012 (in parallel with root biomass at one date), using manual aluminum cylindrical cores (7 cm diameter, 0-15 cm depth). From the center of each plot of two and four species, five randomly-sampled soil cores were extracted, three soil cores were sampled in the same way for monoculture plots (considering lower variability within plots). Soil samples were immediately refrigerated for transport to the laboratory and frozen at -18 °C prior to analyses. In March 2013, soil samples were unfrozen and sieved at 2 mm. Debris and roots were removed. Soil texture was assessed by decantation (Bouyoucos 1962) and soil pH was measured in a prepared 0.01M $CaCl_2$ solution that is added to soil in a 1:2 soil to liquid mixture (Hendershot et al. 1993).

Physically uncomplexed organic matter was isolated according to a modified method (Gregorich and Beare 2008), by dispersing soil in distilled water (1:4 soil: water ratio) and shaken overnight with about 12 glass balls (6 mm diameter) to disrupt aggregates (Poirier et al. 2014). Subsequently, the soil and water mixture was washed over a 53 µm sieve using a fine jet of distilled water, gently crushing aggregates with a rubber spatula. Two fractions were obtained: the particulate organic matter (POM; sand + large particles of organic matter > 53 µm) and the fine particle-size (S&C; silt + clay, <53 µm). The former was retained on the sieve and oven dried at 60°C overnight. The dry mass of this material was recorded before grinding with a ball mill, and homogenized to determine the SOC and N concentrations of the POM (POM-C and POM-N respectively). The SOC and N concentrations of the fine particle-size (S&C; silt + clay, <53 µm) were estimated by subtracting the POM fractions from total soil C and N concentrations. All C and N concentrations were determined by dry combustion with an elemental analyser (CNS-1000, LECO Corp. St. Joseph, MI).

Functional diversity and community-weighted traits calculation

In addition to the initial FD gradient, functional dispersion was calculated using the “FD” R package (Laliberté, Legendre and Shipley 2014) for each trait (FDt) separately, in order to identify the relative importance of each trait on FRP. The average values of functional traits (CWM) - functional mean– were calculated for each community in each plot (36) as the product of the average trait value of a species and its relative abundance in the community, averaged across all species in the community. Both CWM traits and functional variation (FDt) were weighed by species’ relative dominance measured as volume (calculated from diameter and height,(Tobner et al. 2014)). The traits used to calculate the litter and root chemistry, were as much as possible measured *in situ* (Table 3) (Tobner et al. 2014; Jewell et al. 2015).

Statistical analyses

All statistical analyses were performed with R software (version 3.2.1 R Core Team 2015).

Linear mixed-effect models were developed using the *lme* function in the *nlme* package (Pinheiro et al. 2007) to test the effect of SR, FD (when applicable), or tree species identity on fine roots productivity, fine root C and macronutrient concentrations (N, P, K, Ca, Mg) and soil C and N (in soil and in physically uncomplexed organic matter), with treatments and blocks factors included as random factors. Normality of residuals and homogeneity of variance were tested and transformations were performed when necessary. This was followed by means separations between SR levels or tree species identity using Tukey’s HSD tests with the *glht* function in the *multcomp* package (Hothorn et al. 2008). Statistical significance was set at the 0.10 level given the inherent large variability in forest soils.

Results

Tree SR effects on fine root productivity and chemistry

Species richness did not affect FRP (annual fine root production from June 2012- June 2013). Moreover, FRP and did not differ between monocultures and mixed-species stands (Table 1). Fine root C and Ca concentrations were significantly higher in SR4 than in monocultures (*p* = 0.059 and *p* = 0.083 respectively) but did not differ between monocultures and SR2, nor between SR2 and SR4. Fine root concentrations of the macronutrients N, P, K and Mg (Table 1) as well as the root C:N ratio (Table 2) did not vary between monocultures and mixed- species stands.

Table 1. Average values of fine root productivity (FRP) and carbon (C), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) concentrations of fine roots of tree species mixtures. SR1 (single species). SR2 (two species mixtures) and SR4 (four species mixtures) and their identities at the IDENT Montreal site (Québec, Canada). ABBA (*Abies balsamea*), ACRU (*Acer rubrum*), ACSA (*Acer saccharum*), BEAL (*Betula alleghaniensis*), BEPA (*Betula papyrifera*). LALA (*Larix laricina*). PIGL (*Picea glauca*). PIRU (*Picea rubens*). PIRE (*Pinus resinosa*). PIST (*Pinus strobus*). QURU (*Quercus rubra*), THOC (*Thuja occidentalis*). Mean values with the same letter are not significantly different based on the Tukey-HSD post hoc test. Standard errors are given in brackets.

	FRP	C	N	P	K	Ca	Mg
	[g m-^{2} yr-^{1}]	[g kg-1]	[g kg-1]	[g kg-1]	[g kg-1]	[g kg-1]	[g kg-1]
Species richness							
SR1	9.99 (±0.89) a	491.98 (±1.28) a	11.48 (±0.31) a	1.71 (±0.07) a	1.61 (±0.06) a	8.96 (±0.22) a	1.08 (±0.02) a
SR2	10.45 (±0.81) a	483.78 (±8.83) ab	12.13 (±0.23) a	1.57 (±0.08) a	1.59 (±0.05) a	9.35 (±0.14) ab	1.11 (±0.02) a
SR4	9.49 (±0.83) a	495.61 (±1.00) b	11.57 (±0.28) a	1.59 (±0.08) a	1.64 (±0.04) a	9.56 (±0.21) b	1.15 (±0.03) a
Tree species							
ABBA	11.75(±2.78) ab	487.69 (±5.50) a	12.07 (±0.26) be	2.12 (±0.25) de	1.84 (±0.16) ab	10.48 (±0.39) bc	1.26 (±0.07) bc
ACRU	9.35 (±2.88) a	485.65 (±3.36) a	12.13 (±0.47) be	1.91 (±0.16) ce	1.99 (±0.21) b	7.83 (±0.39) ab	1.34 (±0.08) c
ACSA	2.45 (±0.52) a	482.87 (±8.88) a	14.38 (±0.52) e	1.94 (±0.20) ce	1.89 (±0.26) ab	7.4 (±0.27) a	1.2 (±0.10) ac
BEAL	8.68 (±1.93) ab	498.86 (±2.10) a	9.95 (±0.86) ab	1.56 (±0.24) acd	1.52 (±0.10) ab	10.73 (±0.77) c	0.99 (±0.07) ab
BEPA	3.64 (±0.89) a	497.41 (±1.78) a	10.96 (±0.64) bd	1.31 (±0.27) ab	1.45 (±0.08) ab	10.34 (±0.57) bc	0.99 (±0.04) ab
LALA	18.54 (±5.63) b	492.64 (±3.67) a	13.19 (±0.53) cde	1.93 (±0.28) ce	1.48 (±0.07) ab	8.49 (±0.64) ac	1 (±0.05) ac
PIGL	9.64 (±0.63) ab	495.54 (±2.24) a	10.53 (±1.15) bc	2.15 (±0.14) e	1.77 (±0.23) ab	8.46 (±1.03) ac	0.94 (±0.08) ab
PIRE	8.09 (±1.90) b	490.2 (±2.45) a	12.97 (±0.63) bd	1.78 (±0.29) bce	1.24 (±0.05) ab	8.39 (±0.40) ac	1.02 (±0.01) ac
PIRU	19.90 (±2.33) a	495.22 (±2.12) a	10.89 (±0.28) cde	1.7 (±0.27) bce	1.54 (±0.12) a	8.41 (±0.30) ac	1.1 (±0.11) ac
PIST	11.23 (±1.22) ab	482.87 (±3.32) a	13.63 (±0.37) de	1.62 (±0.26) bce	1.16 (±0.15) a	9.58 (±0.54) ac	1.04 (±0.08) ac
QURU	4.52 (±2.99) a	498.02 (±1.36) a	7.5 (±0.60) a	1.49 (±0.38) ac	2.16 (±0.23) b	7.28 (±0.74) a	1.18 (±0.02) ac
THOC	11.31 (±3.27) ab	496.75 (±3.66) a	9.5 (±0.38) ab	1.05 (±0.19) a	1.23 (±0.05) a	10.14 (±0.31) bc	0.9 (±0.06) a

Table 2. Average values of fine root C:N ratio, soil organic carbon (C), soil nitrogen (N), and soil C:N ratio of tree species mixtures SR1 (single species), SR2 (two species mixtures) and SR4 (four species mixtures) and their identities at the IDENT Montreal site (Québec, Canada). ABBA (*Abies balsamea*), ACRU (*Acer rubrum*), ACSA(*Acer saccharum*), BEAL (*Betula alleghaniensis*), BEPA (*Betula papyrifera*), LALA (*Larix laricina*), PIGL (*Picea glauca*), PIRU (*Picea rubens*), PIRE (*Pinus resinosa*), PIST (*Pinus strobus*), QURU (*Quercus rubra*), THOC (*Thuja occidentalis*). Mean values with the same letter are not significantly different based on the Tukey-HSD post hoc test. Standard errors are given in brackets.

	Fine roots	**Soil**						
	C:N ratio	C [g kg-1]	N [g kg-1]	C-POM	C-S&C	N-POM	N-S&C	C:N ratio
				[g SOC $_{POM}$ kg-^{1} soil]				
Species richness								
SR1	44.8(±1.56) a	23.0(±0.03) ab	2.2 (±0.00) b	7.7(±0.17) a	15.2 (±0.36) a	0.47 (±0.02) a	1.69 (±0.06) a	10.9 (±0.2) a
SR2	40.8(±1.12) a	22.4(±0.03) a	2.0 (±0.00) a	7.6(±0.14) a	14.7 (±0.32) a	0.50 (±0.02) a	1.49 (±0.04) b	11.3 (±0.1) b
SR4	43.8(±1.06) a	23.5(±0.05) b	2.2 (±0.00) b	7.9(±0.21) a	15.4 (±0.47) a	0.51 (±0.02) a	1.68 (±0.07) a	10.9 (±0.22) ab
Tree species								
ABBA	40.4(±0.78) ac	23.6(±0.12) a	2.3(±0.03) a	6.8(±0.50) a	16.7(±1.22) a	0.44(±0.06) a	1.87(±0.35) a	10.5(±0.9) a
ACRU	40.3(±1.93) ac	23.1(±0.09) a	2.3(±0.02) a	7.0(±0.77) a	16.0(±0.28) a	0.46(±0.09) a	1.79(±0.22) a	10.4(±0.8) a
ACSA	33.6(±0.61) a	21.4(±0.12) a	2.0(±0.01) a	7.1(±0.76) a	14.3(±1.02) a	0.42(±0.09) a	1.59(±0.23) a	10.7(±0.7) a
BEAL	51.5(±5.16) c	23.8(±0.6) a	2.2(±0.03) a	7.7(±0.41) a	16.0(±1.63) a	0.46(±0.04) a	1.72(±0.34) a	11.3(±0.9) a
BEPA	45.9(±3.11) ac	23.2(±0.20) a	2.2(±0.02) a	8.03(±0.49) a	15.1(±2.14) a	0.50(±0.07) a	1.67(±0.25) a	10.7(±0.6) a
LALA	37.5(±1.39) ac	23.6(±0.15) a	2.3(±0.02) a	8.84(±0.49) a	14.7(±1.40) a	0.51(±0.6) a	1.78(±0.24) a	10.5(±0.7) a
PIGL	49.0(±6.19) bc	23.5(±0.13) a	2.2(±0.01) a	9.17(±0.41) a	14.3(±1.48) a	0.51(±0.03) a	1.67(±0.20) a	10.9(±0.5) a
PIRE	45.9(±2.54) ac	23.3(±0.06) a	2.3(±0.01) a	7.51(±0.35) a	15.8(±1.46) a	0.56(±0.15) a	1.56(±0.22) a	10.1(±0.9) a
PIRU	37.8(±0.90) ac	23.3(±0.14) a	2.1(±0.01) a	8.48(±0.69) a	14.8(±0.54) a	0.50(±0.12) a	1.83(±0.23) a	11.0(±0.5) a
PIST	35.5(±1.05) ab	21.7(±0.14) a	1.9(±0.02) a	6.98(±0.32) a	14.7(±1.31) a	0.38(±0.04) a	1.56(±0.26) a	11.5(±0.8) a
QURU	67.7(±5.68) d	22.9(±0.16) a	2.0(±0.01) a	7.85(±0.69) a	15.0(±1.67) a	0.47(±0.09) a	1.57(±0.22) a	11.3(±0.5) a
THOC	52.5(±2.09) c	22.7(±0.17) a	2.0(±0.02) a	7.18(±0.55) a	15.4(±1.46) a	0.42(±0.08) a	1.59(±0.27) a	11.4(±0.5) a

Tree functional traits effects on fine root productivity

The FRP did not increase along the FD gradient (*p* = 0.304). However, when the variance of each functional trait (FDt) was tested separately, we found a positive effect of root diameter and a negative effect of litter N content, root Ca concentration and tree height on FRP (Table 3).

FRP was positively related to CWM of leaf litter C content, root diameter and root P concentration and negatively related to CWM of leaf litter nitrogen content, specific leaf area, root branching intensity, specific root length, root K concentration, tree height, wood density and seed mass (Table 3).

Table 3. Effect of individual trait means (CWM) and variances (FDt) on fine root productivity (FRP). The values presented are the *p-values* of mixed model regressions and significant positive or negative effects (at α = 0.05) are represented with arrows.

Functional Trait	**FRP**	
	FDt	CWM
Litter nitrogen content	0.028↘	0.026↘
Litter carbon content	0.992	<0.001↗
Leaf dry matter content	0.914	0.842
Specific leaf area	0.171	<0.001↘
Root diameter	0.053↗	<0.001↗
Root banching intensity	0.362	<0.001↘
Specific root length	0.658	<0.001↘
Root nitrogen concentration	0.838	0.187
Root carbon concentration	0.285	0.430
Root phosphorus concentration	0.121	0.017↗
Root potassium concentration	0.741	0.026↘
Root calcium concentration	0.024↘	0.789
Root magnesium concentration	0.598	0.180
Tree height	0.030↘	<0.001↘
Ground diameter	0.870	0.196
Wood density	0.800	<0.001↘
Seed mass	0.153	0.035↘

Table 4. Average values of mean carbon (C) and fine root macronutrients (N, P, K, Ca, Mg) and C:N ratio between conifers and deciduous tree species at the IDENT Montreal site (Québec, Canada). Mean values with the same letter are not significantly different based on the Tukey-HSD post hoc test. Standard errors are given in brackets.

	Conifers	**Deciduous**
C [g kg-1]	492.8(±0.8) a	485.6(±0.3) a
N [g kg-1]	11.9(±0.2) a	11.6(±0.3) a
P [g kg-1]	1.6(±0.1) a	1.6(±0.1) a
K [g kg-1]	1.6(±0.0) a	1.7(±0.0) b
Ca [g kg-1]	9.3(±0.1) a	9.3(±0.2) a
Mg [g kg-1]	1.0(±0.0) a	1.1(±0.0) b
Fine root C:N ratio	42.5(±0.8) a	43.7(±0.5) b

Tree identity effects on fine root productivity and chemistry

We observed differences among tree species in FRP (Fig.1A and Table 1), but the most marked effect of tree species identity on FRP was between tree functional group identity, as conifer tree species were significantly more productive than deciduous tree species (12.92 and 5.72 g m^{-2} yr^{-1} respectively, $p < 0.001$). Comparing our data to those of Tobner et al. (2016) on aboveground productivity (Fig.1B), we noted that deciduous tree species were more productive aboveground than belowground, while we observed the opposite for conifer species. We also observed differences among tree species in all macronutrients (N, P, K, Ca, Mg) concentrations (Table 1), as well as in fine root C:N ratio (Table 2) but not in root C concentration (Table 1). No differences were measured between deciduous and conifer tree species for fine root C concentration, neither for N, P and Ca concentrations (Table 4). However, fine root C:N ratio as well as fine root K and Mg concentrations were significantly higher in deciduous species compared to conifer species ($p = 0.032$, $p = 0.003$ and $p = 0.049$ respectively) (Table 4).

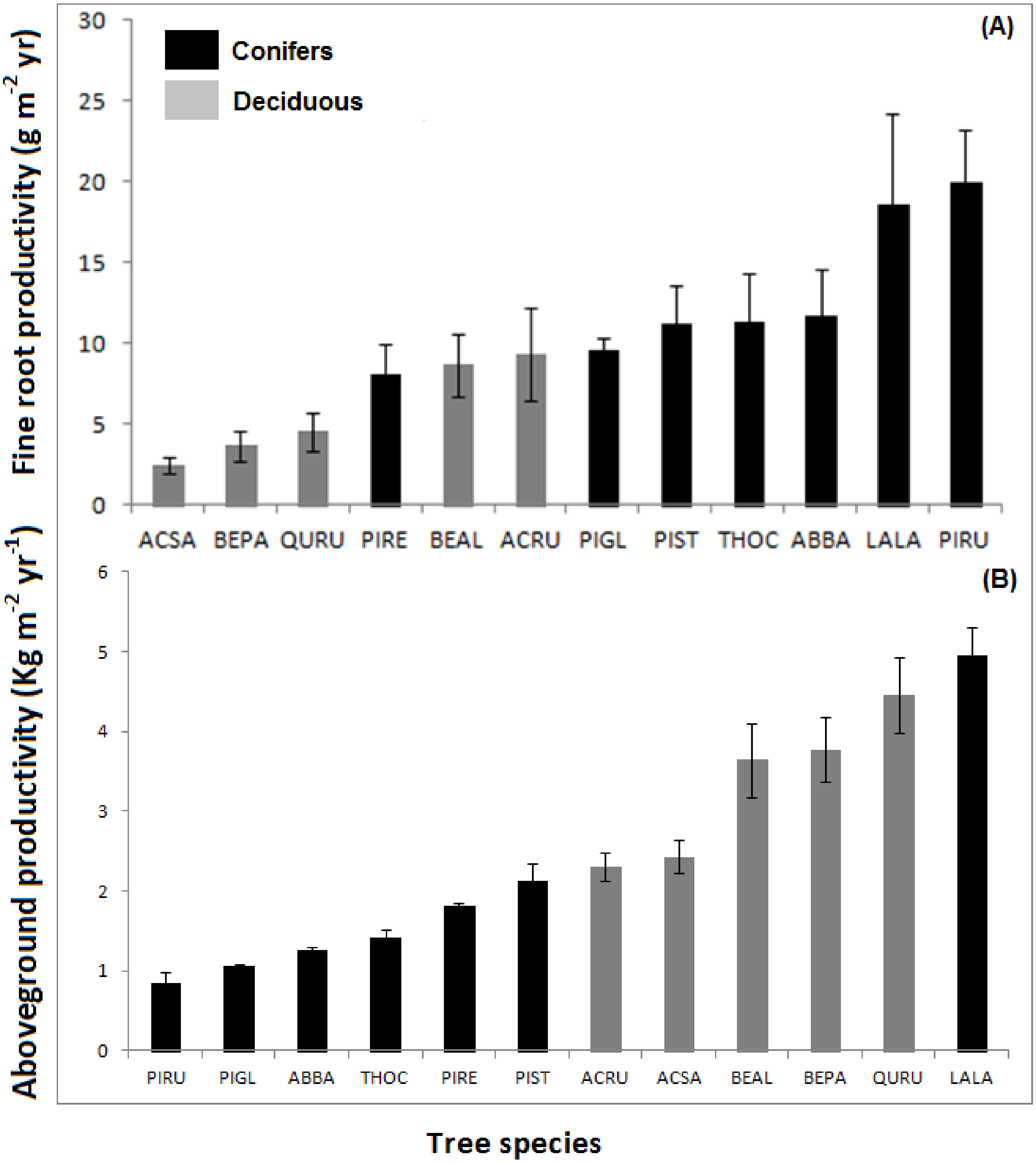

Figure 1. Annual fine root (A) and aboveground (B) productivity (2012-2013) of twelve tree species at the IDENT Montreal site (Québec, Canada). ABBA (*Abies balsamea*), ACRU (*Acer rubrum*), ACSA(*Acer saccharum*), BEAL (*Betula alleghaniensis*), BEPA (*Betula papyrifera*), LALA (*Larix laricina*), PIGL (*Picea glauca*), PIRU (*Picea rubens*), PIRE (*Pinus resinosa*), PIST (*Pinus strobus*), QURU (*Quercus rubra*), THOC (*Thuja occidentalis*). Error bars are standard errors.

Tree SR and identity effects on C and N concentrations in bulk soil and size fractions

Soil organic carbon (SOC) concentration (0-15 cm) was lower in SR2 than in SR4 ($p = 0.102$; Table 2). Mineral soil total N concentration was also significantly lower in SR2 compared to monocultures and SR4 mixtures ($p = 0.002$) (Table 2), while the latter were characterized by similar N concentrations. Soil C:N ratio was significantly higher in SR2 mixtures than in monocultures ($p = 0.0174$; Table 2). No differences in soil total C and N concentrations, nor soil pH were found among tree species (Table 2), neither between deciduous and conifer tree species (data not shown).

Species richness did not affect C and N concentrations in the two size fractions (POM and S&C) (Table 2). Although both C and N concentration in the less protected fractions (POM): POM-C and POM-N did not differ between monocultures and mixed-species stands. The N concentration in the fine fraction (S&C-N) was lower in SR2 compared to monocultures and SR4 mixtures ($p = 0.0012$). Monocultures and SR4 showed similar S&C-N (Table 2). Moreover, we observed no differences among tree species, neither between deciduous and conifers species, in C and N concentrations in the POM and in the S&C fractions (Table 2).

Discussion

Species richness effects on fine root annual productivity and chemistry

SR did not affect fine root annual productivity, which contradicts our first hypothesis. Similarly, Domisch et al. (2015) found no response of fine root production (or root biomass) in mixed stands compared to monocultures in two tree diversity experiments in Finland and Germany (of TreeDivNet and BIOTREE networks, respectively) The authors suggested that previous agricultural use of the experimental sites may have led to relatively high soil nutrient levels, thus limiting root competition. Studies in a young plantation and a mature natural forest (Bauhus et al. 2000, Meinen et al. 2009, respectively) attributed an absence of SR effects on fine root biomass to a lack of spatial separation of roots, and thus a similar soil exploitation strategy and increasing competition rather than complementarity. Similarly, fine root biomass measures in the fall of 2012 on our experimental site showed no effect of SR on rooting depth, fine rooting density, or biomass (Archambault et al. in preparation), suggesting no segregation or differences in exploitation of soils. This absence of segregation could be explained partly by soil fertility, as reported by Valverde-Barrantes et al. (2015) in nutrient rich soils. Our study site is a former agricultural field, with no water limitation (973 mm/yr) and potentially higher nutrient availability than forest soils (mean C/N ratio = 11). In general, SR effects are less pronounced on more fertile sites (Potter and Woodall 2014) and in less stressful (temperate compared to boreal) environments (Paquette and Messier 2011). In contrast, other studies reported positive effects of mixed compared to single-species forests on root biomass, productivity and vertical root distribution (McKay and Malcolm 1988, Erskine et al. 2006, Brassard et al. 2011, 2013). However, these studies were carried out in older forests than the present study. The young age of the stands may partly explanation an absence of response of fine root productivity to SR, as belowground competition may not yet be present, as noted by Domisch et al. (2015). Although Lei et al. (2012a; 2012b) suggested that belowground competition among species in mixed stands (young temperate forest plantation) may occur earlier than aboveground, on our site, a positive net biodiversity effect on aboveground tree growth was measured (Tobner et al. 2016) at the same age. Without nutrient and water limiting conditions, and the absence of herbaceous competition, we suggest that trees will first allocate more biomass to the aboveground compartment (in response to competition for light) before belowground, especially since trees were planted at high-density. This reflects an asymmetry of functioning between aboveground and belowground compartments which is in accordance with the study of Hobbie et al. (2010).We also hypothesized higher fine root nutrient concentrations with increasing tree SR. This was partially verified by higher observed root Ca concentration in SR4 mixtures compared to monocultures. Since Ca concentration was shown to be one of the drivers of leaf litter decomposition (Hobbie et al. (2010), more rapid aboveground leaf decomposition with higher Ca concentrations), our results could suggest a higher fine root decomposition (turnover) in SR4 compared to monocultures which could explain why we did not observe higher annual fine root productivity for SR4 compared to monocultures. However, the fact that tree SR did not affect annual fine root productivity while tree species did, could suggest that higher root Ca concentration in SR4 may be due to the influence of dominant tree species in the mixture rather than a mixture effect *per se*. Other fine root macronutrients (N, P, K, Mg) did not increase with increasing SR levels.

Tree functional diversity and traits effects on fine root productivity

The FDt of root diameter had a positivite effet, while the FDt of litter N content, root Ca concentration and tree height had a negative effect on FRP. On the other hand, FRP was positively related with the CWM of leaf litter C content, root diameter and root P concentration, and negatively related with the CWM of leaf litter nitrogen content, specific leaf area, root branching intensity, specific root length, root K concentration, tree height, wood density and seed mass. Few studies have directly manipulated FD in experiments using trees, therefore comparisons are difficult. Our results indicate that in general, CWM was more strongly correlated to FRP than FDt. This could be due to the close link between CWM and selection effects (Mokany et al. 2008, Roscher et al. 2012) and corroborates studies in herbaceous (Díaz and Cabido 2001, Roscher et al. 2012) and tree systems (Nadrowski et al. 2010) where functional identity rather than diversity better explained productivity.

Moreover, these results are in accordance with studies on the same site; Khlifa et al. (in preparation) showed a dominant effect of CWM versus FD on microbial basal respiration and microbial biomass, and on tree productivity and diversity effects (Tobner et al. 2016). Similarly, Jewell et al. (2015) observed that for early stages of leaf litter decomposition, CWM of leaf traits were more important than FD.

Tree species identity and functional group identity effects on fine root annual productivity and root chemistry

According to our third hypothesis, we did find differences in FRP among tree species. FRP ranged from 2.45 g m^{-2} for the less productive monoculture (*Acer saccharum*) to 19.90 g m^{-2} for the most productive one (*Picea rubens*). Few studies have measured FRP of trees, so here again comparisons are difficult; in general, previous reported values of FRP are higher (for example, Norby et al. 2004, Domisch et al. 2015). However, in these cases the FRP was studied in older stands than the present study. The most visible effect was between functional group identities (leaf habit). Contrary to our expectation, conifer species were characterized by higher belowground productivity than deciduous species. The opposite result was found for aboveground productivity on the same site, at the same age (Tobner et al. 2016), reflecting divergent above and belowground resource allocation strategies between conifers and deciduous tree species. Domisch et al. (2015) found the same trend of higher annual fine root productivity for conifers compared to deciduous species, however, they could not differentiate if this was due to poor development of the deciduous species (beech and oak) or higher growth rates for conifers. The principal difference in root chemistry was also between conifers and deciduous species; we observed a higher root K and Mg under deciduous tree species compared to conifers. This observation agrees with Augusto et al. (2002) who showed a higher foliage nutrient contents (e.g. Ca, Mg, K, N and P) of broadleaf species ecosystems compared to conifer species ecosystems.

We also observed a higher root C:N under deciduous tree species compared to conifers. This was not observed for temperate species at an older age (Withington et al. (2006); no difference in N:C between deciduous and conifers). Again, for 25 temperate species, Comas and Eissenstat (2009) did not measure significant differences in fine root N concentration, nor significant differences between angiosperms and gymnosperms.

Tree species diversity and identity on soil C and N stocks and relative stability

We did not observe higher surface mineral soil total C and N concentration with increasing tree SR, as hypothesized. However, we noted lower total N concentration in soil associated with SR2 compared to monocultures and SR4. We also found no differences between monocultures and mixed stands in C concentrations of the two physical fractions (POM and S&C) nor in POM-N, contrary to our hypothesis about POM showing more rapid response to environmental changes. We did observe lower S&C-N in SR2 mixtures compared to monocultures and SR4. The effects of tree SR were more visible in the fine fraction (S&C) in all cases. The C and N in this fraction (representing the major C and N contents of these soils) are more protected by chemical association with mineral surfaces than those of the POM fraction, indicating that changes due to species richness had a rapid effect on protected soil C. This is surprising, given that after only four years of plantation growth, we expected to see only an effect of SR in the less protected fraction (POM), the fraction that generally responds most rapidly to disturbance or management (Khanna et al. 2001). The rapid shift to greater N in the S&C fraction could be related to the former use of our study site as an agricultural field; microbial response, processing and nutrient turnover may be relatively rapid under these conditions (Vitousek 1982, Pastor et al. 1984, Hobbie 1992). As a consequence, we suggest that the soil C and N in this young temperate plantation are likely to be more rapidly protected (by mineral attachment, at least under SR2), compared to forest soils under natural conditions where the microbial processing and C and N turnover may be slower.

Although deciduous fine roots had higher C:N, tree species identity (and functional group identity) had no effect on total soil C and N contents nor on soil C and N in physical size fractions, which contradicts several studies (Laganière et al. 2011, Gurmesa et al. 2013, Vesterdal et al. 2013). However, the young age could be a reason, since effects of tree species on soil C and N concentrations, stocks or C fractions were generally observed in older stands. Our results tend to indicate that tree SR can influence the soil C and N content and stability earlier than tree species identity (and functional group identity).

Conclusion

The lack of a response of root productivity to SR is perhaps not so much due to the age, but to an asymmetry of functioning between above and belowground under conditions of non-limiting soil fertility, and confirms that studies that focus only on the aboveground compartment are to be generalized with caution. This asymmetry of functioning affecting productivity was most different between conifers and deciduous species, and similarly, differences were noted in root chemistry between the two leaf habits. However higher root C:N of deciduous trees did not feed back to affect soil C and N content, nor C and N in size fractions. Instead, SR2 mixtures resulted in lower total N as well as lower total N in the S&C fraction, indicating a greater response than the POM fraction, which is unexpected. The rapid response of the fine fraction (versus POM) to SR in young plantations, would seem to indicate a rapid microbial processing of litter input and also a relatively rapid protection of this new input. This is important for the potential afforestation of agricultural soils (as carbon sinks), although the reasons for this movement into the S&C fraction need to be investigated on this and other similar afforested sites.

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Chapitre II

Do temperate tree species diversity and identity influence soil microbial community function and composition?[2]

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[2] Article soumis prochainement : Khlifa, R., Paquette, A., Messier, C., Reich, P.B., A.D. Munson. Do temperate tree species diversity and identity influence soil microbial community function and composition?

Abstract

Few studies of biodiversity-ecosystem function have characterized soil microbial community response to gradients of plant species richness and functional diversity, and even fewer in tree communities. We examined how microbial communities in surface mineral soil responded to experimental gradients of tree species richness (SR) and functional diversity (FD), as well as to individual trait variance (FDt), community-weighted mean trait value (CWM) and tree identity. The site was a 4-yr-old common garden experiment near Montreal, Canada, consisting of conifer and hardwood species mixtures and using a replicated design of high-density tree plots of fixed species-richness levels varying in functional diversity (FD); FD was tested both as a gradient as well as a variance of individual functional traits (FDt). Microbial community composition, community-level physiological profiles (CLPP) and respiration were evaluated using Phospholipid fatty acid (PLFA) analysis and the MicroResp^{TM} system. The relationship between species richness and glucose induced respiration (GIR), basal respiration (BR), metabolic quotient (qCO_2) followed a positive but saturating shape indicating functional redundancy. In general, microbial communities associated with species mixtures used a greater number of carbon sources than monocultures, and communities associated with deciduous trees used a greater number of carbon sources than those associated with conifers. There were no differences in total PLFA abundance, nor in fungi, bacteria, or other tested microbial PLFA abundance or ratios, between monocultures and SR mixtures. In monocultures, only microbial communities associated with *Larix laricina* had higher abundance of total PLFA, total bacteria and Gram positive bacteria, compared with some other monocultures. Neither soil microbial community respiration nor composition responded to the FD gradient. However, the FDt and the CWM of several functional traits affected (both positively or negatively) both BR and GIR; in general, CWM of traits affected had stronger effects than did FDt. In conclusion, both tree SR and identity (especially leaf habit) affect soil microbial community respiration and composition, however, no response to the FD gradient was observed. For the first time, we were able to identify functional traits related to life history strategy and to root characteristics that influence soil microbial community function via BR and GIR.

Keywords

Biodiversity; ecosystem functioning; tree species identity; tree species diversity; soil microbial community; MicroResp^{TM}; Phospholipid fatty acids; IDENT; TreeDivNet

Résumé

Peu d'études de la relation entre la biodiversité et fonctionnement de l’écosystème ont caractérisé la réponse des communautés microbiennes du sol à des gradients de richesse spécifique d’espèces végétales (et encore moins d’arbres) et à leur diversité fonctionnelle. Nous avons examiné la manière dont les communautés microbiennes dans le sol minéral de surface ont répondu à des gradients expérimentaux de richesse spécifique d'arbres (SR) et de diversité fonctionnelle (FD), ainsi qu’à la variance individuelle (FDt), la valeur moyenne pondérée sur la communauté (CWM) des traits fonctionnels des arbres et à l'identité de ces arbres. Le site d’étude était une jeune plantation expérimentale d’arbres âgée de 4 ans près de Montréal, Canada. Cette plantation était constituée de conifères, de feuillus et de mélanges à haute densité d’arbres par parcelle. La FD variait sur des niveaux fixes de SR. La FD a été testée à la fois en tant que gradient et en tant que FDt. Le profil physiologique au niveau de la communauté (CLPP) et la respiration des communautés microbiennes du sol ont été évalués à l'aide du système de MicroResp^{TM} et la composition de ces communautés microbiennes a été déterminée à l’aide de la technique d’extraction des acides gras phospholipidiques (PLFA). La relation entre la SR des espèces et la respiration induite par le glucose (GIR), la respiration basale (BR), le quotient métabolique (qCO_2) a suivi une courbe positive, mais saturante indiquant une redondance fonctionnelle. En général, les communautés microbiennes associées à des mélanges d'espèces ont utilisé un plus grand nombre de sources de carbone que les monocultures, et les communautés associées aux feuillus ont utilisé un plus grand nombre de sources de carbone que celles associées aux conifères. Il n'y a pas eu de différences dans l'abondance totale des PLFA, des champignons, des bactéries, ni dans les ratios testés entre les monocultures et les mélanges. Dans les monocultures, seules les communautés microbiennes associées à *Larix laricina* avaient une plus grande abondance totale des PLFA, bactéries totales et des bactéries Gram-positives, par rapport à certaines autres monocultures. Ni la respiration des communautés microbiennes du sol, ni leur composition n’ont répondu au gradient de FD. Toutefois, la FDt et la CWM de plusieurs traits fonctionnels ont eu un effet (que ce soit positif ou négatif) à la fois sur la BR et la GIR. De manière générale, la CWM des traits a eu des effets plus forts que ceux de la FDt. En conclusion, ce sont à la fois la SR des arbres et leur identité (en particulier leur type de feuillage) qui affectent la respiration des communautés microbiennes du sol et leur composition, cependant, aucune réponse au gradient de FD n’a été observée. Nous avons été en mesure pour la première fois d'identifier les traits fonctionnels (liés à la stratégie de vie et aux caractéristiques racinaires) qui influencent le fonctionnement des communautés microbiennes du sol via la BR et la GIR.

Mots-clés

Biodiversité; fonctionnement des écosystèmes; identité des arbres; diversité des arbres; communautés microbiennes du sol; MicroResp; PLFA; IDENT; TreeDivNet

Introduction

Faced with an accelerated decline in biodiversity and the challenge of maintaining the provision of ecosystem goods and services, the last two decades witnessed a growing number of studies exploring the link between biological diversity and ecosystem function. A positive relationship between biodiversity and ecosystem functioning (BEF) was demonstrated in grasslands, aquatic systems, bacterial microcosms and soil communities (Cardinale et al. 2011, Reich et al. 2012, Hooper et al. 2012). A majority of studies observed that this relationship followed a positive but saturating shape indicating functional redundancy (Cardinale et al. 2011). Other studies showed that species identity and diversity may jointly control ecosystem functioning (Cardinale et al. 2012). BEF has been commonly investigated by examining the relationship between the number of species in a community - species richness (SR) - and producer productivity (Balvanera et al. 2006). But other metrics of biodiversity may be as important as SR to explain functioning (Hooper et al. 2005), including genetic, phylogenetic diversity (Cadotte et al. 2009, Paquette et al. 2015), or functional diversity (FD), the latter defined as the value, range, and distribution of functional traits of organisms in a community (Tilman et al. 1997a). Although the SR has been the most widely used measure of diversity in BEF research (Duffy 2009), evidence is growing that it may not be the most efficient predictor of ecosystem functioning and that other metrics such as FD are needed to understand BEF mechanisms (Hooper et al. 2005). Measurements of ecosystem functions have also been enlarged to integrate trophic levels other than producers (Zavaleta et al. 2010), assuming that plants act on ecosystem productivity via multitrophic feedbacks (Wardle et al. 2004). However, the majority of studies have had an aboveground focus, and until recently, the belowground compartment was relatively neglected in BEF research. This hidden belowground diversity is extremely complex; a single ecosystem is characterized by millions of species and billions of individual organisms (Bardgett and van der Putten 2014), and soil microbial communities are increasingly recognized as major drivers of local and global biogeochemical cycles (Wardle et al. 2004, Van Der Heijden et al. 2008). Through controls on organic matter decomposition and nutrient cycling (Six et al. 2006), belowground communities also feed back to many aboveground processes, including plant community assembly (Wardle et al. 2005). Soil microbial communities depend on plant species for carbon input via litter (leaves, stems, roots) and rhizodeposition (Hättenschwiler et al. 2005, Herman et al. 2012), however plant species vary substantially in their litter quality and quantity and in root exudate input (Mitchell et al. 2012, Vesterdal et al. 2012). Plant species may also directly or indirectly impact microbial community via litter input or root activity that modifies abiotic variables such as soil pH (Eviner and Chapin III 2003).

Soil microbial biomass, microbial activity (respiration), and the respiration rate per unit soil microbial biomass - the metabolic quotient (qCO_2) - have long been used as indicators of soil quality and ecosystem functioning (Anderson and Domsch 1993, Zak et al. 2003, Schloter et al. 2003), providing relatively simple measures of function along diversity gradients. Although previous studies showed increases in soil microbial biomass and respiration in response to increased plant diversity in grasslands (Bardgett and Shine 1999, Eisenhauer et al. 2010), Guenay et al. (2013) went a step further to show that along a species diversity gradient, the effect of complementarity was responsible for increased microbial biomass and caused transgressive overyielding. In one of few studies of tree diversity (natural forests), Thoms et al. (2010) also observed an increase in microbial biomass with increasing tree species diversity. Anderson (2003) stipulated that according to Odum’s theory when extrapolated onto the entire microbial community, community respiration (measured by the qCO_2; often used as an indicator of stress) should decrease with species diversity, due to increasing metabolic efficiency. Indeed, Bardgett and Shine (1999) observed a decrease in qCO_2 with the highest diversity of plant litter input in a grassland experiment. Spohn and Chodak (2015) noted that microorganisms increase their qCO_2 with increasing C:P ratio and C concentration of tree litter, therefore tree species with different litter stoichiometry may alter microbial community qCO_2 by above and belowground litter input (tree identity effect).

The study of Scheibe et al. (2015) separated for the first time the effects of tree species diversity and tree identity on microbial communities, in a near-natural temperate forest. They observed that tree species identity and soil conditions were more important in determining microbial community structure than tree species diversity per se. However, this study only manipulated SR and did not include conifer species, nor mixtures of conifer and deciduous trees. Thoms et al. (2010) observed that soils associated with greater tree leaf litter diversity had the largest total amounts of fatty acids (PLFA), but only a common marker for arbuscular mycorrhizal fungi significantly increased. Recently, Fu et al. (2015) observed that both the aboveground biomass of the understory and the diversity of the tree layer positively influenced the fungal:bacterial ratio in subtropical forests. Similarly, in the Jena grassland experiment, Lange et al. (2014) noted an increase of this ratio with plant functional group richness and with SR (related to an increase in mycorrhizal fungi).We still don't know by which specific mechanisms an increase of plant diversity increase the fungal:bacterial ratio, but we suggest that it is likely due to an increase in fungal organisms. In their study in neotropical rain forest, McGuire et al. 2012 explain that since fungi degrade a large portion of the plant- derived compounds, the diverse mixtures of leaf litter on the forest floor of tropical rain forests may enable the coexistence of diverse fungal taxa via resource partitioning. Fungi are more able to decompose recalcitrant litter, and enhance plant productivity by supplying limiting nutrients as well as providing resistance to disease and drought (Van Der Heijden et al. 2008). In the present study, we aimed to better characterize the links between soil microbial communities and both aboveground tree diversity (artificial SR and FD gradients of temperate species) and identity (of the trees and functional traits), and to better understand the mechanisms underlying these links. We used a 4 yr old common garden experiment (IDENT (Tobner et al. 2014)) of high-density tree communities near Montreal, Canada, a part of TreeDivNet (Verheyen et al. 2016), consisting of conifer and hardwood species mixtures along dual gradients of SR and FD. The SR gradient varies from one, two, four to 12 species (12 monocultures; 14 two-species mixtures; 10 four-species mixtures and one mixture containing all 12 species). The FD gradient was developed by choosing species combinations at SR levels of 2 and 4 which varied in FD, enabling independent tests of FD while holding SR constant. The FD metrics were based on functional dispersion (FDis; Laliberté and Legendre 2010). We also tested the effect of the average values of traits (CWM: functional mean) and their variance (FDt) (Ricotta and Moretti 2011).

We hypothesized that:

1. Soil microbial biomass and microbial basal respiration increase with increasing tree SR and FD (following a positive but saturating shape), while respiration rate per unit microbial biomass (qCO_2) decreases (increased metabolic efficiency in C use);
2. Higher tree diversity (SR and FD) is associated with an increase in the total PLFA and in the fungi:bacteria ratio;
3. FD gradient have a stronger effect on soil microbial community function and composition than SR gradient.

4. Independently, soil microbial community functioning and composition are determined by the identity of trees and functional traits.

As studied to date, links between above and belowground diversity are still tenuous, especially for treed ecosystems. We aimed to evaluate these links by taking into account both the number of species (SR) and their assemblage (FD, FDt and CWM) and using soil microbial biomass (by glucose-induced respiration), basal respiration and qCO_2 as indicators of ecosystem function. This approach is crucial to further understand the mechanisms governing forest biogeochemical cycles and ecosystem productivity.

Methods

Site description and experimental design

The site is located at Ste-Anne-de-Bellevue, near Montreal, Québec, Canada, 45.5° N, 73.9° W. Mean annual temperature is 6.2 ºC with a mean annual precipitation of 963 mm (climate.weatheroffice.gc.ca). The experiment is part of the IDENT network (International Diversity Experiment Network with Trees), that includes seven sites in North America and Europe (Tobner et al. 2014), the present study site was established on a former agricultural field in spring, 2009, with seedlings of mostly one (deciduous) or two (conifer) years of age. An area of about 0.6 ha was planted with seedlings of 12 North American temperate tree species, selected to cover a wide range of functional traits, including early and late successional species: *Acer saccharum* Marsh (Sugar Maple); *Acer rubrum* L. (Red Maple); *Betula alleghaniensis* Britton (Yellow Birch); *Betula papyrifera* Marsh (Paper Birch) and *Quercus rubra* L. (Northern Red Oak); *Abies balsamea* (L.) Mill. (Balsam Fir); *Larix laricina* (Du Roi) K. Koch. (Tamarack); *Pinus strobus* L. (Eastern White Pine); *Pinus resinosa* Aiton (Red Pine); *Picea glauca* Voss (White Spruce); *Picea rubens* Sarg. (Red Spruce) and *Thuja occidentalis* L. (Eastern White Cedar). Trees were planted in square plots of ~ 4 by 4 meters; each plot consisted of 64 individual trees (8 × 8 assemblages of planted seedlings at regular intervals) at 50 cm planting distance, so as to create 37 different tree communities, each replicated in 4 blocks. Each block consisted of 12 monocultures (SR1), 14 combinations of two-species mixtures (SR2), 10 combinations of four-species mixtures (SR4) and one mixture with all 12 species (SR12) for a total of 148 plots. In parallel, a FD gradient was established at SR2 and SR4 species mixtures (the values of functional diversity are zero for monocultures) from FD indices calculated prior to planting, using the functional dispersion index (FDis; Laliberté and Legendre 2010). This gradient was rated on a scale of one to eight depending on the degree of a priori functional diversity and was based on 12 above- and belowground traits, to ensure the inclusion of traits linked to numerous ecosystem processes (see Appendix S2 for more details). Within plots, trees in mixtures were planted at random with restrictions, to prevent the clumping of species. Within each block, however, plots were randomly distributed and separated by ~1.25 m corridors to allow circulation within the site and to minimize disturbance and interactions among communities. Tree communities were periodically weeded manually, and corridors between plots were trenched during the 2011 and 2012 seasons, to prevent the roots from growing into adjacent plots. A fence was installed to protect trees from herbivory. To our knowledge, this plantation is the first tree diversity experiment to have continuously eliminated understory vegetation to isolate the effects of tree SR and FD.

Soil sampling

Soils were sampled in June 2012 (during the fourth growing season), using aluminum cylindrical corers (8.5 cm diameter and 3 cm depth). Two soil cores were sampled from the center of a square created by four trees. Location within tree communities was chosen at random from the 25 possible sites after the outer perimeter was excluded. The two soil samples were then pooled. Fresh soil samples were sieved at <2 mm and visible debris were removed before the soils were frozen, prior to MicroResp^{TM} and PLFA analysis.

MicroResp^{TM} physiological profiles

Community Level Physiological Profiles (CLPP) and basal respiration (BR) were measured using the MicroResp™ system (Campbell et al. 2003). Prior to analysis, soil moisture content of all samples was pre-adjusted to 40% of the water holding capacity. Soils were then pre-incubated for one week in microcosms at ambient temperature (23°C ± 2), in dark conditions (Berard et al. 2014). The carbon sources used were L-alanine, L-arabinose, L-arginine, citric acid, D-fructose, D-galactosem D-glucose, L-malic acid, L-lysine, N-acetyl glucosamine, oxalic acid, protocatechuic acidm DL-aspartic acidm γ-aminobutyric acid and D-trehalose.

All measures were done in triplicate and with two soil samples per plate after 6 h incubation in the dark at room temperature (23 ± 1°C). The absorbance of the detection microplate was measured at 570 nm (Biotek EL-800 spectrophotometer). The average amount of CO_2 that evolved per sample was calculated and used to normalize individual C source concentrations before multivariate analysis (Campbell et al. 2003). A calibration curve of absorbance versus head space equilibrium CO_2 concentration (measured by gas chromatography) was fitted to a regression model, and results were expressed in µg C-CO_2 g^{-1}soil h^{-1} (Campbell et al. 2003, Bérard et al. 2011, 2014). A proxy for microbial biomass was determined by glucose-induced respiration (GIR) (Berard et al. 2011, 2014) based on Anderson and Domsch (1978, 1985) and Chapman et al. (2007). The respiration rate per unit soil microbial biomass - the metabolic quotient qCO_2 - is the ratio of BR to GIR (Saul-Tcherkas and Steinberger 2009; Berard et al. 2011, 2014), and is a measure of the ecophysiological status of soil microorganisms (Anderson and Domsch 2010). (See Appendix S1 for more details).

Phospholipid fatty acid extraction (PLFA)

The procedure of Hamel et al. (2006) was performed for lipid extraction and PLFA analyses. Briefly, total soil lipids were extracted by shaking freeze-dried soil in a dichloromethane-methanol-citrate buffer mixture (1:2:0.8). The phospholipids were separated from other lipids on a silicic acid column and esterified forming fatty acid methyl esters (FAME) which were separated and quantified by gas chromatography (Findlay 2004). PLFA identities were assigned based on comparison of retention times to known standards. The biomarkers used to discriminate different PLFA are commonly used for microbial community profiling (Leckie 2005). The abundance of individual PLFAs was expressed as ng PLFA g^{-1} dry soil. (See Appendix S1 for more details).

Functional diversity and community-weighted traits calculation

In addition to the initial FD gradient, a functional dispersion was calculated also using the “FD” R package (Laliberté, Legendre and Shipley 2014) for each trait (FDt) separately to identify the relative importance of each trait on GIR and BR. The average values of functional traits (CWM) - functional mean– were calculated for each community in each plot (37) as the product of the average trait value of a species and its relative abundance in the community, averaged across all species in the community. Both CWM traits and functional variation (FDt) were weighted by species' relative abundance in the community. The traits used to calculate the FDt and the CWM were either related to tree life strategies (such as SLA, wood density) or to litter and root chemistry, and were as much as possible measured *in situ* (Table 2 and Appendix S4, Table S2) (Tobner et al. 2014, Jewell et al. 2015).

Statistical analyses

All statistical analyses were performed within the R environment (version 3.2.1 R Core Team 2015).

Linear mixed-effect models were developed using the lme function in the nlme package (Pinheiro et al. 2007) to test the effect of SR (n = 148), FD (n = 96), FDt or CWM as a fixed factors on microbial parameters (BR, GIR, qCO_2), and PLFA groups as response variables with plot identity (to take into account our unbalanced design) and blocks included as random factors, and soil % of clay as environmental co-variable. Mean separation among SR or FD levels were applied using Tukey's HSD tests with the glht function in the multcomp package (Hothorn et al. 2008). Predicted values and standard errors of the mixed models were computed using the predictSE function in the AICcmodavg package (Mazerolle 2016). In all linear mixed-effect models, the normality of residuals and homogeneity of variance were tested and transformations were performed when necessary. Statistical significance was set at the 0.05 level (see Appendix S3 for more details).

The shape of the relationship between soil microbial parameters (BR, GIR, qCO_2) and tree SR was examined through the fitting of five different functions (linear, exponential, logarithmic, power and Michaelis–Menten (M-M) that involve different biological interpretations (Cardinale et al. 2011, Reich et al. 2012, Delgado-Baquerizo et al. 2016). We selected the best model fits by following Akaike information criteria (AICc; Burnham and Anderson (2003)). The lower the AICc index, the better the model. Here, we consider a ΔAICc > 2 threshold (Burnham and Anderson 2003) to differentiate between substantially different models.

Overyielding, transgressive overyielding and underyielding effects of tree diversity on microbial biomass and respiration were investigated according to Guenay et al. (2013). Overyielding corresponded with higher values of species mixtures than the mean of component monocultures (the opposite in the case of underyielding) and transgressive overyeryielding corresponded with higher values of species mixtures than the most productive monoculture.

The CLPP datasets were standardized by scaling (subtracting the mean SIR of all soils on all substrates, then dividing by the standard deviation) and then subjected to principal component analysis (PCA), followed by a between-class analysis to discriminate among SR levels (Chessel et al. 2004). We also used a PCA to characterize tree species monocultures by their C source utilization (CLPP). The PCA were computed using the dudi.pca function of the ade4 package (Dray and Dufour 2007).

Results

Tree species richness and microbial community parameters and composition

Glucose-induced-respiration (GIR) and microbial basal respiration (BR) were globally higher in mixtures than in monocultures and qCO_2 showed a trend to be higher in SR2 and SR12 (Tukey-HSD tests: $p < 0.05$) (Table 5).

Table 5. Soil microbial parameters as influenced by tree species richness. BR (basal respiration), GIR (active microbial biomass) in µg $C\text{-}CO_2\ g^{-1}soil\ h^{-1}$, qCO_2 (BR / GIR) (metabolic quotient). SR1 (monocultures), SR2 (two species mixtures), SR4 (four species mixtures) and SR12 (twelve species together). Means with the same letter are not significantly different based on the Tukey-HSD post hoc test. Standard errors are presented in brackets.

	BR	**GIR**	**qCO_2**
SR1	0,23(±0,01) **a**	0,99(±0,07) **a**	0,24(±0,02) **a**
SR2	0,32(±0,01) **b**	1,13(±0,06) **ab**	0,28(±0,02) **b**
SR4	0,32(±0,01) **b**	1,25(±0,07) **b**	0,26(±0,03) **ab**
SR12	0,36(±0,04) **b**	0,89(±0,23) **ab**	0,41(±0,08) **b**

The Michaelis–Menten (M-M) function appeared as the best model shaping the relationship between tree SR and BR. However, the models could not distinguish among M-M, logarithmic and power functions for GIR, neither among the five tested functions for qCO_2 (see Appendix S4, Table S4). Since M-M was the best model for BR and was either the best or close to the best (but not distinguishable) for GIR and qCO_2, we used the M-M function for describing the relationship between all three soil microbial parameters and tree SR (Fig. 2). All tree species mixtures overyielded on BR. Moreover, 21% and 30% of SR2 and SR4 mixtures (and the sole SR12 treatment), respectively, showed transgressive overyielding (Fig. 3a). For GIR, 8/14 tree species mixtures showed overyielding, 2/14 showed transgressive overyielding and 6/14 showed underyielding in SR2. 9/10 tree species mixtures in SR4 showed overyielding and 1/10 showed underyielding but no transgressive overyielding, while the sole SR12 community underyielded (Fig. 3b). In SR2, AbAr (*Abies balsamea- Acer rubrum*), BpQr (*Betula papyrifera - Quercus rubra*), ArBa (*Acer rubrum - Betula alleghaniensis*) and BpPs (*Betula papyrifera- Pinus strobus*), BaQr (*Betula alleghaniensis- Quercus rubra*) were the mixtures involved in transgressive overyielding of BR and GIR respectively (Fig. 3a, b). These SR2 mixtures also showed the highest and lowest values of qCO_2 respectively. Similarly, PrePsPruPg (*Pinus resinosa- Pinus strobus- Picea rubens- Picea glauca*), AbBpLlPg (*Abies balsamea- Betula papyrifera- Larix laricina- Picea glauca*) and BaPrePruPs (*Betula alleghaniensis- Pinus resinosa- Picea rubens- Pinus strobus*), which were the mixtures responsible for transgressive overyielding of BR in SR4 mixtures, showed the highest values of qCO_2 (Fig. 3a, c).

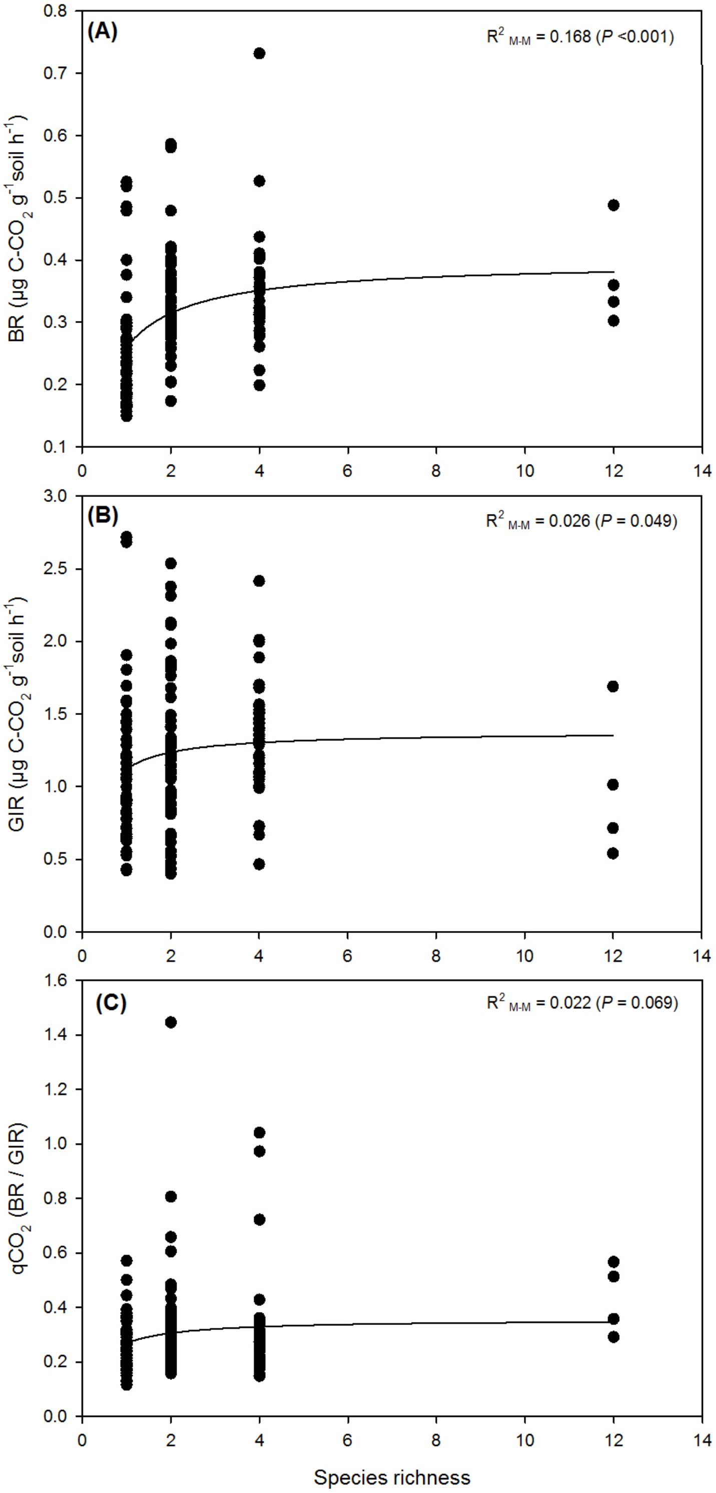

Figure 2. Relationship between soil microbial parameters and tree species richness. (A) BR (basal respiration), (B) GIR (active microbial biomass) expressed in µg C-CO_2 g^{-1}soil h^{-1}, (C) qCO_2 (metabolic quotient) (n = 4 replications for each identity plot for a total of 148 plots).The solid lines represent fitted regressions for the best model.

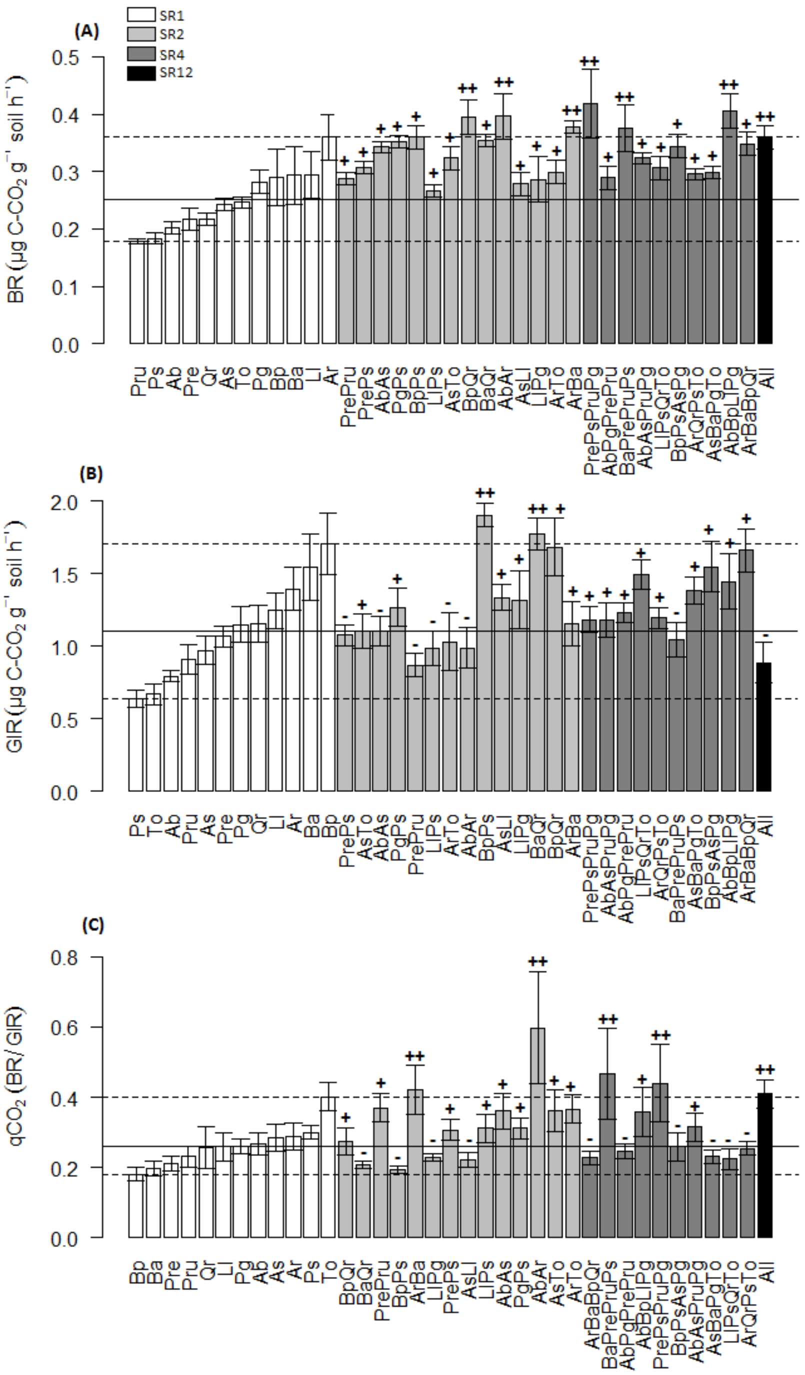

Figure 3. Histogram of soil microbial parameters as influenced by tree species richness and tree identities. (A) BR (basal respiration), (B) GIR (active microbial biomass) and (C) qCO_2 (metabolic quotient). The grand mean of all monocultures mean values are highlighted by the black solid line and minimal and maximal monoculture values are highlighted by dotted lines. Higher values of species mixtures than the mean of monocultures show overyielding (symbol (+)), values above the higher dotted line show transgressive overyielding (symbol (++)), values below the lower dotted line show underyielding (symbol (-)). SR1 (monocultures), SR2 (two species mixtures), SR4 (four species mixtures) and SR12 (all twelve species together). Ab (*Abies balsamea*), Ar (*Acer rubrum*), As (*Acer saccharum*), Ba (*Betula alleghaniensis*), Bp (*Betula papyrifera*), Ll (*Larix laricina*), Pg (*Picea glauca*), Pru (*Picea rubens*), Pre (*Pinus resinosa*), Ps (*Pinus strobus*), Qr (*Quercus rubra*), To (*Thuja occidentalis*). Error bars are standard error.

The first two axes of the PCA conducted on the 15 different C sources measured on all SR levels (accounting for 99.3% of the total variability, Fig. 4A) revealed a clear separation of monocultures from mixtures (Monte-Carlo tests; p = 0.001). Dim1 (66.3% of total variability) appeared to separate SR1 from the other SR levels, demonstrating that SR1 is characterized by lower catabolic activity, whereas Dim2 (33% of total variability) appeared to separate SR12 from SR2 and SR4 mixtures. The number of the C sources catabolized was greater in SR4 mixtures.

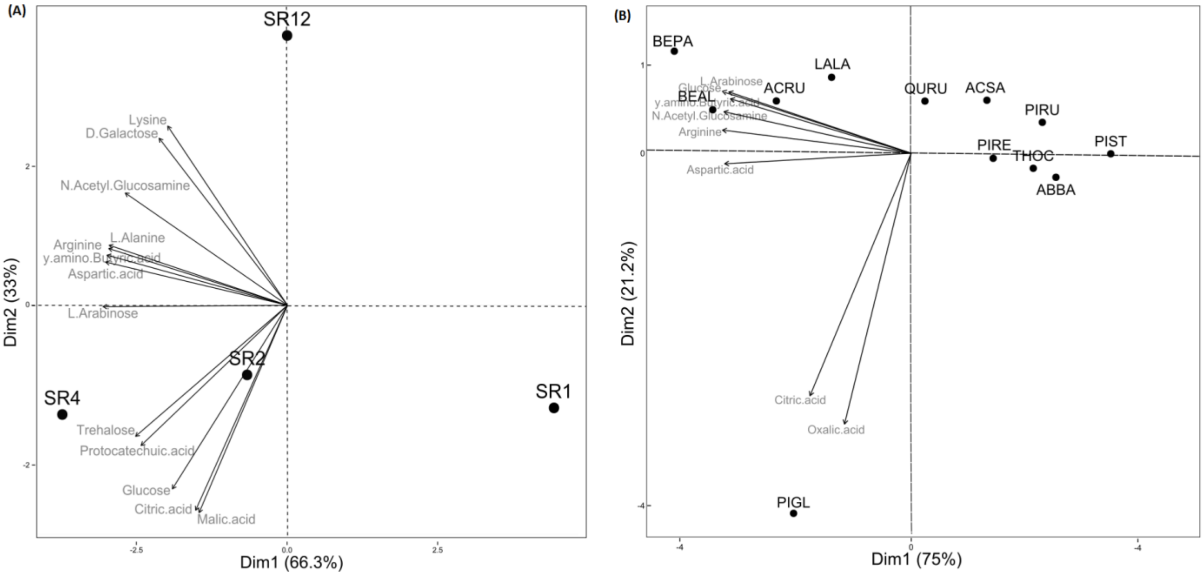

Figure 4. (A) Biplot of the principle components analysis based on the community level physiological profiles (CLPP) measurements of the different soils sampled: ordination of four tree species richness levels, and correlation plot between the PCA axes and the 13 different substrates catabolized that best explained the variations. (B) Biplot of the principle components analysis based on CLPP measurements of the different soils sampled: ordination of twelve tree monocultures, and correlation plot between the PCA axes and the 8 different substrates catabolized that best explained the variations. SR1 (monocultures); SR2 (two species mixtures); SR4 (four species mixtures) and SR12 (all twelve species together). ABBA (*Abies balsamea*); ACRU (*Acer rubrum*); ACSA (*Acer saccharum*); BEAL (*Betula alleghaniensis*); BEPA (*Betula papyrifera*); LALA (*Larix laricina*); PIGL (*Picea glauca*); PIRU (*Picea rubens*); PIRE (*Pinus resinosa*); PIST (*Pinus strobus*); QURU (*Quercus rubra*); THOC (*Thuja occidentalis*).

SR did not influence total PLFA ($PLFA_{tot}$), PLFA for different taxonomic groups (fungi ($PLFA_{fun}$), bacteria ($PLFA_{bact}$), actinobacteria ($PLFA_{act}$), Gram positive ($PLFA_{pos}$) and Gram negative bacteria ($PLFA_{neg}$)) (data not shown), neither was the fungi: bacteria ratio affected by SR (Appendix S4, Table S3).

Functional diversity and microbial community parameters and composition

The GIR and BR did not increase along the FD gradient nor did this FD gradient affect any PLFA group (data not shown). However, when the variance of each functional trait (FDt) was tested separately (in a full model adjusted for SR levels), we found a positive effect of root calcium and litter nitrogen on BR (Table 6). We also found a positive effect of the variance in seed mass, and a negative effect of the variance in root diameter and specific root length on GIR. The variance in wood density and root carbon also had a negative effect on GIR although these results did not obtain significance at the 5% threshold (Table 6).

Table 6. Effect of individual trait means (CWM) and variances (FD) on soil microbial basal respiration (BR) and soil microbial biomass (GIR). The values presented are the *p-values* of mixed model regressions and significant positive or negative effects (at $\alpha = 0.05$) are represented with arrows.

Functional Trait	**BR**		**GIR**	
	FD	CWM	FD	CWM
Litter Nitrogen Content	0.025↗	0.436	0.722	0.004↗
Litter Carbon Content	0.952	0.002↘	0.316	0.027↘
Leaf Dry Matter Content	0.936	0.440	0.222	0.067
Specific Leaf Area	0.173	<0.001↗	0.630	<0.001↗
Root Diameter	0.647	0.018↗	0.038↘	<0.001↘
Root Banching Intensity	0.687	0.021↗	0.490	<0.001↗
Specific Root Length	0.895	0.003↗	0.043↘	<0.001↗
Root Nitrogen Concentration	0.704	0.393	0.429	0.009↘
Root Carbon Concentration	0.901	0.329	0.082	<0.001↗
Root Phosphorus Concentration	0.784	0.259	0.420	0.042↘
Root Potassium Concentration	0.935	0.184	0.314	0.184
Root Calcium Concentration	0.019↗	0.496	0.350	0.256
Root Magnesium Concentration	0.998	0.662	0.979	0.253
Tree Height	0.603	0.004↗	0.507	<0.001↗
Ground Diameter	0.322	0.023↗	0.483	<0.001↗
Wood Density	0.309	0.005↗	0.069	<0.001↗
Seed Mass	0.576	0.831	0.007↗	0.047↗

Identity effects and microbial community parameters and composition

The PCA analyses (first two axes accounted for > 96% of total variability) on CLPP measurements for the 12 monocultures discriminated evergreen species from deciduous trees (Fig. 4B). Dim1 (75% of total variability) showed that a higher number of catabolized C sources were used by microbial communities associated with deciduous tree mixtures, compared to conifers. Dim2 (21.2% of total variability) revealed that microbial communities associated with *Picea glauca* (Fig. 4B) appeared to use dominantly carboxylic acid substrates (oxalic and citric acids), compared to C sources used by other monocultures. Moreover, conifers were characterized by lower values of GIR ($p < 0.001$) and BR ($p = 0.052$) compared to deciduous species (data not shown).

The CWM influenced GIR and BR (Table 6), GIR was affected by a greater number of traits than BR. Both parameters were positively related to the CWM of specific leaf area, root branching intensity, root specific length, tree height, tree ground diameter and wood density, and negatively related to litter carbon content. BR was positively related with root diameter while GIR was negatively correlated with the same trait.

Globally, species identity (monocultures) did not influence microbial community structure (no differences in individual PLFAs). The exception was *Larix laricina*, which showed higher abundance of total PLFAs and total bacteria compared with *Thuja occidentalis* ($p = 0.044$ and $p = 0.041$ respectively), and higher abundance of Gram positive bacteria compared with *Betula alleghaniensis* ($p = 0.089$), *Picea glauca* ($p = 0.044$), *Picea rubens* ($p = 0.047$) and *Thuja occidentalis* ($p < 0.001$) (Appendix S4, Table S3).

Discussion

Effects of tree species diversity (SR) on the functional structure and composition of soil microbial communities

Our first hypothesis stated that soil glucose-induced-respiration (GIR, a proxy for microbial biomass) and basal respiration (BR) would increase, while the metabolic quotient (qCO_2) would decrease with increasing tree species richness (SR) and diversity (FD). This was partially validated, despite low R^2 values (which could be partly explained by the gap of SR mixtures between SR4 and SR12), the relationship between soil microbial parameters and tree SR followed the expected positive but saturating shape indicating functional redundancy (Cardinale et al. 2011). Functional redundancy commonly suggests that some fraction of species can be lost with minimal effects on microbial community functioning, however, redundancy may not be assured if the function is related to certain single species that uses resources in a unique way (Tilman et al. 1997b, Cardinale et al. 2004). The number of soil C sources catabolized was higher in SR2 and SR4 mixtures compared to monocultures and to SR12. The higher microbial activity observed at higher SR levels may be due to increased rhizospheric C inputs, as observed by Lange et al. (2015), who also observed greater soil C storage with increased diversity (SR). These positive effects of tree mixtures on soil GIR and BR concord with Stefanowicz et al. (2012), who found that in metal-contaminated soils, plant SR had a relatively strong positive effect on all microbial parameters (microbial biomass, BR and functional richness) except for the fungal component. The qCO_2 which is usually used as a sensitive ecophysiological indicator of community stress induced by soil environmental conditions (Anderson 2003) was significantly higher under soils associated with SR2 and SR12 mixtures, indicating that the associated microbial communities allocate more energy to maintenance rather than biomass growth (Anderson and Domsch 1993, Anderson 2003, Butenschoen et al. 2011). The same community that uses more C sources (SR4 mixtures) use C more efficiently, perhaps by exploiting a higher number of compounds.

SR promoted overyielding and transgressive overyielding in soil GIR and soil microbial BR, in accordance with our first hypothesis. The observed effects were stronger for BR than for GIR, since no transgressive overyielding or underyielding were observed for the latter. This partially contradicts our expectations of principally overyielding and suggests the occurrence of stronger interactions among tree species and potentially between trees and soil microbes (Guenay et al. 2013), when increasing SR levels to more than two species. This could also suggest that BR is less affected by above and belowground interactions than is GIR (microbial biomass).

Our hypothesis that total PLFA and fungal:bacterial ratio will increase with increasing tree species diversity was not validated. This lack of response of community structure to tree diversity is in line with other studies in boreal (Lucas-Borja et al. 2012) and temperate broad-leaved forests (Scheibe et al. 2015), while contradicting results for other tree diversity studies

(Thoms et al. 2010, Fu et al. 2015) and for manipulated diversity gradients in grasslands (Scherber et al. 2010, Eisenhauer et al. 2010).

Effects of tree species diversity through functional variance and functional mean

Since microbial community parameters were not related to the a priori FD gradient (12 above- and belowground traits), we calculated a gradient that was more pertinent to soil microbial processes under study (based on the traits that best explained GIR and BR). Here again, the results were not significant which does not allow us to validate our third hypothesis. However, we analyzed the effect of the variance in belowground and life-history strategy traits (FDt) on microbial GIR and BR. Seed mass had a positive effect, while root diameter and specific root length had a negative effect on GIR. Leaf litter nitrogen and root calcium positively affected BR. This indicates that different mechanisms are operating for these two community functions (biomass versus respiration). Few studies have directly manipulated FD in experiments using trees, therefore comparisons are difficult. Moreover, all such experiments to date report on early stages of forest development (Tobner et al. 2016). Stefanowicz et al. (2012) which used plant functional richness (based on the presence/absence in the mixture of non-leguminous forbs, legumes and graminoids) also found negligible effects of functional richness on microbial community parameters.

When we evaluated the CWM of traits in relation to function, GIR and BR were related to several similar, but also different tree functional traits (Table 2). In general, CWM was more important than FDt for both functions, and GIR was more strongly related to CWM than was BR. In contrast to FDt response, GIR is more related to mean litter and root chemistry traits (positive effects of litter N and root C, and negative effects of root N and root P). Both GIR and BR were similarly related to specific leaf area, root branching intensity, specific root length, tree height, ground diameter, wood density (positive effects) and litter C (negative effects), considered to be life strategy traits (Reich 2014).

Recent studies on the same site showed that CWM better explained tree productivity and diversity effects than did FD (Tobner et al. 2016). Similarly, Jewell et al. (2015) observed that for early stages of leaf litter decomposition, CWM of leaf traits were more important than FD, while surface soil respiration increased with increasing tree FD, independently of average trait values.

Identity effects on the functional structure and composition of soil microbial communities

We also hypothesized that tree and trait identity will influence soil microbial community parameters. Generally, we found an effect of leaf habit, microbial communities associated with deciduous species were characterized by higher values of GIR and BR compared with those of conifers. On the same site, Rivest et al. (2015) observed that soils under *Larix laricina* presented higher values of microbial biomass and BR compared to *Acer saccharum*. They hypothesized that a greater availability of metabolically accessible compounds resulted from higher growth rates and inputs in plots containing *Larix laricina*. Mixtures dominated by deciduous species were associated with transgressive overyielding in BR and GIR in two species mixtures, while mixtures dominated by conifer species explained transgressive overyielding in soil microbial BR in SR4 mixtures. This suggests that transgressive overyielding effects in soil BR and GIR could be mostly driven by species identity rather than FD.

The PCA identified groups of tree species that differed in terms of their utilization of C sources (MicroResp^{TM}). Deciduous tree species were generally able to utilize a wider spectrum of organic C compounds than slow growing conifer species (Fig. 3b). Within tree species, soil microbial communities associated with *Betula papyrifera* and *Betula alleghaniensis* appear (and mixtures including these species) to be able to metabolize a larger number of carbon sources, suggesting a greater soil C storage capacity under these mixtures. These two tree species were also among the principal species involved in overyielding and transgressive overyielding for both BR and GIR. In contrast, the soil microbial community associated with *Picea glauca* used more complex C sources such as carboxylic acid (oxalic acid and citric acid). Among the organic substrates tested here, easily assimilable compounds such as fructose and alanine were shown to induce strong positive priming effects, while oxalic acid (more complex organic compound) was associated to positive as well as negative priming effects (Hamer and Marschner 2002).

Unlike with the microbial community structure (MicroResp^{TM}), we did observe minor shifts in PLFA composition with tree species. Although we sampled in summer, which is considered the more favorable season to observe compositional shifts (Grayston et al. 2001, Thoms and

Gleixner 2013), only *Larix laricina* showed higher abundances of total PLFAs and total bacteria compared with *Thuja occidentalis*, and higher abundances of Gram positive bacteria compared with *Betula alleghaniensis*, *Picea glauca*, *Picea rubens* and *Thuja occidentalis* (Table B3, Appendix B). Gram positive bacteria have the capacity to tolerate drier seasons (Harrison and Bardgett 2010, Štursová and Baldrian 2011, Escobar et al. 2015) due to their thicker cell walls and capacity to form spores (Berard et al. 2011, Nessner Kavamura et al. 2013). On the same study site (Collin et al. in preparation), found that soil moisture (via volumetric water content measurement) was significantly lower under *Larix laricina* mixtures compared to other monocultures, this might explain the higher amount of Gram positive bacteria associated with this species.

Numerous studies exploring species diversity and forest soil microbial community structure (using PLFA profiles) highlighted the importance of abiotic factors: soil pH, soil texture and soil moisture in driving community structure (Merilä et al. 2010, Brockett et al. 2012, Wu et al. 2012a,b). Microbial community structure can also be influenced by seasonal variation (Leckie 2005, Thoms and Gleixner 2013, Escobar et al. 2015). Since the present design is a young tree community in a common garden experiment, and we sampled at one point in time, these factors did not vary significantly (Table B1, Appendix B), minimizing the probability to observe major changes in soil microbial community structure. However, we need to be careful when drawing conclusions based on data from a single time point. Although a number of studies emphasized the importance of long-term BEF studies (Cardinale et al. 2007, Reich et al. 2012) to validate relationships, after only four years we observed an effect of tree mixtures and tree identity on the microbial community parameters. Since plant root and soil microbe interactions can occur over short timescales of hours to days (Wardle 2002, Bardgett et al. 2005), the four year period of our common garden experiment is relevant for microbial response in the early phases of succession.

Conclusion

Globally, increasing tree species richness (SR) had a positive effect on soil microbial community parameters (GIR, BR and qCO_2) compared to monocultures. This is the first study to show a positive but decelerating relationship between plant SR and associated microbial community function, without significant changes in community composition. Tree species identity modestly influenced soil microbial composition (PLFA). Moreover, tree species identity (especially the effect of leaf habit) also influenced soil microbial functional structure, revealing specific species effects of *Betula papyrifera* and *Betula alleghaniensis* as species able to metabolize a greater number of C sources; in general, deciduous species metabolized a higher number of C sources compared to conifers. We know of no other study demonstrating such differences between deciduous and conifer species in the use of C sources (among twelve common North American tree species) Another identity effect was associated with *Larix laricina*; soil microbial total PLFA, total bacteria and Gram positive bacterial species were higher compared to some other species. We note that CWM of traits is more important than FDt to link traits to functions of GIR (soil microbial biomass) and BR. Taken together, our results suggest that both the identity of trees and their diversity influence soil microbial functioning and composition in young tree communities.

To our knowledge, this is the first tree diversity experiment to isolate the effects of tree SR and FD on soil microbial community function and to identify tree species as well as tree species mixtures that contribute to GIR (microbial biomass) and BR. Our study also contributes to enlightening a part of the complex and poorly understood relationship between aboveground biodiversity and belowground ecosystem functioning with the identification of tree functional traits that directly affect GIR (microbial biomass) and respiration.

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Chapitre III

Understory vegetation is the strongest driver of fine root decomposition in a temperate plantation[3]

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[3] Article soumis prochainement : Khlifa, R., Angers, D., and A.D. Munson. Understory vegetation is the strongest driver of fine root decomposition in a temperate plantation.

Abstract

The different paths of vegetation succession after contrasting silvicultural treatments for plantation establishment may feedback to carbon (C) and nitrogen (N) cycles by mechanisms that are still poorly understood. Although critical to understanding the potential role of plantation ecosystems for C sequestration, few studies have focused on belowground processes such as root decomposition. Here we investigated how silvicultural treatments (factorial combinations of blade scarification, fertilization, and vegetation control with herbicide) and subsequent vegetation affected fine root decomposition (< 2mm; FRD), fine root chemistry (C, N, P, K, Ca, Mg) and surface mineral SOC and N concentrations (in bulk soil and in two size fractions: particulate organic matter – POM, and silt and clay associated – S&C), 27 years after plantation of native white pine in Petawawa, Ontario, Canada. We observed a domination and higher basal area of conifers when vegetation control was applied, while deciduous species and higher species richness were observed when vegetation control was not applied. Blade scarification was characterized by the highest understory species diversity while vegetation control recorded the lowest. FRD (measured as mass loss after 8 months *in-situ*) was highest when blade scarification and fertilization were combined, and the lowest when vegetation control was applied alone. Mineral SOC (in bulk soil, in POM and S&C fractions) significantly decreased in response to blade scarification, while mineral N was not affected by silvicultural treatments in the bulk soil and two fractions. We found no correlation between species diversity and FRD, neither between FRD and SOC and N in mineral soil nor between FRD and soil pH. Instead, we found that understory vegetation rather than overstory vegetation, root chemistry and soil properties best explained FRD. We conclude that silvicultural treatments affect both FRD and SOC through subsequent vegetation, but the effect on FRD is more likely due to species-specific effects that induced a favorable soil environment (i.e. higher leaf litter quality, higher soil moisture) of understory vegetation than to a higher species diversity *per se*, and that the negative effects of silvicultural treatments and subsequent vegetation on SOC seem not be offset by a higher FRD in this temperate forest plantation.

Keys words

Root decomposition; understory vegetation; silvicultural treatments; species diversity; soil organic carbon

Résumé

Les différents chemins que peut emprunter la succession végétale suite à l’application de traitements sylvicoles dans le cadre de l’établissement de plantations, peuvent avoir une rétroaction sur les cycles du carbone (C) et de l'azote (N) par des mécanismes qui sont encore mal compris. Malgré l’importance de la compréhension du rôle potentiel des plantations dans la séquestration du C, peu d'études ont porté sur les processus souterrains tels que la décomposition des racines. Ici, nous avons étudié la manière dont des traitements sylvicoles (combinaisons factorielles de scarifiage, de fertilisation, et de contrôle de la végétation à l’aide d’un phytocide) et la végétation qui leur a fait suite, ont affecté la décomposition des racines fines (FRD; <2mm), la chimie des racines fines (C, N, P, K, Ca, Mg) et les concentrations en carbone organique (SOC) et en N de l’horizon minéral de surface (dans le sol entier et dans deux fractions: la matière organique particulaire (POM), et le limon et argile (S&C), 27 ans après l’établissement d’une plantation de pin blanc à Petawawa, Ontario, Canada. Nous avons observé une dominance des conifères et une surface terrière plus élevée, là où le contrôle de la végétation a été appliqué, tandis qu’une dominance de feuillus et une diversité d’espèces plus élevée étaient retrouvés là où ce dernier n’a pas été appliqué. La plus grande diversité d’espèces du sous-bois a été retrouvée là où le scarifiage a été appliqué, inversement, la plus faible diversité d’espèces du sous-bois a été enregistrée là où il y a eu contrôle de la végétation. La FRD (mesurée en terme de perte de masse après 8 mois *in situ*) était plus élevée lorsque le scarifiage et la fertilisation étaient combinés, et plus basse là où le contrôle de la végétation été appliqué seul. Le SOC (dans le sol entier et dans les fractions POM et S&C) a été significativement diminué par l’application de scarifiage, tandis que l'azote minéral n'a été affectée par aucun des trois traitements sylvicoles que cela soit dans le sol entier ou dans les fractions. Nous n’avons trouvé aucune corrélation entre la diversité des espèces et la FRD, la FRD et les SOC et N dans le sol minéral, ni entre la FRD et le pH du sol. En revanche, nous avons constaté que la végétation du sous-bois plus que les arbres, la chimie des racines et les propriétés du sol expliquait le mieux la FRD. Nous en concluons que les traitements sylvicoles affectent à la fois la FRD et le SOC à travers la végétation qui leur fait suite. Ces effets sur la FRD sont plus probablement due à des effets spécifiques des espèces du sous-bois qui induisent un environnement de sol favorable à la décomposition (i.e. meilleure qualité de la litière des feuilles, humidité du sol plus élevée) qu’à une plus grande diversité en soi. De plus, les effets négatifs des traitements sylvicoles et de la végétation qui leur fait suite sur le SOC ne semblent pas être compensés par une FRD plus élevée dans cette plantation de forêt tempérée.

Mots-clés

Décomposition racinaire; végétation du sous-bois; traitements sylvicoles; diversité des espèces; carbone organique du sol

Introduction

Land devoted to tree plantations is expected to increase in coming years (Carle and Holmgren 2008), whether for primarily protective functions or for intensive timber production (FAO 2006). Afforestation is also seen as a potential way to mitigate climate change by sequestering carbon in both tree biomass and forest soils (Paul et al. 2002, Arevalo et al. 2009). In the context where global temperatures are expected to rise by 1-3°C during the next century (IPCC 2013), there is no better time to more fully evaluate the mechanisms of soil carbon (C) loss and gain due to forest management practices. Silvicultural practices are likely to influence C storage directly through soil perturbation and changes in microclimate, but also indirectly, through multiple factors such as changes in vegetation structure and composition, litter input and subsequent litter decomposition rate.

Intensive silvicultural practices may lead to soil C losses, Maillard et al. (2010) found that intensive blade scarification had the most negative impact on soil organic C stock and stability, twenty years after plantation establishment (on the present study site), while studies reported decreases in soil C with increasing organic matter removal 8 to 16 yr after planting (Smaill et al. 2008), with complete forest floor removal (Powers et al. 2005, Smaill et al. 2008), and vegetation control through herbicide application (Shan et al. 2001, Echeverria et al. 2004). Nonetheless, fertilization can contribute to increased soil C in different forest types (Johnson and Curtis 2001) or may mitigate the negative impact of blade scarification (Maillard et al. 2010). However, several studies have noted no change in soil C storage after fertilization (Shan et al. 2001).

Tree species composition (either in plantations or natural forests) influences biogeochemical cycling of soil organic carbon (SOC) (Côté et al. 2000, Laganiere et al. 2013) and thereby partly determines forest ecosystem functioning. For example, Laganiere et al. (2013) showed that SOC was in more stable form in trembling aspen stands, while black spruce stands promoted greater SOC accumulation (partly due to low quality C inputs). Moreover, several studies explored the effects of tree species or forest cover type on different C fractions (Paré et al. 2006, He et al. 2008, Yang et al. 2009). Laganière et al. (2011) measured C in different soil aggregate size classes and soil physical fractions, they observed that black spruce soils accumulated more uncomplexed organic matter (OM) compared to aspen. This uncomplexed OM is composed of particles of OM that are not bound to mineral particles, and separated by density (using heavy liquids) and/or size (by sieving) (Gregorich et al. 2006). These two fractions are highly responsive to changes in C inputs to the soil and can provide a measurable change before any such change in the total soil organic matter (Gregorich and Janzen 1996). The proportion of total soil C and N accounted for in the uncomplexed OM can be substantial, and the amount of soil C and N accounted for in the size fraction is often greater than that in the density fraction (Gregorich et al. 2006). This uncomplexed OM is thought to be less protected against microbial degradation compared with the mineral-associated fraction because it is more accessible to microbes and their enzymes, and usually less recalcitrant biochemically (Lützow et al. 2006). Although often overlooked in comparison to tree species, understory species are a key component in the forest C cycle, due to associated high proportional litter input and litter turnover rate (Nilsson and Wardle 2005). Globally, litter decomposition in a number of biomes was observed to be more influenced by the high variability of green leaf traits of different species than by global climatic variation (Cornwell et al. 2008). Fontaine et al. (2004) demonstrated that the supply of fresh C (from plant litter decomposition) may accelerate the decomposition of soil C and induce a negative C balance (a phenomenon called the priming effect). The effect of root litter inputs is less studied compared to aboveground litter, however Cheng et al. (2003) measured increases in respiration of soil-derived C as high as 383% greater than respiration in the no-plant control soil, indicating that rhizosphere priming can substantially intensify SOC losses related to decomposition.

Silvicultural management practices may alter the diversity and abundance of canopy and understory species (Greene et al. 2016), which could in turn affect nutrient cycling and soil C storage. For example, Poirier et al. (2016) observed that silvicultural treatments and subsequent succession strongly regulated C and N cycling in the mineral soil of mixedwood forest (present study site), blade scarification and subsequent succession to hardwood overstory and herbaceous species cover, contributed to increased mineral soil pH, exchangeable base cations, net NO_3^- -N production, and favored total soil N and SOC in the surface mineral soil. Consequently, silvicultural treatment that induces changes in plant species composition (either canopy or understory) could substantially alter SOC cycling.

Here we investigate the influence of intensive silvicultural treatments: blade scarification, fertilization, and vegetation control with herbicide 27 yrs after plantation. Particularly, the effect of over- and understory vegetation diversity induced by these silvicultural treatments on fine root decomposition and concentration of soil C and N in two size fractions in the surface mineral soil,. We hypothesized that:

1. Silvicultural treatments have oriented forest overstory volume and understory vegetation diversity (especially the vegetation control treatment; plots with no vegetation control will present the highest species richness).
2. Based on the positive link between biodiversity and ecosystem functioning (BEF) (Cardinale et al. 2012), a higher diversity will contribute to higher rates of fine root decomposition (of entire community: trees and understory vegetation), higher macronutrient concentrations (N, P, K, Ca, Mg) in fine roots and higher SOC and N in the surface mineral soil, especially in the POM size fraction.

Methods

Site description

The plantation is located within the Petawawa National Research Forest in the Great Lakes St-Lawrence Forest Region, on the north shore of Cartier Lake (45°57′50″N, 77°34′45″W) at an elevation of 170 m above sea level. The climate of the region is characterized by mean annual temperature of 4.3°C and an average rainfall of 853 mm (Environment Canada 2016). The soils consist of deep, well-drained loam to sandy loam developed from acidic glaciofluvial till (Munson et al. 1993) and are classified as an Orthic Humo-Ferric Podzol (1998) or Haplorthod (Soil Survey Staff 2010).

Experimental design

The site at Cartier Lake was clearcut in the summer of 1985 and, the experimental design was established in 1986. Before the clear-cutting, the forest on the experimental site was composed of aspen (*Populus tremuloides* Michaux), white birch (*Betula papyrifera* Marshall) and white spruce (*Picea glauca* (Moench) Voss), with lesser components of yellow birch (*Betula alleghaniensis* Britton), basswood (*Tilia americana* L.), white pine and balsam fir (*Abies balsamea* (L.) Miller) (Munson et al. 1993). The experimental design consisted of four randomized complete blocks. Whole plots (20 by 40 m plots with 5 m buffers) were 2^3 factorial: two levels of three treatments. The control plots were first clearcut and planted without subsequent silvicultural treatment. The main treatments were blade scarification (S: levels 0 and 1), fertilization (F: levels 0 and 1) and vegetation control treatment (V: levels 0 and 1). Level 0 represented undisturbed condition following harvest and Level 1 the application of the treatment. Blade scarification at Level 1 represented complete removal of the organic forest floor. Fertilization at Level 1 consisted of an annual application of the slow-release fertilizer Osmocote (17:6:10 N-P-K plus micronutrients; N as 9.1% NH_4^+ and 7.9% NO_3^-). In the first growing season, 30 g of Osmocote were spread on the soil surface around each tree and this amount was increased each year to 40, 60, 80, 135 and 200 g in the second, third, fourth, fifth and sixth growing season, respectively. The vegetation control treatment at Level 1 represented an annual midsummer application of glyphosate isopropylamine salt (trade name Roundup) at a rate of 2.0 $kg.ha^{-1}$ of active ingredient for each of the 4 years following the establishment of the plantation. In April 1986, each 20 by 40 m whole plot was divided in two 20 by 20 m subplots and planted with 100 trees each of white spruce and white pine. In the present study only the subplots with white pine were studied.

Vegetation

Evaluation of the herb cover was made in July, 2013, using three 50 by 50 cm quadrats randomly distributed on each plot (see Appendix S4; Table S5). Basal area (in m^2 ha^{-1}) was determined in summer 2014 for each plot using a two-factor metric wedge prism (2 m^2 /ha basal area factor). All the trees that were validated by the prism were counted. The basal area was then estimated by multiplying the number of counted trees by the basal area factor. This information was used to estimate deciduous versus conifer basal area for each of the plots.

Soil sampling and analyses

The surface mineral soil (0-15 cm depth) was sampled by taking three randomly located cores per plot with an 8-cm-diameter soil corer, at the end of July, 2013. Soils were sieved through a 2 mm stainless steel sieve. Fine roots were removed, washed and dried, then stored protected from humidity previous to the decomposition experiment.

Soil texture was assessed by decantation (Bouyoucos 1962) and soil pH was measured in a prepared 0.01M $CaCl_2$ solution that is added to soil in a 1:2 soil to liquid mixture (Canadian Society of Soil Science, 2007). Cation exchange capacity (CEC) and base cations (Ca, Mg, K and Na) were assessed through an unbuffered NH_4Cl-$BaCl_2$ 0.1 M solution (Amacher et al. 1990) and measured by inductively coupled plasma emission (Optima 4300 DV ICP-OES, Perkin-Elmer Inc., Waltham, MA, USA).

Soil organic carbon was determined by the Walkley-Black method and total soil N was determined using the Kjeldhal method, to be consistent with previous studies on the same site (Munson et al. 1993). The analyses were performed on air-dried soil samples, finely ground (<150 µm) with a ball-mill mixer MM 200 (Retsch©, Haan, Germany).

Physically uncomplexed organic matter was isolated by size fractionation according to a modified (Gregorich and Beare 2008) method, by dispersing soil in distilled water (1:4 soil: water ratio) and shaking overnight with about 12 glass balls (6 mm diameter) to disrupt aggregates (Poirier et al. 2014). Subsequently, the soil and water mixture was washed over a 53 µm sieve using a fine jet of distilled water, and gently crushing any aggregates with rubber spatula. Two fractions were obtained: the particulate organic matter (POM; sand + large particles of organic matter > 53 µm) and the fine particle-size (S&C; silt + clay; <53 µm). The former was retained on the sieve and oven dried at 60°C overnight. The dry weight of this material was recorded before grinding, and homogenized to determine the SOC and N concentrations of the POM (POM-C and POM-N respectively). The SOC and N concentrations of the fine particle-size (S&C; silt + clay; <53 µm) were estimated by subtracting the POM fractions from total soil C and N concentrations. All C and N concentrations were determined by dry combustion with an elemental analyser (Trumac CNS-1000, LECO Corp. St. Joseph, MI).

Fine root decomposition and root chemistry

Roots removed from the soil samples of each plot (representing roots from all overstory and understory vegetation) were carefully washed, then dried in a forced-air oven at 65°C to constant weight. The dried roots were placed in mesh bags, 0.35 ± 0.02 g of root dry mass in each mesh bag (5 × 10 cm each) with a pore size of 50 microns (ANKOM Technology, Macedon, NY). On October 4th, 2013, the mesh bags were buried (inserted vertically in the soil) between 5 and 10 cm soil depth in the respective plot where originally sampled, and collected on May 21st, 2014. After collection, roots were carefully removed from mesh bags. Visible fungal hyphae were removed after drying roots at 65 °C to constant weight. Each root sample was weighed and finely ground using a ball-mill mixer MM 200 (Retsch©, Haan, Germany). For root chemistry, C was determined by dry combustion (Trumac CNS-1000, LECO Corp. St. Joseph, MI) and the concentrations of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) were determined by digestion (Parkinson and Allen 1975) followed by atomic absorption spectroscopy.

Statistical analyses

All statistical analyses were performed with R software (version 3.2.4 R Core Team 2016).

To test the effect of silvicultural treatments on all studied variables (vegetation diversity, basal area of the overstory, mass loss for root decomposition and soil properties), we applied analysis of variance (ANOVA) with a linear mixed-effect model, developed using the *lme* function in the *nlme* package (Pinheiro et al. 2007), with the three silvicultural treatments as fixed factors and blocks and plot identity as random factors. In all linear mixed-effect models, the normality of residuals and homogeneity of variance were tested and transformations were performed when necessary. Statistical significance was set at the 0.10 level given the inherent high variability in forest soil properties. When there were significant treatment interactions, the averages of treatment-level combinations were compared with the combination of interaction treatment Level 0 with the α level adjusted for the number of comparisons using Bonferonni's adjustment.

Principal component analysis (PCA) was performed to relate silvicultural treatments to over- and understory vegetation characteristics (species richness, basal area, leaf habit) using the *rda* function of the *vegan* package (Oksanen et al. 2016).Variance partitioning was used to determine the unique, shared, and total explained variation contributed by each set of explanatory variables using the varpart function of the *vegan* package and represented schematically by Venn diagrams. The significance of each partition was determined by permutation testing using partial redundancy analysis (rda function available in the vegan package) and ANOVA.

Results

Effects of silvicultural treatments on overstory and understory vegetation

Twenty-seven years after plantation establishment, the three silvicultural treatments led to distinct communities, characterized by contrasting vegetation structure and diversity. We observed a main effect of vegetation control (Fig.5), a domination and higher basal area of conifers when vegetation control was applied, while deciduous species and higher species richness were observed when vegetation control was not applied. Vegetation control alone decreased the species richness of the understory vegetation (*p* <0.054), while increasing the total basal area of the overstory canopy (white pine; *p* <0.001) (Table 8).

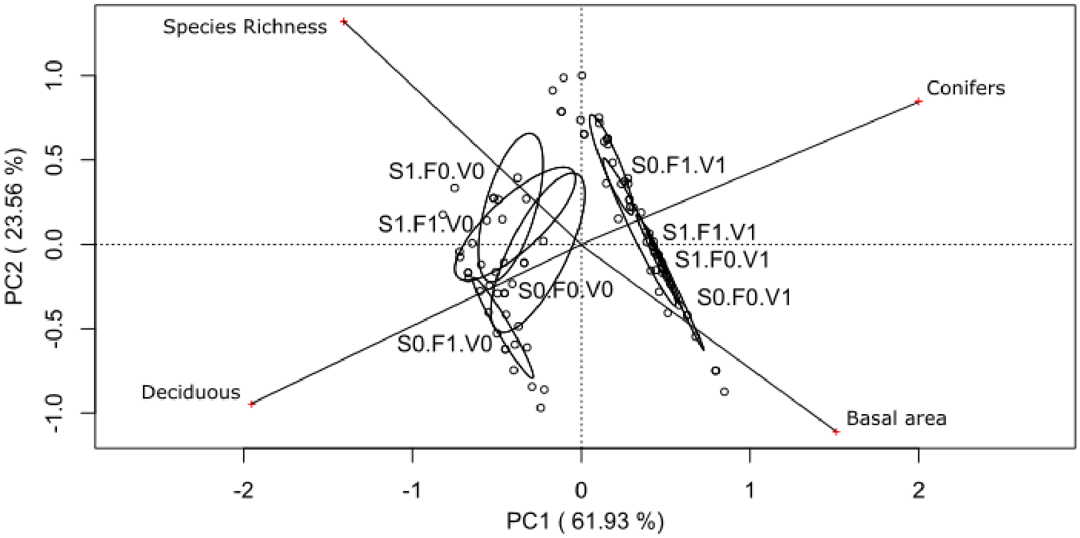

Figure 5. Principle component analysis joint plot ordination of over- and understory vegetation characteristics (species richness, basal area and leaf habit) and the three silvicultural treatments studied (S = scarification, F = fertilization, V = vegetation control, 0 = no treatment, 1 = application of treatment) 27 years after white pine planted.

Table 7. Impact of silvicultural treatments on surface mineral soil (0-15 cm) properties among plots 27 years after white pine planted. (S = scarification, F = fertilization, V = vegetation control, 0 = no treatment, 1 = application of treatment). The values are mean values. Means with the same letter are not significantly different based on the Tukey-HSD post hoc test. Standard errors are presented in brackets.

	pH	**Exch.Ca (mmol(+) kg^{-1} soil)**	**Exch.Mg (mmol(+) kg^{-1} soil)**	**Exch.K (mmol(+) kg^{-1} soil)**	**Total C (g kg-1)**	**Total N (g kg-1)**	**Soil C:N**	**C-POM**	**C-S&C**	**N-POM**	**N-S&C**
								(g SOC $_{POM}$ kg-^{1} soil)			
S0F0V0	4.3(±0.1)	2.36(±0.63)	0.23(±0.05)	0.26(±0.03)	18.25(±3.12)	1.32(±0.14)	13.2(±1.1)	7.67(±1.43)	10.59(±1.99)	0.35(±0.06)	0.97(±0.10)
S1F0V0	4.2(±0.1)	1.62(±0.25)	0.21(±0.04)	0.23(±0.02)	15.59(±1.84)	1.32(±0.07)	11.5(±1.0)	7.51(±1.3)	8.08(±1.85)	0.35(±0.06)	0.98(±0.09)
S0F1V0	4.6(±0.1)	3.88(±0.97)	0.34(±0.08)	0.31(±0.03)	19.23(±3.83)	1.35(±0.16)	13.3(±1.1)	7.98(±1.48)	11.26(±2.46)	0.38(±0.06)	0.97(±0.11)
S1F1V0	4.1(±0.1)	1.22(±0.21)	0.17(±0.03)	0.21(±0.02)	14.25(±0.87)	1.24(±0.06)	11.7(±0.8)	6.32(±0.49)	7.93(±0.77)	0.29(±0.04)	0.95(±0.04)
S0F0V1	4.2(±0.2)	2.21(±0.38)	0.13(±0.02)	0.17(±0.01)	16.06(±1.40)	1.19(±0.07)	13.3(±0.5)	8.19(±1.47)	7.88(±0.91)	0.35(±0.08)	0.85(±0.04)
S1F0V1	4.1(±0.1)	1.07(±0.22)	0.13(±0.02)	0.18(±0.01)	12.99(±1.22)	1.20(±0.05)	10.7(±0.7)	5.59(±0.49)	7.41(±0.86)	0.24(±0.02)	0.97(±0.06)
S0F1V1	4.3(±0.1)	2.56(±0.48)	0.21(±0.04)	0.20(±0.02)	19.33(±2.20)	1.32(±0.08)	14.3(±1.0)	8.03(±1.12)	11.09(±1.3)	0.34(±0.04)	0.98(±0.07)
S1F1V1	4.3(±0.0)	1.73(±0.24)	0.22(±0.04)	0.20(±0.03)	14.78(±1.70)	1.11(±0.08)	13.6(±1.4)	6.28(±0.76)	8.50(±1.33)	0.27(±0.03)	0.84(±0.09)
	S*	S***	V**	V*** S × V*	S*	NS	S*	S*	S*	NS	NS

*, **, *** the symbols indicate the significance at 0.1, 0.05 and 0.01 probability levels, respectively.

NS indicates not significant

Surface mineral soil chemical properties

The different silvicultural treatments influenced the mineral soil chemical properties in different ways. Scarification treatment decreased soil pH ($p = 0.077$), exchangeable Ca ($p = 0.002$), while exchangeable Mg ($p = 0.037$), and K ($p < 0.001$) were decreased by vegetation control. Exchangeable K was also decreased by the combined effect of scarification and vegetation control ($p = 0.098$) (Table 7).

Soil organic C and N in the whole soil and in size fractions

Scarification decreased SOC ($p = 0.051$) but no silvicultural treatment affected total soil N. The soil C:N ratio was significantly decreased by scarification ($p = 0.063$) (Table 7). Total SOC, C-POM and C- S&C decreased with scarification ($p = 0.059$, $p = 0.100$, $p = 0.066$ respectively) while soil total N and N in size fractions were unaffected by treatments.

Fine root decomposition and chemistry

Globally, fine root decomposition (mass loss) over 8 months (October 2013- May 2014) decreased significantly under the vegetation control treatment ($p = 0.043$) (Table 8). The interaction of the three treatments also significantly affected fine root decomposition, the highest decomposition occurred when scarification and fertilization treatments were applied without vegetation control and the lowest was recorded when vegetation control was applied alone (Table 8).

Table 8. Impact of silvicultural treatments on fine roots decomposition (FRD- mass loss) and fine roots C and macronutrients among plots 27 years after white pine planted. (S = scarification, F = fertilization, V = vegetation control, 0 = no treatment, 1 = application of treatment). The values are mean values. Means with the same letter are not significantly different based on the Tukey-HSD post hoc test. Standard errors are presented in brackets. Means in bold represent an effect of the treatment alone, otherwise it is a main effect of the treatment.

	Species richness	**Basal area (m^2 ha^{-1})**	**FRD (mg)**	**C (g kg^{-1})**	**N (g kg^{-1})**	**Root C:N**	**P (g kg^{-1})**	**K (g kg^{-1})**	**Ca (g kg^{-1})**	**Mg (g kg^{-1})**
S0F0V0	4.2(±0.3)	28.0(±1.9)	72.00(±0.01)	411.7(±11.8)	7.4(±0.5)	58.6(±4.2)	1.27(±0.15)	3.35(±0.35)	11.1(±0.96)	0.93(±0.12)
S1F0V0	5.7(±0.6)	23.0(±1.8)	66.62(±0.01)	392.6(±15.2)	7.9(±0.5)	51.0(±3.4)	1.23(±0.16)	4.55(±0.71)	9.17(±0.79)	0.86(±0.10)
S0F1V0	3.8(±0.5)	31.5(±2.1)	63.41(±0.01)	414.5(±13.8)	6.7(±0.5)	65.3(±4.5)	1.27(±0.12)	4.2(±0.58)	12.67(±0.7)	0.85(±0.05)
S1F1V0	5.6(±0.3)	26.0(±1.5)	91.50(±0.01)	425.6(±7.8)	9.0(±0.7)	49.7(±3.5)	1.6(±0.19)	4.55(±0.58)	8.75(±0.83)	1.05(±0.20)
S0F0V1	1.7(±0.5)	46.5(±3.1)	47.46(±0.00)	429.4(±10.2)	7.5(±0.6)	61.4(±4.9)	1.23(±0.12)	2.47(±0.17)	4.96(±0.36)	0.76(±0.07)
S1F0V1	2.2(±0.5)	41.5(±1.7)	63.60(±0.01)	436.1(±8.9)	7.2(±0.4)	62.9(±3.9)	1.31(±0.11)	2.78(±0.22)	4.94(±0.81)	0.63(±0.03)
S0F1V1	4.0(±0.8)	27.0(±1.1)	56.15(±0.01)	431.4(±11.0)	7.4(±0.4)	60.2(±3.5)	1.27(±0.09)	3.31(±0.2)	8.28(±1.01)	0.83(±0.06)
S1F1V1	2.8(±0.7)	37.0(±2.4)	64.78(±0.01)	417.4(±12.6)	7.9(±0.5)	55.0(±3.5)	1.35(±0.14)	3.54(±0.44)	5.7(±0.86)	0.75(±0.04)
	V*	V***	V**	V**	S**	S**	NS	V***	V***	V**
			S×F×V**					F**	S***	
									F**	

*. **. *** the symbols indicate the significance at 0.1. 0.05 and 0.01 probability levels. respectively.

NS indicates not significant

Moreover, a variation partitioning analysis identified the understory vegetation as a stronger driver of fine root decomposition than fine root chemistry, soil properties (soil pH, exchangeable base cations, sand and clay, data not shown), overstory vegetation or silvicultural treatment (Fig.6A). Understory vegetation abundance alone explained 39.8% of the fine root decomposition rate. Within understory vegetation, the same variation partitioning analysis identified a stronger contribution of fern cover (26.4%) compared to herb and shrub cover, in controlling fine root decomposition (Fig.6B). Going a step further, we undertook a forward selection of explanatory variables to identify the understory vegetation species that best explained fine root decomposition rate. Abundance (cover) of 8 of the 32 referenced species best explained fine root decomposition (Table 9).

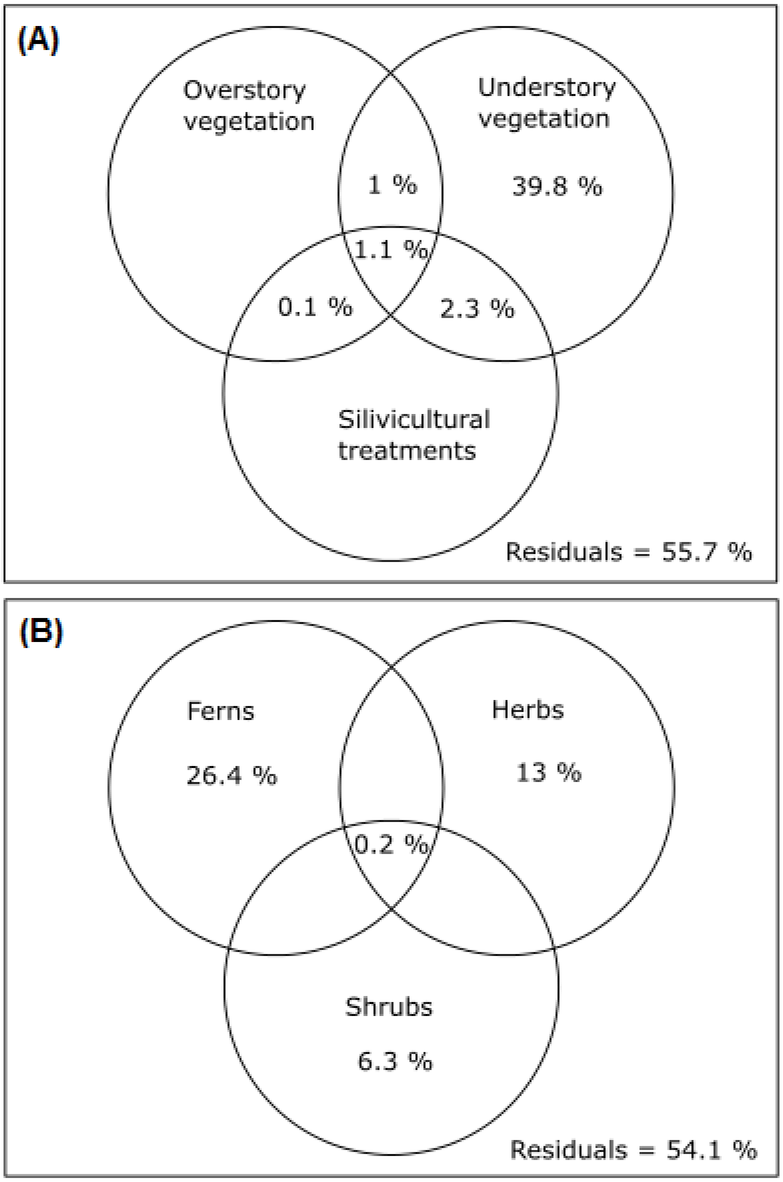

Figure 6. Venn diagram displaying the results of unique, shared, and total variation of the fine root decomposition explained by (A) the three silvicultural treatments and over- and understory vegetation percentage of cover, (B) understory vegetation composition.

Table 9. Understory vegetation species that best explained fine roots decomposition process according to a forward selection of explanatory variables. The (+) sign represent a positive relationship between the abundance of the species and fine root decomposition (FRD).

	Correlation with FRD	**Adj R^2**	**Adj R^2 Cum**	**F**	***p*-values**
Onoclea sensibilis	(+)	0.104	0.104	12.034	0.010
Trientalis borealis	(+)	0.106	0.210	13.553	0.006
Polytrichum sp	(+)	0.123	0.332	18.077	0.002
Rubus idaeus	(+)	0.052	0.385	8.841	0.015
Maianthemum canadense	(+)	0.031	0.415	5.771	0.016
Lycopodium obscurum	(+)	0.025	0.440	4.954	0.050
Diervilla lonicera	(+)	0.020	0.460	4.329	0.039
Fougere sp	(+)	0.021	0.481	4.623	0.037

Fine root C, C:N and macronutrients responded to silvicultural treatments (Table 8). Vegetation control increased root C (p = 0.045) and decreased root Mg (p = 0.031), while scarification increased root N (p = 0.034). Root K increased with fertilization (p = 0.040) and decreased with vegetation control (p = 0.006). Individually, the three treatments had an effect on root Ca, which decreased with scarification (p = 0.001) and vegetation control (p <0.001) and increased with fertilization (p = 0.031). The root C:N ratio was decreased by the scarification alone (p = 0.021). The applied treatments did not affect root P.

Discussion

Silvicultural treatment effects on understory species diversity, tree leaf habit and overstory tree volumes

As we expected, silvicultural treatments altered the diversity of understory vegetation. Twenty-seven years after treatment, vegetation control had the greatest effect on species diversity, with a higher diversity in plots without vegetation control (1.7 species on average with vegetation control species compared to 4.2 species without vegetation control). In an earlier study at the same site, Maillard et al. (2010) showed that twenty years after treatment, conifer species were dominant on vegetation control plots, while deciduous trees were dominant without vegetation control, the cover of herbs was also higher without vegetation control. This is confirmed by our measures of basal area, which was higher in plots with vegetation control (46.5 m^{-2} ha^{-1}) compared to plots without vegetation control (28 m^{-2} ha^{-1}).

Silvicutural treatment effects on soil properties

After 27 years, blade scarification (which represents a complete removal of organic layer) led to the development of hardwood herb-rich community with the highest cover of hardwood (mainly aspen) and herbs (Poirier et al. 2016). Although this vegetation community was characterized by higher levels of SOC, total N, Ca, Mg and K in the surface mineral soil, due to aspen presence on this relatively coarse-textured soil (Ste-Marie et al. 2007; Laganiere et al. 2013), we measured decreased soil pH, soil exchangeable Ca, total SOC, as well as SOC in both labile (C-POM) and fine (S&C) fractions. This decrease is in accordance with Maillard et al. (2010), twenty years after plantation on the same site, they observed that blade scarification decreased C concentration in the organic forest floor, while the effect of blade scarification on the mineral soil was less evident and partly mitigated by the addition of fertilizer. These observations suggest that it takes considerable time to observe the negative effect of blade scarification on the mineral soil and underlines the need to be careful when applying new silvicultural treatments for anticipated mitigation to increase soil C storage. In contrast, we found no effects of the three silvicutural treatments singly or combination on total N in surface mineral soil, nor on N in size fractions. Similarly, earlier studies on the same site measured no change in surface mineral soil total N after 6 years (Burgess et al. 1995) nor after 20 years (Maillard and Munson unpublished results).

Silvicutural treatments and subsequent vegetation effects on annual fine root decomposition

Although the greater herb cover and species diversity observed on the blade scarification plots was expected to be associated with a higher litter decomposition (Handa et al. 2014), we found no significant correlation between the diversity of understory vegetation (species richness) and fine root decomposition (FRD, as mass loss in 8 months). However, the highest FRD was observed when blade scarification and fertilization were combined without vegetation control (25.6% mass loss). Conversely, the lowest FRD was observed on plots where vegetation control was applied alone (13.6% mass loss), this treatment is also associated with lower species diversity. This suggests increasing species richness does not contribute to increased FRD, however, a reduction in diversity (lower understory species richness and monocultures in vegetation control plots) reduces FRD, compared to the control.

The lack of species diversity effects on FRD in our study is consistent with other studies such as Guerrero-Ramírez et al. (2016) who observed that tree root decomposition in tropical forests (experimental forest sites in central Panama) is mediated by root quality (structural, anatomical, and chemical characteristics) and soil environment rather than by species richness. Similarly, on the same experimental site, Scherer-Lorenzen et al. (2007) found only species-specific effects on decomposition when studying leaf litter mixing. In our study, we did not find correlations between fine root chemistry (C, N, P, K, Ca, Mg) nor soil properties and FRD. Instead, variance partitioning identified that understory vegetation cover, especially fern and herb cover, rather than overstory vegetation, was the stronger driver of FRD. In a boreal forest in northern Sweden, De Long et al. (2016) also demonstrated a stronger effect of understory plant functional group removal on litter decomposition rate, compared to successional stage and increased soil temperature. The understory vegetation importance seems not to be limited to effects on decomposition, since Poirier et al. (2016) on the same site observed a preponderant role of herb cover rather than overstory functional character (hardwood vs softwood), in mineral and subsurface soil properties, particularly N cycling.

As Guerrero-Ramírez et al. (2016), we believe our observations support recent evidence that the effects of diversity on decomposition, when present, are less important than diversity effects on primary productivity (Cardinale et al. 2011, Hooper et al. 2012). In our case, the stronger effect of cover of understory functional types compared to species diversity on FRD could be explained by the presence of particular plant functional types within litter mixtures (Handa et al. 2014). Principally, percent cover of ferns (*Onoclea sensibilis*), herbs (*Trientalis borealis*), and moss (*Polytrichum sp.*) contributed to explain FRD. Given the absence of a root chemistry effect we suggest that the increased FRD associated with these species could be mostly due to indirect effects of these species on FRD such as providing a favorable decomposition environment (i.e. by increasing soil moisture or altering pH (Finér et al. 2016, De Long et al. 2016) since microbes are very sensitive to soil pH (Rousk et al. 2009), or a species-specific responses related to litter quality (proximate carbon chemistry: lignin, lignin/N, solubles and fibre; not measured in the present study).

Conclusion

This study continues a series of studies conducted at this Petawawa Research Forest plantation since its establishment in 1986. Twenty-seven years after plantation, silvicultural treatments represent disturbances that clearly induced changes in species diversity and composition, which in turn impacted both positively or negatively belowground processes. Fine root decomposition (FRD) increased under blade scarification and fertilization and decreased following vegetation control, which is nevertheless recognized to control competing vegetation and ensure the survival and growth of planted white pines. Moreover, twenty-seven years after conifer plantation establishment in a temperate forest, SOC losses in the surface mineral soil (0-15 cm) following blade scarification have not recovered to the levels of control plots. Finally, the experiment revealed that understory vegetation rather than overstory vegetation, root chemistry or soil properties (soil pH, exchangeable base cations) had the greatest effect on FRD. This is a rare study to identify to the species level the understory species controlling belowground decomposition, however we are not able to identify the mechanism of control. The fact that percentage cover is influential would seem to indicate a global biomass rather than diversity effect on this process. In a context where studies exploring the relationship between species diversity and belowground processes are rare, especially in treed ecosystems, our study advances knowledge on factors driving processes contributing to soil carbon accumulation. The study also demonstrates the silvicultural and vegetation succession impacts are important to consider over relatively long periods, in order to understand vegetation feedback on these processes.

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Conclusion générale

L'objectif de ce projet de doctorat était de développer les connaissances sur le lien entre la biodiversité des compartiments aériens et le fonctionnement des compartiments souterrains des écosystèmes forestiers artificiels (plantations en climat tempéré), afin de mieux comprendre les mécanismes qui interviennent dans les processus biogéochimiques des sols, influençant particulièrement le cycle du C.

Diversité vs identité des arbres

Malgré l'intérêt croissant des chercheurs depuis les deux dernières décennies pour l'étude du lien entre la biodiversité et le fonctionnement des écosystèmes (BEF) (Cardinale et al. 2012), les connaissances sur les parties souterraines (racines, communautés microbiènnes, cycles des éléments nutritifs) sont à ce jour bien moins connues et étudiées que celles des parties aériennes. La principale raison de cette méconnaissance tient au fait de la difficulté à y accéder, surtout dans les écosystèmes forestiers. Ainsi, durant ce projet de doctorat, je me suis particulièrement concentrée sur les compartiments souterrains afin d'apporter des connaissances cruciales manquantes pour l'étude de la BEF.

Nous savons aujourd'hui que selon le processus écosystémique étudié ou la mesure de biodiversité utilisée, cette dernière peut influencer positivement ou négativement le processus ou encore ne pas avoir d'effet. L'identité des espèces d'arbres est elle aussi très importante pour expliquer la relation entre BEF. En effet, les deux mécanismes proposés pour expliquer une relation positive entre BEF sont basés à la fois sur la diversité et sur l'identité, la complémentarité des niches basée sur l'augmentation de la diversité des traits fonctionnels (FD), et les effets de sélection dus à la présence d'espèces dominantes qui présentent des traits particuliers (Roscher et al. 2012). Dans le chapitre I de ce projet de doctorat, les résultats ont révélé une absence d'effet des gradients de diversité (i.e. l'augmentation croissante de richesse spécifique (SR) et de diversité fonctionnelle (FD)) sur la productivité annuelle des racines fines, mais un effet de la diversité (SR) sur l'N du sol (plus bas dans SR2 que dans les monocultures et SR4). L'inverse a été observé en ce qui concerne l'identité des arbres qui a eu un effet plus important sur la productivité annuelle des racines fines mais pas sur les concentrations C et N du sol superficiel. Dans le chapitre II, ce sont à la fois la diversité et l'identité des arbres qui ont eu un effet sur le fonctionnement et la composition des communautés microbiennes du sol, alors que dans le chapitre III, l'identité de la végétation du sous-bois l'emportait sur la diversité (SR) pour expliquer le processus de décomposition des racines fines. Ces résultats viennent donc renforcer l'importance de tenir compte à la fois de la diversité et de l'identité des espèces d'arbres et les autres composantes de la végétation, dans le cadre d'une gestion optimale et durable des écosystèmes forestiers de climat tempéré.

L'approche des traits fonctionnels

Il est désormais bien établi que la biodiversité a un effet sur le fonctionnement des écosystèmes, ce que l'on connait moins c'est comment est-ce que la biodiversité influence ce fonctionnement ? Dans le but de participer à combler le manque de connaissances à ce sujet, nous avons eu recours à l'approche des traits fonctionnels dont le rôle est fondamental pour expliquer les corrélations sous-jacentes à la relation entre BEF. En particulier en ce qui concerne la complémentarité des niches (la plus grande exploitation des ressources due à une plus grande diversité des traits) tel qu'évoqué un peu plus haut.

C'est ainsi que dans les chapitre I et II, la plantation expérimentale de Sainte-Anne-de-Bellevue près de Montréal (projet IDENT) a permis pour la première fois de tester les effets de la FD sur un processus donné de manière indépendante de la SR. L'objectif a été d'étudier la relation entre BEF en utilisant les traits fonctionnels sous différents aspects obtenus à partir d'indices de dispersion calculés selon la méthode décrite par Laliberté and Legendre (2010), i.e. d'abord selon un gradient de FD puis, en utilisant les traits de manière individuelle en tenant compte de la variance du trait ou encore de sa valeur moyenne. Dans tous les cas (i.e. productivité annuelle des racines fines, respiration basale et biomasse microbienne), la valeur moyenne individuelle des traits a eu un effet plus marqué que celui de la variance de ces mêmes traits (i.e. un plus grand nombre de traits ont eu un effet sur les processus étudiés). Ce résultat peut s'expliquer par le lien étroit entre la valeur moyenne individuelle des traits et les effets de sélection (Mokany et al. 2008, Roscher et al. 2012). Par ailleurs, nos résultats sont les premiers à avoir permis d'identifier les traits qui ont une influence (positive ou négative) sur les différents processus étudiés dans les chapitres I et II. Cette découverte permet d'éclairer une partie de la relation complexe et mal connue de la relation entre la diversité de la végétation et le fonctionnement des compartiments souterrains, en ce qui concerne la productivité annuelle des racines fines ainsi que la respiration et la biomasse des communautés microbiennes du sol.

Cependant, lors de l'étude de l'effet de la FD en tant que gradient sur les processus de productivité annuelle des racines fines et sur les paramètres des communautés microbiennes du sol, aucun effet n'a été observé. L'absence d'effets du gradient de FD dans la présente étude vient appuyer la tendance observée par des études précédentes. En effet, à ce jour toutes les autres études menées sur le même site et employant le même gradient de FD n'ont relevé aucun effet de ce gradient quelque soit le processus étudié.

Il est donc légitime de se questionner sur la validité de la méthode employée pour le calcul du gradient de FD. En effet, de par le manque de connaissances actuelle empêchant une généralisation de la méthode, le calcul d'un gradient de FD fait appel à de nombreux choix souvent arbitraires, e.g. quels traits choisir, combien de traits choisir, est-ce judicieux que certains de ces traits proviennent de la littérature, comment tenir compte de la dynamique et de la plasticité des traits dans ce calcul, etc. Dans cette étude, 12 traits ont été utilisés pour le calcul du gradient de FD initial (gradient *a priori*). Ces traits ont été choisi afin d'être reliés à plusieurs processus écosystémiques différents. Le fait que ces traits ne soient pas reliés plus particulièrement au processus étudié (dans cette étude, la productivité des racines fines, biomasse microbienne – par GIR, respiration de la communauté microbienne) pourrait être l'une des raisons pour lesquelles un potentiel effet du gradient de FD n'a pas été observé. Cependant, dans le chapitre II du présent projet, j'ai entrepris de recalculer un second gradient de FD qui cette fois contenait des traits plus adaptés au processus étudié. Là encore, je n'ai pas observé d'effet du gradient de FD. Ceci confirme la complexité liée au calcul d'un tel gradient et rappelle que des efforts doivent encore être faits dans cette direction dans le but de pouvoir être en mesure de généraliser et d'extrapoler les résultats à l'échelle de l'écosystème.

Conifères vs feuillus

De ce projet de doctorat ont émergé plusieurs autres résultats innovants. Le Chapitre I a permis de révéler des différences de fonctionnement marquées entre les espèces de conifères et les feuillus au niveau de la productivité annuelle des racines fines, les conifères produisent plus de racines fines que les feuillus. La différence entre ces deux types de feuillages ne se limite pas à la productivité annuelle des racines fines. En effet, dans le chapitre II nos résultats ont indiqués que les feuillus métabolisaient un plus grand nombre de sources de C (parmi les sources de C testées dans cette étude) que les conifères, révélant un effet spécifique de certains feuillus (e.g. bouleau jaune et bouleau à papier). Nous n'avons pas connaissance d'autres études ayant démontré une telle différence de productivité annuelle de racines fines et d'utilisation des sources de C entre feuillus et conifères d'autant plus que notre étude portait sur un grand nombre d'espèces (i.e. 12 espèces d'arbres Nord américain).

D'autre part, la différence de productivité entre parties aériennes et parties souterraines observées ici chez les conifères et les feuillus vient renforcer les résultats d'études qui soulignent l'existence de dissymétrie de fonctionnement entre ces deux compartiments (Hobbie et al. 2010) et l'importance de tenir compte de la biomasse de l'arbre entier lorsqu'il s'agit d'émettre des conclusions générales. Ce fait ne concerne pas uniquement la biomasse totale de l'arbre et pourrait être extrapolé à tous les processus ainsi qu'aux communautés microbiennes. En effet, nous en savons actuellement très peu sur le lien entre les communautés microbiennes des parties aériennes des arbres et celles associées à leurs rhizosphères. Une dissymétrie de fonctionnement entre ces deux communautés pourrait être l'une des raisons expliquant les différences de fonctionnement en ce qui concerne l'allocation des ressources entre parties aériennes et parties souterraines des arbres. Notons également que dans la présente étude, la composition des communautés microbiennes a été étudiée via la méthode d'extraction des acides gras phospholipidiques (PLFA) qui présente certaines limites telles que le fait de ne pas être assez sensible (Frostegård et al. 2011), et que seul un changement dans la communauté entière des microorganismes est détecté (or plusieurs acides gras sont communs à plusieurs organismes). De plus, des acides gras d'organismes morts peuvent également être pris en compte. D'autres méthodes plus précises existent, tel que le séquençage génomique de ces communautés. Cette méthode présente par l'avantage de tenir compte des changements dans les communautés de microorganismes à l'échelle de l'individu et non plus à l'échelle de la communauté toute entière.

Il pourrait être intéressant à l'avenir d'avoir recours à ce type de techniques lors de l'étude des communautés microbiennes.

Implications potentielles pour l'aménagement forestier et la mitigation du CO_2 atmosphérique

Lors de l'étude de l'effet de la diversité sur la productivité annuelle des racines fines dans le chapitre I, nos résultats ont indiqué que la diversité des arbres a plus affecté la fraction S&C (fraction fine présumément plus stable) que dans la fraction POM (fraction labile et plus grossière). Ce résultat est surprenant compte tenu du jeune âge de la plantation (i.e. 4 ans au moment de l'étude) puisque l'on s'attendait à ce que ce soit la fraction POM qui contienne le plus de C et d'N, car cette fraction est supposée répondre le plus rapidement aux propriétés physiques, chimiques, et aux changements microclimatiques du sol (Khanna et al. 2001) et donc aux changements dans l'utilisation des sols (e.g. augmentation de la diversité des arbres). Ce résultat pourrait néanmoins être utilisé pour proposer aux gestionnaires forestiers l'implantation d'arbres forestiers (tels que des bouleaux jaunes ou à papier) sur d'anciens champs agricoles comme c'est le cas dans la présente. En effet, ceci pourrait permettre d'augmenter le « turnover » du C et de l'N sur ces sites tout en augmentant la stabilité de ces deux éléments dans les sols. Cependant, une étude de la séquestration et de la stabilité de ces deux éléments sur ce site à plus long terme serait requise afin de valider nos suggestions aux gestionnaires.

D'autre part, le chapitre III s'est focalisé sur l'étude de l'impact de trois traitements sylvicoles appliqués il y a 27 ans sur la décomposition des racines fines ainsi que sur le C et l'N du sol (totaux et dans les fractions). Cette étude a montré qu'avec le temps (i.e. 27 ans) ces traitements sylvicoles ont modifié la composition des espèces d'arbres et de végétation du sous-bois. Nos résultats ont révélé que la végétation du sous-bois influençait le plus la décomposition des racines fines que les espèces d'arbres, les propriétés du sol ou encore la chimie des racines dans cette plantation âgée de forêt tempérée. Une investigation plus poussée a permis pour la première fois d'identifier les espèces du sous-bois qui ont le mieux expliqué les variations de décomposition des racines fines. En effet, certaines espèces du sous-bois (e.g. onoclée sensible et trientale boréale) ont été associées à l'augmentation de la décomposition des racines fines sans que cela ne soit accompagné d'un phénomène de « priming effect » (i.e. l'accélération de la décomposition du C du sol suite à l'incorporation de C provenant de la litière des plantes). Il pourrait néanmoins être intéressant dans le cadre de la poursuite de cette étude, de compléter cette dernière par une analyse de pistes. Ceci permettrait de reconstituer le cheminement des effets induits par les trois traitements sylvicoles sur la décomposition des racines fines tout en intégrant chacun des intervenants responsables de ces effets (e.g. potentiel effet hiérarchique du traitement sur les arbres, des arbres sur la végétation du sous-bois, finalement de la végétation du sous-bois sur le sol).

De manière générale, les résultats de cette thèse ont permis de découvrir et de mettre en évidence des aspects jusqu'alors inconnus du lien entre BEF, notamment en ce qui concerne le lien entre la diversité des parties aériennes et le fonctionnement des parties souterraines. Nos résultats ont permis d'identifier avec précision les espèces d'arbres, de végétation du sous-bois ou encore les traits fonctionnels et les processus sur lesquels ils interviennent. Ceci pourrait permettre d'affiner les modèles de prédiction des cycles du C et de l'N ou encore de prodiguer des conseils avisés aux gestionnaires forestiers.

Bibliographie

Alain, K., and J. Querellou. 2009. Cultivating the uncultured: limits, advances and future challenges. Extremophiles 13:583–594.

Amacher, M. C., R. E. Henderson, M. D. Breithaupt, C. L. Seale, and J. M. LaBauve. 1990. Unbuffered and buffered salt methods for exchangeable cations and effective cation-exchange capacity. Soil Science Society of America Journal 54:1036–1042.

Amoroso, M. M., and E. C. Turnblom. 2006. Comparing productivity of pure and mixed Douglas-fir and western hemlock plantations in the Pacific Northwest. Canadian Journal of Forest Research 36:1484–1496.

Amundson, R. 2001. The carbon budget in soils. Annual Review of Earth and Planetary Sciences 29:535–562.

Anderson, J. P. E., and K. H. Domsch. 1978. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biology and Biochemistry 10:215–221.

Anderson, T. H. 2003. Microbial eco-physiological indicators to asses soil quality. Agriculture, Ecosystems and Environment 98:285–293.

Anderson, T. H., and K. H. Domsch. 1985. Determination of ecophysiological maintenance carbon requirements of soil microorganisms in a dormant state. Biology and Fertility of Soils 1:81–89.

Anderson, T. H., and K. H. Domsch. 1993. The metabolic quotient for CO_2 (qCO_2) as a specific activity parameter to assess the effects of environmental conditions, such as pH, on the microbial biomass of forest soils. Soil Biology and Biochemistry 25:393–395.

Anderson, T. H., and K. H. Domsch. 2010. Soil microbial biomass: The eco-physiological approach. Soil Biology and Biochemistry 42:2039–2043.

Arevalo, C. B. M., J. S. Bhatti, S. X. Chang, and D. Sidders. 2009. Ecosystem carbon stocks and distribution under different land-uses in north central Alberta, Canada. Forest Ecology and Management 257:1776–1785.

Augusto, L., J. Ranger, D. Binkley, and A. Rothe. 2002. Impact of several common tree species of European temperate forests on soil fertility. Annals of Forest Science 59:233–253.

Augusto, L., A. De Schrijver, L. Vesterdal, A. Smolander, C. Prescott, and J. Ranger. 2015. Influences of evergreen gymnosperm and deciduous angiosperm tree species on the functioning of temperate and boreal forests. Biological Reviews 90:444–466.

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

Baisden, W. T., R. Amundson, A. C. Cook, and D. L. Brenner. 2002. Turnover and storage of C and N in five density fractions from California annual grassland surface soils. Global Biogeochemical Cycles 16(4).

Balvanera, P., A. B. Pfisterer, N. Buchmann, J. S. He, T. Nakashizuka, D. Raffaelli, and B. Schmid. 2006. Quantifying the evidence for biodiversity effects on ecosystem functioning and services. Ecology Letters 9:1146–1156.

Bardgett, R. D., W. D. Bowman, R. Kaufmann, and S. K. Schmidt. 2005. A temporal approach to linking aboveground and belowground ecology. Trends in Ecology and Evolution 20:634–641.

Bardgett, R. D., and W. H. van der Putten. 2014. Belowground biodiversity and ecosystem functioning. Nature 515:505–511.

Bardgett, R. D., and A. Shine. 1999. Linkages between plant litter diversity, soil microbial biomass and ecosystem function in temperate grasslands. Soil Biology and Biochemistry 31:317–321.

Bauhus, J., P. K. Khanna, and N. Menden. 2000. Aboveground and belowground interactions in mixed plantations of *Eucalyptus globulus* and *Acacia mearnsii*. Canadian Journal of Forest Research 30:1886–1894.

Berard, A., T. Bouchet, G. Sévenier, A. L. Pablo, and R. Gros. 2011. Resilience of soil microbial communities impacted by severe drought and high temperature in the context of Mediterranean heat waves. European Journal of Soil Biology 47:333–342.

Berard, A., C. Mazzia, V. Sappin Didier, L. Capowiez, and Y. Capowiez. 2014. Use of the MicroResp$^{\mathrm{TM}}$ method to assess pollution-induced community tolerance in the context of metal soil contamination. Ecological Indicators 40:27–33.

Berish, C. W., and J. J. Ewel. 1988. Root development in simple and complex tropical successional ecosystems. Plant and Soil 106:73–84.

Birouste, M., E. Kazakou, A. Blanchard, and C. Roumet. 2012. Plant traits and decomposition: are the relationships for roots comparable to those for leaves? Annals of Botany 109:463–472.

Bouyoucos, G. J. 1962. Hydrometer method improved for making particle size analyses of soils. Agronomy Journal 54:464–465.

Bragazza, L., C. Freeman, T. Jones, H. Rydin, J. Limpens, N. Fenner, T. Ellis, R. Gerdol, M. Hájek, T. Hájek, P. Iacumin, L. Kutnar, T. Tahvanainen, and H. Toberman. 2006. Atmospheric nitrogen deposition promotes carbon loss from peat bogs. Proceedings of the National Academy of Sciences 103:19386-19389.

Brassard, B. W., H. Y. H. Chen, Y. Bergeron, and D. Paré. 2011. Differences in fine root productivity between mixed- and single-species stands. Functional Ecology 25:238–246.

Brassard, B. W., H. Y. H. Chen, X. Cavard, J. Laganière, P. B. Reich, Y. Bergeron, D. Paré, and Z. Yuan. 2013. Tree species diversity increases fine root productivity through increased soil volume filling. Journal of Ecology 101:210–219.

Brockett, B. F. T., C. E. Prescott, and S. J. Grayston. 2012. Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada. Soil Biology and Biochemistry 44:9–20.

Bunker, D. E., F. Declerck, J. C. Bradford, R. K. Colwell, I. Perfecto, O. L. Phillips, M. Sankaran, and S. Naeem. 2005. Species loss and aboveground carbon storage in a tropical forest. Science 310:1029–1031.

Burnham, K. P., and D. R. Anderson. 2003. Model selection and multimodel inference: a practical information-theoretic approach. Springer Science and Business Media.

Butenschoen, O., S. Scheu, and N. Eisenhauer. 2011. Interactive effects of warming, soil humidity and plant diversity on litter decomposition and microbial activity. Soil Biology and Biochemistry 43:1902–1907.

Cadotte, M. W., J. Cavender Bares, D. Tilman, and T. H. Oakley. 2009. Using phylogenetic, functional and trait diversity to understand patterns of plant community productivity. PLoS One 4 (5):e5695.

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

Campbell, C. D., S. J. Grayston, and D. J. Hirst. 1997. Use of rhizosphere carbon sources in sole carbon source tests to discriminate soil microbial communities. Journal of Microbiological Methods 30:33–41.

Canada, E. 2016. Canadian Climate Normal 1971–2000, Petawawa National Forestry Center. http://climat.meteo.gc.ca/climate_normals/results_f.html?searchType=stnNameandtxtStationName=petawawaandsearchMethod=containsandtxtCentralLatMin=0andtxtCentralLatSec=0andtxtCentralLongMin=0andtxtCentralLongSec=0andstnID=4353anddispBack=0.

Cardinale, B. J., J. E. Duffy, A. Gonzalez, D. U. Hooper, C. Perrings, P. Venail, A. Narwani, G. M. Mace, D. Tilman, D. A.Wardle, A. P. Kinzig, G. C. Daily, M. Loreau, J. B. Grace, A. Larigauderie, D. S. Srivastava, and S. Naeem. 2012. Corrigendum: Biodiversity loss and its impact on humanity. Nature 489:326–326.

Cardinale, B. J., A. R. Ives, and P. Inchausti. 2004. Effects of species diversity on the primary productivity of ecosystems: Extending our spatial and temporal scales of inference. Oikos 104:437 – 450.

Cardinale, B. J., K. L. Matulich, D. U. Hooper, J. E. Byrnes, E. Duffy, L. Gamfeldt, P. Balvanera, M. I. O'Connor, and A. Gonzalez. 2011. The functional role of producer diversity in ecosystems. American Journal of Botany 98:572–592.

Cardinale, B. J., J. P. Wright, M. W. Cadotte, I. T. Carroll, A. Hector, D. S. Srivastava, M. Loreau, and J. J. Weis. 2007. Impacts of plant diversity on biomass production increase through time because of species complementarity. Proceedings of the National Academy of Sciences of the United States of America 104:18123–18128.

Carle, J., and P. Holmgren. 2008. Wood from planted forests: a global outlook 2005-2030. Forest Products Journal 58:6–18.

Cavard, X., Y. Bergeron, H. Y. H. Chen, and D. Paré. 2010. Mixed-species effect on tree aboveground carbon pools in the east-central boreal forests. Canadian Journal of Forest Research 40:37–47.

Cebrian, J. 1999. Patterns in the Fate of Production in Plant Communities. The American Naturalist 154:449–468.

Chapman, S. J., C. D. Campbell, and R. R. E. Artz. 2007. Assessing CLPPs using MicroResp^{TM}. Journal of Soils and Sediments 7:406–410.

Cheng, W., D. W. Johnson, and S. Fu. 2003. Rhizosphere effects on decomposition. Soil Science Society of America Journal 67:1418–1427.

Chessel, D., A. B. Thioulouse, and D. Jean. 2004. The ade4 package. I. One-table methods. R News 4:5–10.

Comas, L. H., and D. M. Eissenstat. 2009. Patterns in root trait variation among 25 coexisting North American forest species. New Phytologist 182:919–928.

Coq, S., J. M. Souquet, E. Meudec, V. Cheynier, and S. Hättenschwiler. 2010. Interspecific variation in leaf litter tannins drives decomposition in a tropical rain forest of French Guiana. Ecology 91:2080–2091.

Cornwell, W. K., J. H. C. Cornelissen, K. Amatangelo, E. Dorrepaal, V. T. Eviner, O. Godoy, S. E. Hobbie, B. Hoorens, H. Kurokawa, N. Pérez-Harguindeguy, H. M. Quested, L. S. Santiago, D. A. Wardle, I. J. Wright, R. Aerts, S. D. Allison, P. van Bodegom, V. Brovkin, A. Chatain, T. V Callaghan, S. Díaz, E. Garnier, D. E. Gurvich, E. Kazakou, J. A. Klein, J. Read, P. B. Reich, N. A. Soudzilovskaia, M. V. Vaieretti, and M. Westoby. 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. Ecology Letters 11:1065–1071.

Côté, L., S. Brown, D. Paré, J. Fyles, and J. Bauhus. 2000. Dynamics of carbon and nitrogen mineralization in relation to stand type, stand age and soil texture in the boreal mixedwood. Soil Biology and Biochemistry 32:1079–1090.

Cuevas, E., S. Brown, and A. E. Lugo. 1991. Above- and belowground organic matter storage and production in a tropical pine plantation and a paired broadleaf secondary forest. Plant and Soil 135:257–268.

Degens, B. P., L. A. Schipper, G. P. Sparling, and M. V. 2000. Decreases in organic C reserves in soils can reduce the catabolic diversity of soil microbial communities. Soil Biology and Biochemistry 32:189–196.

Delgado-Baquerizo, M., L. Giaramida, P. B. Reich, A. N. Khachane, K. Hamonts, C. Edwards, L. Lawton, and B. K. Singh. 2016. Lack of functional redundancy in the relationship between microbial diversity and ecosystem functioning. Journal of Ecology:1–11.

De Deyn, G. B., H. Quirk, and R. D. Bardgett. 2011. Plant species richness, identity and productivity differentially influence key groups of microbes in grassland soils of contrasting fertility. Biology Letters 7:75–78.

Dias, A. T. C., M. P. Berg, F. Bello, A. R. Oosten, K. Bíla, and M. Moretti. 2013. An experimental framework to identify community functional components driving ecosystem processes and services delivery. Journal of Ecology 101:29–37.

Díaz, S., and M. Cabido. 2001. Vive la difference: plant functional diversity matters to ecosystem processes. Trends in Ecology and Evolution 16:646–655.

Dijkstra, F. A., S. E. Hobbie, and P. B. Reich. 2006. Soil processes affected by sixteen grassland species grown under different environmental conditions. Soil Science Society of America Journal 70:770–777.

Domisch, T., L. Finér, S. M. Dawud, L. Vesterdal, and K. Raulund-Rasmussen. 2015. Does species richness affect fine root biomass and production in young forest plantations? Oecologia 177:581–594.

Dray, S., and A. B. Dufour. 2007. The ade4 package: implementing the duality diagram for ecologists. Journal of Statistical Software 22:1–20.

Duffy, J. E. 2009. Why biodiversity is important to the functioning of real-world ecosystems. Frontiers in Ecology and the Environment 7:437-444.

Echeverria, M. E., D. Markewitz, L. A. Morris, and R. L. Hendrick. 2004. Soil organic matter fractions under managed pine plantations of the southeastern USA. Soil Science Society of America Journal 68:950–958.

Edgar, C. B., and T. E. Burk. 2001. Productivity of aspen forests in northeastern Minnesota, U.S.A., as related to stand composition and canopy structure. Canadian Journal of Forest Research 31:1019–1029.

Eisenhauer, N., H. Beßler, C. Engels, G. Gleixner, M. Habekost, A. Milcu, S. Partsch, A. C. W. Sabais, C. Scherber, and S. Steinbeiss. 2010. Plant diversity effects on soil microorganisms support the singular hypothesis. Ecology 91:485–496.

Erskine, P. D., D. Lamb, and M. Bristow. 2006. Tree species diversity and ecosystem function: Can tropical multi-species plantations generate greater productivity? Forest Ecology and Management 233:205–210.

Escobar, I. E. C., V. M. Santos, D. K. A. da Silva, M. F. Fernandes, U. M. T. Cavalcante, and L. C. Maia. 2015. Changes in microbial community structure and soil biological properties in mined dune areas during re-vegetation. Environmental Management 55:1433–1445.

Eviner, V. T., and F. S. Chapin III. 2003. Functional matrix: a conceptual framework for predicting multiple plant effects on ecosystem processes. Annual Review of Ecology, Evolution, and Systematics 34:455–485.

FAO. 2006. Global planted forests thematc study: results and analysis. In: Del Lungo, A., Ball, J., Carle, J. (Eds.). FAO.

Findlay, R. H. 2004. Determination of microbial community structure using phospholipid fatty acid profiles. Pages 983–1004 Molecular Microbial Ecology Manual, 2nd Edition. Kluwer Academic Publishers, Netherlands.

Finér, L., M. Jurgensen, M. Palviainen, S. Piirainen, and D. Page Dumroese. 2016. Does clear-cut harvesting accelerate initial wood decomposition? A five-year study with standard wood material. Forest Ecology and Management 372:10–18.

Fissore, C., C. P. Giardina, C. W. Swanston, G. M. King, and R. K. Kolka. 2009. Variable temperature sensitivity of soil organic carbon in North American forests. Global Change Biology 15:2295–2310.

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

Fornara, D. A., and D. Tilman. 2008. Plant functional composition influences rates of soil carbon and nitrogen accumulation. Journal of Ecology 96:314–322.

Fornara, D. A., and D. Tilman. 2009. Ecological mechanisms associated with the positive diversity–productivity relationship in an N-limited grassland. Ecology 90:408–418.

Fornara, D. A., D. Tilman, and S. E. Hobbie. 2009. Linkages between plant functional composition, fine root processes and potential soil N mineralization rates. Journal of Ecology 97:48–56.

Frostegård, Å., A. Tunlid, and E. Bååth. 2011. Use and misuse of PLFA measurements in soils.

Soil Biology and Biochemistry 43:1621–1625.

Fu, X., F. Yang, J. Wang, Y. Di, X. Dai, X. Zhang, and H. Wang. 2015. Understory vegetation leads to changes in soil acidity and in microbial communities 27 years after reforestation. Science of the Total Environment 502:280–286.

Gaillard, V., C. Chenu, and S. Recous. 2003. Carbon mineralisation in soil adjacent to plant residues of contrasting biochemical quality. Soil Biology and Biochemistry 35:93–99.

Gartner, T. B., and Z. G. Cardon. 2004. Decomposition dynamics in mixed-species leaf litter. Oikos 104:230–246.

Grayston, S. J., G. S. Griffith, J. L. Mawdsley, C. D. Campbell, and R. D. Bardgett. 2001. Accounting for variability in soil microbial communities of temperate upland grassland ecosystems. Soil Biology and Biochemistry 33:533–551.

Greene, R. E., R. B. Iglay, K. O. Evans, D. A. Miller, T. B. Wigley, and S. K. Riffell. 2016. A meta-analysis of biodiversity responses to management of southeastern pine forestsopportunities for open pine conservation. Forest Ecology and Management 360:30–39.

Gregorich, E. G., and M. H. Beare.2008. Physically uncomplexed organic matter. Soil sampling and methods of analysis. 2nd ed. CRC Press, Boca Raton, FL, pp. 607-616.

Gregorich, E. G., M. H. Beare, U. F. McKim, and J. O. Skjemstad. 2006. Chemical and biological characteristics of physically uncomplexed organic matter. Soil Science Society of America Journal 70:975–985.

Gregorich, E. G., and H. H. Janzen. 1996. Storage of soil carbon in the light fraction and macroorganic matter. Structure and organic matter storage in agricultural soils,167–190.

Griffiths, B. ., K. Ritz, R. Wheatley, H. Kuan, B. Boag, S. Christensen, F. Ekelund, S. Sørensen, S. Muller, and J. Bloem. 2001. An examination of the biodiversity–ecosystem function relationship in arable soil microbial communities. Soil Biology and Biochemistry 33:1713–1722.

Grime, J. P. 1998. Benefits of plant diversity to ecosystems: immediate, filter and founder effects. Journal of Ecology 86:902–910.

Guckland, A., M. Jacob, H. Flessa, F. M. Thomas, and C. Leuschne. 2009. Acidity, nutrient stocks, and organic-matter content in soils of a temperate deciduous forest with different abundance of European beech (Fagus sylvatica L.). Journal of Plant Nutrition and Soil Science 172:500–511.

Guenay, Y., A. Ebeling, K. Steinauer, W. W. Weisser, and N. Eisenhauer. 2013. Transgressive overyielding of soil microbial biomass in a grassland plant diversity gradient. Soil Biology and Biochemistry 60:122–124.

Guerrero-Ramírez, N. R., D. Craven, C. Messier, C. Potvin, B. L. Turner, and I. T. Handa. 2016. Root quality and decomposition environment, but not tree species richness, drive root decomposition in tropical forests. Plant and Soil:1–15.

Gurmesa, G. A., I. K. Schmidt, P. Gundersen, and L. Vesterdal. 2013. Soil carbon accumulation and nitrogen retention traits of four tree species grown in common gardens. Forest Ecology and Management 309:47–57.

Hadden, D., and A. Grelle. 2016. Changing temperature response of respiration turns boreal forest from carbon sink into carbon source. Agricultural and Forest Meteorology 223:30–38.

Hamel, C., K. Hanson, F. Selles, A. F. Cruz, R. Lemke, B. McConkey, and R. Zentner. 2006. Seasonal and long-term resource-related variations in soil microbial communities in wheat-based rotations of the Canadian prairie. Soil Biology and Biochemistry 38:2104–2116.

Hamer, U., and B. Marschner. 2002. Priming effects of sugars, amino acids, organic acids and catechol on the mineralisation of lignin and peat. Journal of Plant Nutrition and Soil Science 165:261.

Handa, I. T., R. Aerts, F. Berendse, M. P. Berg, A. Bruder, O. Butenschoen, E. Chauvet, M. O. Gessner, J. Jabiol, and M. Makkonen. 2014. Consequences of biodiversity loss for litter decomposition across biomes. Nature 509:218–221.

Handershot, W. H., H. Lalande, and M. Duquette. 1993. Soil Reaction and Exchangeable Acidity. Soil Sampling and Methods of Analysis. Martin R. Carter (Ed.). Canadian Society of Soil Science.

Harden, J. W., K. P. O'neill, S. E. Trumbore, H. Veldhuis, and B. J. Stocks. 1997. Moss and soil contributions to the annual net carbon flux of a maturing boreal forest. Journal of Geophysical Research 102(D24).

Harrison, K. A., and R. D. Bardgett. 2010. Influence of plant species and soil conditions on plant–soil feedback in mixed grassland communities. Journal of Ecology 98:384–395.

Hättenschwiler, S., and P. Gasser. 2005. Soil animals alter plant litter diversity effects on decomposition. Proceedings of the National Academy of Sciences of the United States of America 102:1519–1524.

Hättenschwiler, S., A. V. Tiunov, and S. Scheu. 2005. Biodiversity and litter decomposition in terrestrial ecosystems. Annual Review of Ecology, Evolution, and Systematics 36:191–218.

He, Y., Z. Xu, C. Chen, J. Burton, Q. Ma, Y. Ge, and J. Xu. 2008. Using light fraction and macroaggregate associated organic matters as early indicators for management-induced changes in soil chemical and biological properties in adjacent native and plantation forests of subtropical Australia. Geoderma 147:116–125.

Hector, A., B. Schmid, C. Beierkuhnlein, M. C. Caldeira, M. Diemer, P. G. Dimitrakopoulos, J. A. Finn, H. Freitas, P. S. Giller, J. Good, R. Harris, P. Högberg, K. Huss Danell, J. Joshi, A. Jumpponen, C. Körner, P. W. Leadley, M. Loreau, A. Minns. 1999. Plant Diversity Experiments and in Productivity European Grasslands. Science 286:1123–1127.

Herman, D. J., M. K. Firestone, E. Nuccio, and A. Hodge. 2012. Interactions between an arbuscular mycorrhizal fungus and a soil microbial community mediating litter decomposition. FEMS Microbiology Ecology 80:236–247.

Hobbie, S. E. 1992. Effects of plant species on nutrient cycling. Trends in Ecology and Evolution 7:336–339.

Hobbie, S. E., M. Ogdahl, J. Chorover, O. A. Chadwick, J. Oleksyn, R. Zytkowiak, and P. B. Reich. 2007. Tree species effects on soil organic matter dynamics: the role of soil cation composition. Ecosystems 10:999–1018.

Hobbie, S. E., J. Oleksyn, D. M. Eissenstat, and P. B. Reich. 2010. Fine root decomposition rates do not mirror those of leaf litter among temperate tree species. Oecologia 162:505–513.

Hooper, D. U. 1998. The role of complementarity and competition in ecosystem responses to variation in plant diversity. Ecology 79:704–719.

Hooper, D. U., E. C. Adair, B. J. Cardinale, J. E. K. Byrnes, B. A. Hungate, K. L. Matulich, A. Gonzalez, J. E. Duffy, L. Gamfeldt, and M. I. O'Connor. 2012. A global synthesis reveals biodiversity loss as a major driver of ecosystem change. Nature 486:105–108.

Hooper, D. U., F. S. Chapin, J. J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J. H. Lawton, D. M. Lodge, M. Loreau, S. Naeem, B. Schmid, H. Setälä, A. J. Symstad, J. Vandermeer, and D. A. Wardle. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecological Monographs 75:3–35.

Hooper, D. U., and P. M. Vitousek. 1998. Effects of plant composition and diversity on nutrient cycling. Ecological Monographs 68:121–149.

Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general parametric models. Biometrical Journal 50:346–363.

Ilstedt, U., A. Nordgren, and A. Malmer. 2000. Optimum soil water for soil respiration before and after amendment with glucose in humid tropical acrisols and a boreal mor layer. Soil Biology and Biochemistry 32:1591–1599.

Ingram, J. S. I., and E. C. M. Fernandes. 2001. Managing carbon sequestration in soils: concepts and terminology. Agriculture, Ecosystems and Environment 87:111–117.

Ingwersen, J., C. Poll, T. Streck, and E. Kandeler. 2008. Micro-scale modelling of carbon turnover driven by microbial succession at a biogeochemical interface. Soil Biology and Biochemistry 40:864–878.

Insam, H., and K. Haselwandter. 1989. Metabolic quotient of the soil microflora in relation to plant succession. Oecologia 79:174–178.

IPCC, 2013. Climate change 2013: the physical science basis. In: Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M. (Eds.), Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Jacob, A., D. Hertel, and C. Leuschner. 2014. Diversity and species identity effects on fine root productivity and turnover in a species-rich temperate broad-leaved forest. Functional Plant Biology 41:678–689.

Jandl, R., M. Lindner, L. Vesterdal, B. Bauwens, R. Baritz, F. Hagedorn, D. W. Johnson, K. Minkkinen, and K. A. Byrne. 2007. How strongly can forest management influence soil carbon sequestration? Geoderma 137:253–268.

Janzen, H. H. 2004. Carbon cycling in earth systems-a soil science perspective. Agriculture, Ecosystems and Environment 104:399–417.

Jenkinson, D. S. 1966. Studies on the decomposition of plant material in soil. Journal of Soil Science 17:280–302.

Jewell, M. D., B. Shipley, A. Paquette, C. Messier, and P. B. Reich. 2015. A traits-based test of the home-field advantage in mixed-species tree litter decomposition. Annals of Botany 116:781–788.

Jobbágy, E. G., and R. B. Jackson. 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. Ecological Applications 10:423–436.

Johnson, D. W., and P. S. Curtis. 2001. Effects of forest management on soil C and N storage: meta analysis. Forest Ecology and Management 140:227–238.

Jones, P. D., S. L. Edwards, S. Demarais, and A. W. Ezell. 2009. Vegetation community responses to different establishment regimes in loblolly pine (*Pinus taeda*) plantations in southern MS, USA. Forest Ecology and Management 257:553–560.

Kandeler, E., M. Stemmer, and E. M. Klimanek. 1999. Response of soil microbial biomass, urease and xylanase within particle size fractions to long-term soil management. Soil Biology and Biochemistry 31:261–273.

Khanna, P. K., B. Ludwig, J. Bauhus, and C. O'Hara. 2001. Assessment and significance of labile organic C pools in forest soils. Pages 167–182 R. Lal et al. (ed.) Assessment methods for soil carbon. Lewis Publ., Boca Raton, FL.

Krishnan, P., T. A. Black, A. G. Barr, N. J. Grant, D. Gaumont Guay, and Z. Nesic. 2008. Factors controlling the interannual variability in the carbon balance of a southern boreal black spruce forest. Journal of Geophysical Research: Atmospheres 113(D9).

Kurz, W. A., C. C. Dymond, G. Stinson, G. J. Rampley, E. T. Neilson, A. L. Carroll, T. Ebata, and L. Safranyik. 2008. Mountain pine beetle and forest carbon feedback to climate change. Nature 452:987–990.

Laganière, J., D. A. Angers, D. Paré, Y. Bergeron, and H. Y. H. Chen. 2011. Black spruce soils accumulate more uncomplexed organic matter than aspen soils. Soil Science Society of America Journal 75:1125–1132.

Laganière, J., X. Cavard, B. W. Brassard, D. Paré, Y. Bergeron, and H. Y. H. Chen. 2015. The influence of boreal tree species mixtures on ecosystem carbon storage and fluxes. Forest Ecology and Management 354:119–129.

Laganiere, J., D. Paré, Y. Bergeron, H. Y. H. Chen, B. W. Brassard, and X. Cavard. 2013. Stability of soil carbon stocks varies with forest composition in the Canadian boreal biome. Ecosystems 16:852–865.

Lal, R. 2010. Sequestration of carbon in soils and biota. Bioscience:708–721.

Laliberté, E., P. Legendre, and B. Shipley. 2014. FD: measuring functional diversity from multiple traits, and other tools for functional ecology. R package version 1.0-12.

Laliberté, E., and P. Legendre. 2010. A distance-based framework for measuring functional diversity from multiple traits. Ecology 91:299–305.

Lange, M., M. Habekost, N. Eisenhauer, C. Roscher, H. Bessler, C. Engels, Y. Oelmann, S. Scheu, W. Wilcke, E.D. Schulze, and G. Gleixner. 2014. Biotic and abiotic properties mediating plant diversity effects on soil microbial communities in an experimental grassland. PLoS One, 9(5), e96182.

Lange, M., N. Eisenhauer, C. A. Sierra, H. Bessler, C. Engels, R. I. Griffiths, P. G. Mellado

Vázquez, A. A. Malik, J. Roy, S. Scheu, S. Steinbeiss, B. C. Thomson, S. E. Trumbore, and G. Gleixner. 2015. Plant diversity increases soil microbial activity and soil carbon storage. Nature Communications 6:6707.

Leckie, S. E. 2005. Methods of microbial community profiling and their application to forest soils. Forest Ecology and Management 220:88–106.

Lei, P., M. Scherer-Lorenzen, and J. Bauhus. 2012a. Belowground facilitation and competition in young tree species mixtures. Forest Ecology and Management 265:191–200.

Lei, P., M. Scherer-Lorenzen, and J. Bauhus. 2012b. The effect of tree species diversity on fine-root production in a young temperate forest. Oecologia 169:1105–1115.

Lejon, D. P. H., R. Chaussod, J. Ranger, and L. Ranjard. 2005. Microbial community structure and density under different tree species in an acid forest soil (Morvan, France). Microbial Ecology 50:614–625.

Leuschner C, H. D. 2003. Fine root biomass of temperate forests in relation to soil acidity and fertility, climate, age and species. Pages 405–438 *in* K. Esser, U. Lüttge, W. Beyschlag, and F. Hellwig, editors. Progress in Botany. Springer Berlin Heidelberg.

De Long, J. R., E. Dorrepaal, P. Kardol, M. C. Nilsson, L. M. Teuber, and D. A. Wardle. 2016. Understory plant functional groups and litter species identity are stronger drivers of litter decomposition than warming along a boreal forest post-fire successional gradient. Soil Biology and Biochemistry 98:159–170.

Loreau, M., and A. Hector. 2001. Partitioning selection and complementarity in biodiversity experiments. Nature 412:72–76.

Lucas Borja, M. E., D. Candel Pérez, F. R. López Serrano, M. Andrés, and F. Bastida. 2012. Altitude-related factors but not Pinus community exert a dominant role over chemical and microbiological properties of a Mediterranean humid soil. European Journal of Soil Science 63:541–549.

Lund, Z. F., R. W. Pearson, and G. A. Buchanan. 1970. An implanted soil mass technique to study herbicide effects on root growth. Weed Science 18:279–281.

Lundquist, E. ., L. Jackson, K. Scow, and C. Hsu. 1999. Changes in microbial biomass and community composition, and soil carbon and nitrogen pools after incorporation of rye into three California agricultural soils. Soil Biology and Biochemistry 31:221–236.

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

Maillard, É., D. Paré, and A. D. Munson. 2010. Soil carbon stocks and carbon stability in a twenty-year-old temperate plantation. Soil Science Society of America Journal 74:1775–1785.

Marschner, P., K. E, and M. B. 2003. Structure and function of the soil microbial community in a long-term fertilizer experiment. Soil Biology and Biochemistry 35:453–461.

Mazerolle, M. J. 2016. AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). R package version 1.

McGuire, K. L., N. Fierer, C. Bateman, K. K. Treseder, and B. L. Turner. 2012. Fungal community composition in neotropical rain forests: the influence of tree diversity and precipitation. Microbial Ecology, 63:804-812.

McKay, H. M., and D. C. Malcolm. 1988. A comparison of the fine root component of a pure and a mixed coniferous stand. Canadian Journal of Forest Research 18:1416–1426.

McMahon, S. K., M. A. Williams, P. J. Bottomley, and D. D. Myrold. 2005. Dynamics of microbial communities during decomposition of carbon-13 labeled ryegrass fractions in soil. Soil Science Society of America Journal 69:1238–1247.

Meinen, C., D. Hertel, and C. Leuschner. 2009. Biomass and morphology of fine roots in temperate broad-leaved forests differing in tree species diversity: is there evidence of below-ground overyielding? Oecologia 161:99–111.

Merilä, P., M. Malmivaara-Lämsä, P. Spetz, S. Stark, K. Vierikko, J. Derome, and H. Fritze. 2010. Soil organic matter quality as a link between microbial community structure and vegetation composition along a successional gradient in a boreal forest. Applied Soil Ecology 46:259–267.

Mitchell, R. J., A. M. Keith, J. M. Potts, J. Ross, E. Reid, and L. A. Dawson. 2012. Overstory and understory vegetation interact to alter soil community composition and activity. Plant and Soil 352:65–84.

Mokany, K., J. Ash, and S. Roxburgh. 2008. Functional identity is more important than diversity in influencing ecosystem processes in a temperate native grassland. Journal of Ecology 96:884–893.

Mommer, L., J. van Ruijven, H. de Caluwe, A. E. Smit-Tiekstra, C. A. M. Wagemaker, N. Joop Ouborg, G. M. Bögemann, G. M. van der Weerden, F. Berendse, and H. de Kroon. 2010. Unveiling below-ground species abundance in a biodiversity experiment: A test of vertical niche differentiation among grassland species. Journal of Ecology 98:1117–1127.

Moreno, J. L., T. Hernández, A. Pérez, and C. García. 2002. Toxicity of cadmium to soil microbial activity: effect of sewage sludge addition to soil on the ecological dose. Applied Soil Ecology 21:149–158.

Mouchet, M. A., S. Villéger, N. W. H. Mason, and D. Mouillot. 2010. Functional diversity measures: an overview of their redundancy and their ability to discriminate community assembly rules. Functional Ecology 24:867–876.

Munson, A. D., H. A. Margolis, and D. G. Brand. 1993. Intensive silvicultural treatment: impacts on soil fertility and planted conifer response. Soil Science Society of America Journal 57:246–255.

Nadrowski, K., C. Wirth, and M. Scherer-Lorenzen. 2010. Is forest diversity driving ecosystem function and service? Current Opinion in Environmental Sustainability 2:75–79.

Naeem, S., and J. P. Wright. 2003. Disentangling biodiversity effects on ecosystem functioning: deriving solutions to a seemingly insurmountable problem. Ecology Letters 6:567–579.

Nannipieri, P., J. Ascher, M. T. Ceccherini, L. Landi, G. Pietramellara, and G. Renella. 2003. Microbial diversity and soil functions. European Journal of Soil Science 54:655–670.

Nessner Kavamura, V., R. G. Taketani, M. D. Lançoni, F. D. Andreote, R. Mendes, and I. Soares de Melo. 2013. Water regime influences bulk soil and rhizosphere of *Cereus jamacaru* bacterial communities in the Brazilian Caatinga biome. PloS One 8:e73606.

Nilsson, M. C., and D. A. Wardle. 2005. Understory vegetation as a forest ecosystem driver: evidence from the northern Swedish boreal forest. Frontiers in Ecology and the Environment 3:421–428.

NOAA/ESRL. 2015. http://www.esrl.noaa.gov/gmd/ccgg/trends/.

Norby, R. J., J. Ledford, C. D. Reilly, N. E. Miller, and E. G. O'Neill. 2004. Fine-root production dominates response of a deciduous forest to atmospheric CO_2 enrichment. Proceedings of the National Academy of Sciences of the United States of America 101:9689–9693.

Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. Henry, H. Stevens, and H. Wagner. 2016. vegan: Community Ecology Package.

Pan, Y., R. A. Birdsey, J. Fang, R. Houghton, P. E. Kauppi, W. A. Kurz, O. L. Phillips, A. Shvidenko, S. L. Lewis, and J. G. Canadell. 2011. A large and persistent carbon sink in the world's forests. Science 333:988–993.

Paquette, A., S. Joly, and C. Messier. 2015. Explaining forest productivity using tree functional traits and phylogenetic information: two sides of the same coin over evolutionary scale? Ecology and Evolution 5:1774–1783.

Paquette, A., and C. Messier. 2011. The effect of biodiversity on tree productivity: From temperate to boreal forests. Global Ecology and Biogeography 20:170–180.

Paré, D., R. Boutin, G. R. Larocque, and F. Raulier. 2006. Effect of temperature on soil organic matter decomposition in three forest biomes of eastern Canada. Canadian Journal of Soil Science 86:247–256.

Pastor, J., J. D. Aber, C. A. McClaugherty, and J. M. Melillo. 1984. Aboveground production and N and P cycling along a nitrogen mineralization gradient on Blackhawk Island, Wisconsin. Ecology 65:256–268.

Paul, K. I., P. J. Polglase, J. G. Nyakuengama, and P. K. Khanna. 2002. Change in soil carbon following afforestation. Forest Ecology and Management 168:241–257.

Petchey, O. L. 2003. Integrating methods that investigate how complementarity influences ecosystem functioning. Oikos 101:323–330.

Petchey, O. L., and K. J. Gaston. 2006. Functional diversity: back to basics and looking forward. Ecology Letters 9:741–58.

Piao, S., P. Ciais, P. Friedlingstein, P. Peylin, M. Reichstein, S. Luyssaert, H. Margolis, J. Fang, A. Barr, and A. Chen. 2008. Net carbon dioxide losses of northern ecosystems in response to autumn warming. Nature 451:49–52.

Pinheiro, J., D. Bates, S. DebRoy, and D. Sarkar. 2007. Linear and nonlinear mixed effects models. R package version 3:57.

Poirier, V., D. A. Angers, and J. K. Whalen. 2014. Formation of millimetric-scale aggregates and associated retention of 13C–15N-labelled residues are greater in subsoil than topsoil. Soil Biology and Biochemistry 75:45–53.

Poirier, V., M. R. Coyea, D. A. Angers, and A. D. Munson. 2016. Silvicultural treatments and subsequent vegetation impact long-term mineral. Forest Ecology and Management 368:140–150.

Potter, K. M., and C. W. Woodall. 2014. Does biodiversity make a difference? Relationships between species richness, evolutionary diversity, and aboveground live tree biomass across US forests. Forest Ecology and Management 321:117–129.

Potvin, C., and N. J. Gotelli. 2008. Biodiversity enhances individual performance but does not affect survivorship in tropical trees. Ecology Letters 11:217–223.

Powers, R. F., D. Andrew Scott, F. G. Sanchez, R. A. Voldseth, D. Page Dumroese, J. D. Elioff, and D. M. Stone. 2005. The North American long-term soil productivity experiment: Findings from the first decade of research. Forest Ecology and Management 220:31–50.

Pretzsch, H., and G. Schütze. 2009. Transgressive overyielding in mixed compared with pure stands of Norway spruce and European beech in Central Europe: Evidence on stand level and explanation on individual tree level. European Journal of Forest Research 128:183–204.

R Core Team. 2015. R: A Language and Environment for Statistical Computing.

Ranjard, L., A. Echairi, V. Nowak, D. P. H. Lejon, R. Nouaïm, and R. Chaussod. 2006. Field and microcosm experiments to evaluate the effects of agricultural Cu treatment on the density and genetic structure of microbial communities in two different soils. FEMS Microbiology Ecology 58:303–315.

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

Ravenek, J. M., H. Bessler, C. Engels, M. Scherer-Lorenzen, A. Gessler, A. Gockele, E. De Luca, V. M. Temperton, A. Ebeling, C. Roscher, B. Schmid, W. W. Weisser, C. Wirth, H. de Kroon, A. Weigelt, and L. Mommer. 2014. Long-term study of root biomass in a biodiversity experiment reveals shifts in diversity effects over time. Oikos:1528–1536.

Reich, P. B. 2014. The world-wide "fast–slow" plant economics spectrum: a traits manifesto. Journal of Ecology 102:275–301.

Reich, P. B., D. Tilman, F. Isbell, K. Mueller, S. E. Hobbie, D. F. B. Flynn, and N. Eisenhauer. 2012. Impacts of biodiversity loss escalate through time as redundancy fades. Science 336:589–592.

Ricotta, C., and M. Moretti. 2011. CWM and Rao's quadratic diversity: A unified framework for functional ecology. Oecologia 167:181–188.

Rivest, D., A. Paquette, B. Shipley, P. B. Reich, and C. Messier. 2015. Tree communities rapidly alter soil microbial resistance and resilience to drought. Functional Ecology 29:570–578.

Roscher, C., J. Schumacher, M. Gubsch, A. Lipowsky, A. Weigelt, N. Buchmann, B. Schmid, and E. D. Schulze. 2012. Using plant functional traits to explain diversity–productivity relationships. PLoS One 7:e36760.

Rousk, J., P. C. Brookes, and E. Bååth. 2009. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. Applied and Environmental Microbiology 75:1589–1596.

S Díaz, and M. Cabido. 2001. Vive la différence: plant functional diversity matters to ecosystem processes. Trends in Ecology and Evolution 16:646–655.

Saha, S. K., P. K. R. Nair, V. D. Nair, and B. M. Kumar. 2009. Soil carbon stock in relation to plant diversity of homegardens in Kerala, India. Agroforestry Systems 76:53–65.

Sassi, M. Ben, J. Dollinger, P. Renault, A. Tlili, and A. Bérard. 2012. The FungiResp method: An application of the MicroResp^{TM} method to assess fungi in microbial communities as soil biological indicators. Ecological Indicators 23:482–490.

Saul Tcherkas, V., and Y. Steinberger. 2009. Substrate utilization patterns of desert soil microbial communities in response to xeric and mesic conditions. Soil Biology and Biochemistry 41:1882–1893.

Scheibe, A., C. Steffens, J. Seven, A. Jacob, D. Hertel, C. Leuschner, and G. Gleixner. 2015. Effects of tree identity dominate over tree diversity on the soil microbial community structure. Soil Biology and Biochemistry 81:219–227.

Schenk, H. J., and R. B. Jackson. 2002. Rooting depths, lateral root spreads and below-ground/above-ground allometries of plants in water-limited ecosystems. Journal of Ecology 90:480–494.

Scherber, C., N. Eisenhauer, W. W. Weisser, B. Schmid, W. Voigt, M. Fischer, E. D. Schulze, C. Roscher, A. Weigelt, and E. Allan. 2010. Bottom-up effects of plant diversity on multitrophic interactions in a biodiversity experiment. Nature 468:553–556.

Scherer-Lorenzen, M., J. Luis Bonilla, and C. Potvin. 2007. Tree species richness affects litter production and decomposition rates in a tropical biodiversity experiment. Oikos 116:2108–2124.

Schloter, M., O. Dilly, and J. Munch. 2003. Indicators for evaluating soil quality. Agriculture, Ecosystems and Environment 98:255–262.

Schmid, I., and M. Kazda. 2002. Root distribution of Norway spruce in monospecific and mixed stands on differtent soils. Forest Ecology and Management 159:37–47.

Scott, D. A., J. Novosad, and G. Golddsmith. 2007. Ten-year results from the North American long-term soil productivity study in the western gulf coastal plain. Advancing the fundamental sciences: proceedings of the Forest Service national earth sciences conference: 331-340

Shan, J., L. A. Morris, and R. L. Hendrick. 2001. The effects of management on soil and plant carbon sequestration in slash pine plantations. Journal of Applied Ecology 38:932–941.

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

Smaill, S. J., P. W. Clinton, and L. G. Greenfield. 2008. Postharvest organic matter removal effects on FH layer and mineral soil characteristics in four New Zealand *Pinus radiata* plantations. Forest Ecology and Management 256:558–563.

Soil Classification Working Group. 1998. The Canadian system of soil classification. 3rd ed. Agric. and Agri-Food Canada Publ. 1646. NRC Research Press, Ottawa, ON.

Soil Survey Division Staff. 1993. Soil survey manual. Agric. Handbk. 18. U.S. Gov. Print. Office, Washington, DC.

Spohn, M., and M. Chodak. 2015. Microbial respiration per unit biomass increases with carbon-to-nutrient ratios in forest soils. Soil Biology and Biochemistry 81:128–133.

Spycher, G., P. Sollins, and S. Rose. 1983. Carbon and nitrogen in the light fraction of a forest soil: vertical distribution and seasonal patterns. Soil Science 135:79–87.

Stefanowicz, A. M., P. Kapusta, G. Szarek Lukaszewska, K. Grodzińska, M. Niklińska, and R. D. Vogt. 2012. Soil fertility and plant diversity enhance microbial performance in metal-polluted soils. Science of the Total Environment 439:211–219.

Steinbeiss, S., H. Beßler, C. Engels, V. M. Temperton, N. Buchmann, C. Roscher, Y. Kreutziger, J. Baade, M. Habekost, and G. Gleixner. 2008. Plant diversity positively affects short-term soil carbon storage in experimental grasslands. Global Change Biology 14:2937–2949.

Ste-Marie, C., D. Paré, and D. Gagnon. 2007. The contrasting effects of aspen and jack pine on soil nutritional properties depend on parent material. Ecosystems 10:1299–1310.

Stoy, P. C., A. D. Richardson, D. D. Baldocchi, G. G. Katul, J. Stanovick, M. D. Mahecha, M. Reichstein, M. Detto, B. E. Law, and G. Wohlfahrt. 2009. Biosphere-atmosphere exchange of CO 2 in relation to climate: a cross-biome analysis across multiple time scales. Biogeosciences 6:2297–2312.

Štursová, M., and P. Baldrian. 2011. Effects of soil properties and management on the activity of soil organic matter transforming enzymes and the quantification of soil-bound and free activity. Plant and Soil 338:99–110.

Swift, M. J., O. W. Heal, and J. M. Anderson. 1979. Decomposition in terrestrial ecosystems. Blackwell Scientific Publications, Oxford.

Symstad, A. J., and D. Tilman. 2001. Diversity loss, recruitment limitation, and ecosystem functioning: lessons learned from a removal experiment. Oikos 92:424–435.

Tanja, S., F. Berninger, T. Vesala, T. Markkanen, P. Hari, A. Mäkelä, H. Ilvesniemi, H. Hänninen, E. Nikinmaa, and T. Huttula. 2003. Air temperature triggers the recovery of evergreen boreal forest photosynthesis in spring. Global Change Biology 9:1410–1426.

Thoms, C., A. Gattinger, M. Jacob, F. M. Thomas, and G. Gleixner. 2010. Direct and indirect effects of tree diversity drive soil microbial diversity in temperate deciduous forest. Soil Biology and Biochemistry 42:1558–1565.

Thoms, C., and G. Gleixner. 2013. Seasonal differences in tree species' influence on soil microbial communities. Soil Biology and Biochemistry 66:239–248.

Tilman, D. 2001. Functional diversity. Encyclopedia of Biodiversity 3:109–120.

Tilman, D., J. Knops, D. Wedin, P. Reich, M. Ritchie, and E. Siemann. 1997a. The influence of functional diversity and composition on ecosystem processes. Science 277:1300–1302.

Tilman, D., D. Lehman, and K. Thompson. 1997b. Plant diversity and ecosystem productivity: Theoretical considerations. Proceedings of the National Academy of Sciences, USA 94:1857 – 1861.

Tlili, A., M. Marechal, B. Montuelle, B. Volat, U. Dorigo, and A. Bérard. 2011. Use of the MicroResp^{TM} method to assess pollution-induced community tolerance to metals for lotic biofilms. Environmental Pollution 159:18–24.

Tobner, C. M., A. Paquette, D. Gravel, P. B. Reich, L. J. Williams, and C. Messier. 2016.

Functional identity is the main driver of diversity effects in young tree communities. Ecology Letters 19:638–647.

Tobner, C. M., A. Paquette, P. B. Reich, D. Gravel, and C. Messier. 2014. Advancing biodiversity-ecosystem functioning science using high-density tree-based experiments over functional diversity gradients. Oecologia 174:609–621.

Torsvik, V., and L. Øvreås. 2002. Microbial diversity and function in soil: from genes to ecosystems. Current Opinion in Microbiology 5:240–245.

Ueyama, M., H. Iwata, and Y. Harazono. 2014. Autumn warming reduces the CO2 sink of a black spruce forest in interior Alaska based on a nine-year eddy covariance measurement. Global Change Biology 20:1161–1173.

Valverde Barrantes, O. J., K. A. Smemo, L. M. Feinstein, M. W. Kershner, and C. B. Blackwood. 2015. Aggregated and complementary: Symmetric proliferation, overyielding, and mass effects explain fine-root biomass in soil patches in a diverse temperate deciduous forest landscape. New Phytologist 205:731–742.

Van DerHeijden, M. G., R. D. Bardgett, and N. M. Van Straalen. 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters 11:296–310.

Verheyen, K., M. Vanhellemont, H. Auge, L. Baeten, C. Baraloto, N. Barsoum, S. Bilodeau Gauthier, H. Bruelheide, B. Castagneyrol, D. Godbold, J. Haase, A. Hector, H. Jactel, J. Koricheva, M. Loreau, S. Mereu, C. Messier, B. Muys, P. Nolet, A. Paquette, J. Parker, M. Perring, Q. Ponette, C. Potvin, P. B. Reich, A. Smith, M. Weih, and M. Scherer-Lorenzen. 2016. Contributions of a global network of tree diversity experiments to sustainable forest plantations. Ambio 45:29–41.

Vesala, T., S. Launiainen, P. Kolari, J. Pumpanen, S. Sevanto, P. Hari, E. Nikinmaa, P. Kaski, H. Mannila, and E. Ukkonen. 2010. Autumn temperature and carbon balance of a boreal Scots pine forest in Southern Finland. Biogeosciences 7:163–176.

Vesterdal, L., N. Clarke, B. D. Sigurdsson, and P. Gundersen. 2013. Do tree species influence soil carbon stocks in temperate and boreal forests? Forest Ecology and Management 309:4–18.

Vesterdal, L., B. Elberling, J. R. Christiansen, I. Callesen, and I. K. Schmidt. 2012. Soil respiration and rates of soil carbon turnover differ among six common European tree species. Forest Ecology and Management 264:185–196.

Villéger, S., N. W. H. Mason, and D. Mouillot. 2008. New multidimensional functional diversity indices for a multifaceted framework in functional ecology. Ecology 89:2290–2301.

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

Vitousek, P. 1982. Nutrient cycling and nutrient use efficiency. American Naturalist:553–572.

Vivanco, L., and A. T. Austin. 2006. Intrinsic effects of species on leaf litter and root decomposition: a comparison of temperate grasses from North and South America. Oecologia 150:97–107.

Wardle, D. A, R. D. Bardgett, J. N. Klironomos, H. Setälä, W. H. van der Putten, and D. H.

Wall. 2004. Ecological linkages between aboveground and belowground biota. Science 304:1629–1633.

Wardle, D. A. 2002. Communities and ecosystems: linking the aboveground and belowground components. Princeton University Press.

Wardle, D. A., R. D. Bardgett, M. B. Usher, and D. W. Hopkins. 2005. How plant communities influence decomposer communities. Biological diversity and function in soils:119–138.

Withington, J. M., P. B. Reich, J. Oleksyn, and D. M. Eissenstat. 2006. Comparisons of structure and life span in roots and leaves among temperate trees. Ecological Monographs 76:381–397.

Wu, J., L. van der Linden, G. Lasslop, N. Carvalhais, K. Pilegaard, C. Beier, and A. Ibrom. 2012a. Effects of climate variability and functional changes on the interannual variation of the carbon balance in a temperate deciduous forest. Biogeosciences 9:13–28.

Wu, Y. T., J. Gutknecht, K. Nadrowski, C. Geißler, P. Kühn, T. Scholten, S. Both, A. Erfmeier, M. Böhnke, and H. Bruelheide. 2012b. Relationships between soil microorganisms, plant communities, and soil characteristics in Chinese subtropical forests. Ecosystems 15:624–636.

Yang, Y., J. Guo, G. Chen, Y. Yin, R. Gao, and C. Lin. 2009. Effects of forest conversion on soil labile organic carbon fractions and aggregate stability in subtropical China. Plant and Soil 323:153–162.

Yuan, W., Y. Luo, A. D. Richardson, R. A. M. Oren, S. Luyssaert, I. A. Janssens, R. Ceulemans, X. Zhou, T. Grünwald, and M. Aubinet. 2009. Latitudinal patterns of magnitude and interannual variability in net ecosystem exchange regulated by biological and environmental variables. Global Change Biology 15:2905–2920.

Zak, D. R., Holmes, W. E., White, D. C., Peacock, A. D., and D. Tilman. 2003. Plant diversity, soil microbial communities, and ecosystem function: are there any links? Ecology 84:2042–2050.

Zavaleta, E. S., J. R. Pasari, K. B. Hulvey, and D. Tilman. 2010. Sustaining multiple ecosystem functions in grassland communities requires higher biodiversity. Proceedings of the National Academy of Sciences 107 :1443–1446.

Zelenev, V. V., A. H. C. van Bruggen, and A. M. Semenov. 2005. Short-term wavelike dynamics of bacterial populations in response to nutrient input from fresh plant residues. Microbial Ecology 49:83–93.

Zelles, L. 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. Biology and Fertility of Soils 29:111–129.

Annexes

Supplementary information

Appendix S1. Supplementary methods

MicroResp^{TM} physiological profiles. The MicroResp™ method allows soil respiration and microbial community physiological profiles to be determined colorimetrically in microplates (Campbell et al. 2003), in a wide range of applications (Tlili et al. 2011; Sassi et al. 2012). Soil moisture content of all samples was pre-adjusted to 40% of the water holding capacity (WHC), which is in the optimal range of WHC for microbial respiration (Ilstedt et al. 2000; Moreno et al. 2002; Campbell et al. 2003). Soils were then pre-incubated for one week in microcosms at ambient temperature (23°C ± 2), in dark conditions (Berard et al. 2014). Community Level Physiological Profiles (CLPP) and basal respiration (BR) were measured using the MicroResp™ system (Campbell et al. 2003). The system consists of a detection microplate, containing 150 ml purified agar (1%), cresol red indicator dye (12.5 µg ml^{-1}), potassium chloride (150 mM) and sodium bicarbonate (2.5 mM), attached to a 96-deep-well microplate (1.2 ml volume). The microplate is filled with ~ 0.35 g of fresh soil weight per well; only water is added to wells (25 µl) for BR measurements, or one of 15 different C sources are added, based on their ecologically relevance to soil and root ecology (Campbell et al. 1997). Carbon sources were applied in 25 ml to achieve a final concentration of 30 mg g^{-1} soil water for Substrate-induced respiration (SIR) measurements. The carbon sources used were L-alanine, L-arabinose, L-arginine, citric acid, D-fructose, D-galactose, D-glucose, L-malic acid, L-lysine, N-acetyl glucosamine, oxalic acid, protocatechuic acid, DL-aspartic acid, γ-aminobutyric acid and D-trehalose.

All measures were done in triplicate and with two soil samples per plate after 6 h incubation in the dark at room temperature (23 ± 1°C). The absorbance of the detection microplate was measured at 570 nm (Biotek EL-800 spectrophotometer). The average amount of CO_2 that evolved per sample was calculated and used to normalize individual C source concentrations before multivariate analysis (Campbell et al. 2003). A calibration curve of absorbance versus head space equilibrium CO_2 concentration (measured by gas chromatography) was fitted to a regression model, and results were expressed in µg C-CO_2 g^{-1}soil h^{-1} (Campbell et al. 2003; Bérard et al. 2011; 2014). Microbial biomass was determined by glucose-induced respiration (GIR) (Berard et al. 2011; 2014) based on Anderson and Domsch (1978; 1985) and Chapman et al. (2007). The respiration rate per unit soil microbial biomass - the metabolic quotient qCO_2 - is the ratio of basal respiration (BR) to microbial biomass (GIR) (Saul-Tcherkas and Steinberger 2009; Berard et al. 2011; 2014), and is a measure of the ecophysiological status of soil microorganisms (Anderson and Domsch 2010). It has been used in a large number of studies and for different purposes, for example, microbe status in relation to plant succession (Insam and Haselwandter 1989) or to metal soil contamination (Berard et al. 2014).

Phospholipid fatty acid extraction (PLFA). The determination of the phospholipid fatty acid (PLFA) pattern of soil organisms is one of the most commonly used methods to represent living soil microbial biomass and to identify the presence or absence of various functional groups of interest through known PLFA biomarkers (Frostegård et al. 2011). The procedure of Hamel et al. (2006) was performed for lipid extraction and PLFA analyses. Briefly, total soil lipids were extracted by shaking about 2 g of freeze-dried soil in a dichloromethane-methanol-citrate buffer mixture (1:2:0.8) for 1 h. The phospholipids were separated from other lipids on a silicic acid column and esterified forming fatty acid methyl esters (FAME) which were separated and quantified by gas chromatography (Findlay 2004). Gas chromatography was carried out on an Agilent 7890A equipped with a 7693 Autosampler and a flame ionisation detector, the carrier gas used was hydrogen. PLFA identities were assigned based on comparison of retention times to known standards. Fatty acids were named according to the ω-designation described as follows: total number of carbons followed by a colon, the number of double bonds, the symbol ω, the position of the first double bond from the methyl end of the molecule. The biomarkers used to discriminate different PLFA are commonly used for microbial community profiling (Leckie 2005). The abundance of individual PLFAs was expressed as ng PLFA g^{-1} dry soil.

Fine root chemistry (root traits). Fine roots (≤ 2mm diameter) were sampled in October 2012 in a central point between four trees to maximise species interactions among local tree neighbourhoods. Five and three soil cores were sampled per plot in mixed communities and monocultures, respectively, to a depth of 15 cm. The fine roots were then rinsed, dried in a forced-air oven at 65°C to constant weight, then finely ground using a ball mill apparatus (Retsch mixer mill, MM200, Retsch GmbH, Haan, Germany). For root chemistry, C was determined by dry combustion (Trumac CNS, LECO Corp., MI, USA) and the concentrations of nitrogen (N), phosphorus (P) potassium (K), calcium (Ca) and magnesium (Mg) were determined by digestion (Parkinson and Allen 1975) followed by atomic absorption spectroscopy (Optima 4300 DV ICP-OES, Perkin-Elmer Inc., Waltham MA, USA).

Appendix S2. *A priori* functional diversity gradient. The *A priori* functional diversity gradient was based on 12 above- and belowground traits, to ensure the inclusion of traits linked to numerous ecosystem processes. The 12 traits were: maximal tree height, growth rate, wood density, seed mass, wood decomposition rate, rooting depth, fine root diameter, mycorrhization type, specific leaf area, leaf longevity, mass-based leaf nitrogen and leaf size.

Appendix S3. Statistical analyses. ANOVA analyses. All statistical analyses were performed within the R environment (version 3.2.1 R Core Team 2015). First, a linear mixed-effect model was developed using the *lme* function in the *nlme* package (Pinheiro et al. 2007) to test the effect of SR (n = 148) as a fixed factor on microbial parameters (BR, GIR, qCO_2), and PLFA groups as response variables with plot identity (to take into account our unbalanced design) and blocks included as random factors, and soil % of clay as environmental co-variable. In the same way, a second linear mixed-effect model was used to test the effect of SR and FD (n= 96) on microbial parameters (BR, GIR, qCO_2) and PLFA groups. Monocultures (FD = 0) and SR12 were therefore excluded from that and further analysis. The interactions of each diversity variable with environmental co-variables were in all cases non-significant and so were removed from further analyses. Mean separations between SR or FD levels were applied using Tukey's HSD tests with the *glht* function in the *multcomp* package (Hothorn et al. 2008). Predicted values and standard errors of the mixed models were computed using the *perdictSE* function in the *AICcmodavg* package (Mazerolle 2016). Then, we applied a third linear mixed-effect model using each trait separately, whether functional variance (FDt) or community-weighted mean trait value (CWM) on soil GIR and BR, to identify traits responsible for the observed diversity effects (where applicable). In all linear mixed-effect models, the normality of residuals and homogeneity of variance were tested and transformations were performed when necessary. Statistical significance was set at the 0.05 level.

Appendix S4. Tables

Table S1. Soil characteristics under tree species mixtures and their identities at the IDENT Montreal site (Québec, Canada). The numbers are mean values. SR1 (monocultures); SR2 (two species mixtures); SR4 (four species mixtures) and SR12 (all twelve species together). Ab (*Abies balsamea*);Ar (*Acer rubrum*); As (*Acer saccharum*); Ba (*Betula alleghaniensis*); Bp (*Betula papyrifera*); Ll (*Larix laricina*); Pg (*Picea glauca*); Pru (*Picea rubens*); Pre (*Pinus resinosa*); Ps (*Pinus strobus*); Qr (*Quercus rubra*); To (*Thuja occidentalis*).

Treatment	SR levels	FDclass levels	pH	Clay %	Sand %
SR1	1	0	5.40	10.15	86.53
SR2	2	-	5.40	10.43	86.35
SR4	4	-	5.38	11.45	85.39
SR12	12	-	-	-	-
Ab	1	0	5.43	23.50	71.80
Ar	1	0	5.29	17.41	76.86
As	1	0	5.23	8.05	90.95
Ba	1	0	5.33	4.24	93.01
Bp	1	0	5.59	9.81	86.69
Ll	1	0	5.32	10.84	85.96
Pg	1	0	5.79	6.98	90.78
Pre	1	0	5.22	8.98	87.77
Pru	1	0	5.44	11.26	85.24
Ps	1	0	5.33	2.48	94.78
Qr	1	0	5.33	12.79	82.23
To	1	0	5.49	5.49	92.26
PrePru	2	1	5.39	4.26	92.97
LlPs	2	2	5.47	5.72	91.28
BaQr	2	3	5.39	10.46	87.30
BpQr	2	4	5.32	5.23	90.81
PgPs	2	5	5.43	12.61	83.19
AbAr	2	6	5.23	16.37	79.41
AsLl	2	7	5.60	9.54	87.23
AsTo	2	8	5.42	16.61	79.17
ArBa	2	2	5.46	7.26	91.25
PrePs	2	2	5.43	7.69	89.82
LlPg	2	4	5.43	8.58	88.89
BpPs	2	5	5.32	10.27	87.01
AbAs	2	7	5.44	20.40	75.37
ArTo	2	7	5.34	11.01	85.24

AbPgPrePru	4	1	5.42	5.25	91.75
ArBaBpQr	4	2	5.46	5.53	91.69
BaPrePruPs	4	3	5.37	11.36	84.86
AbBpLlPg	4	4	5.39	9.75	87.74
AbAsPruPg	4	5	5.18	17.75	78.00
LlPsQrTo	4	6	5.35	13.08	83.89
ArQrPsTo	4	7	5.31	7.79	89.71
AsBaPgTo	4	8	5.49	11.02	86.24
PrePsPruPg	4	2	5.40	11.54	86.45
BpPsAsPg	4	7	5.40	21.41	73.61

Table S2. Mean values and units of tree functional traits used to compute functional diversity indices (FD_t). Ab (*Abiesbalsamea*);Ar (*Acer rubrum*); As (*Acer saccharum*); Ba (*Betula alleghaniensis*); Bp (*Betula papyrifera*); Ll (*Larix laricina*); Pg (*Picea glauca*); Pru (*Picea rubens*); Pre (*Pinus resinosa*); Ps (*Pinus strobus*); Qr (*Quercus rubra*); To (*Thuja occidentalis*). (1) *in situ* Jewell; M. (2014). MSc thesis; (2) *in situ* Tobner et al (2013). Frontiers (incl. unpublished); (3) *in situ* Khlifa et al.; 2016; (4) *in situ* Laura Williams (unpublished); (5) Literature Chave; (6) Literature Kew

Functional traits	**Units**	**Species**												
		Ab	**Ar**	**As**	**Ba**	**Bp**	**Ll**	**Pg**	**Pr**	**Pru**	**Pre**	**Qr**	**To**	**Sources**
Litter nitrogen	mg N/N	14.84	6.87	6.91	12.16	13.18	6.91	7.53	5.74	10.48	7.43	9.11	8.59	(1)
Litter carbon	mg C/C	514.55	460.78	435.42	479.88	481.29	511.62	491.50	519.70	510.52	525.40	466.64	498.79	(1)
Leaf dry matter content	g/g	414.35	427.34	411.87	325.57	287.20	295.36	408.93	344.20	408.06	343.84	424.20	274.50	(1)
Specific leaf area	m^2/g	6.23	11.85	11.27	13.34	13.09	8.26	4.35	4.54	4.68	6.54	9.58	5.36	(1)
Root diameter	mm	0.45	0.35	0.33	0.28	0.26	0.38	0.33	0.37	0.27	0.56	0.27	0.57	(2)
Root branching intensity	N°of root tips/cm	1.90	3.10	2.70	4.00	4.50	2.80	3.10	3.90	2.90	3.20	4.60	1.20	(2)
Specific root length	m/g	23.90	64.50	57.80	90.30	74.00	41.30	48.30	39.50	68.30	16.10	71.90	13.90	(2)
Tree height	cm	131.84	232.96	235.03	260.66	414.00	341.05	129.57	145.65	127.37	165.16	303.94	154.89	(2)
Ground diameter	cm	27.59	34.37	28.49	31.07	42.97	42.99	28.62	34.60	25.49	31.99	34.82	31.16	(2)
Root nitrogen	g/g	1.21	1.21	1.44	0.99	1.10	1.32	1.05	1.30	1.09	1.36	0.75	0.95	(3)
Root carbon	g/g	48.77	48.57	48.29	49.89	49.74	49.26	49.55	49.02	49.52	48.29	49.80	49.68	(3)
Root phosphorus	g/g	2121.05	1905.18	1938.48	1560.05	1305.34	1926.25	2154.93	1776.96	1703.59	1624.48	1489.91	1052.85	(3)
Root potassium	g/g	1840.23	1993.06	1887.41	1520.21	1451.21	1479.08	1773.07	1236.46	1542.30	1164.92	2161.28	1232.81	(3)
Root calcium	g/g	10476.10	7826.26	7400.97	10733.65	10344.60	8486.19	8462.96	8390.21	8408.66	9576.35	7280.13	10138.24	(3)
Root magnesium	g/g	1261.22	1340.46	1204.49	988.87	991.19	999.48	936.19	1023.89	1095.60	1044.25	1181.48	902.73	(3)
Wood density	g/cm^3	0.33	0.49	0.56	0.55	0.48	0.49	0.33	0.41	0.37	0.34	0.56	0.3	(5)
Seed mass	g/1000	7.6	23.7	55.2	1	0.3	2	2.4	9	3.3	17	3143	1.5	(6)

Table S3. Average values in (ng g^{-1}) of total PLFA (PLFAtot) and PLFAs for different taxonomic groups fungi (PLFAfun); bacteria (PLFAbact); Gram positive (PLFApos) and negative bacteria (PLFAneg) for soils from plots of tree species mixtures (1, 2, 4 and 12 species) and their identities at the IDENT Montreal site (Québec, Canada). SR1 (monocultures); SR2 (two species mixtures); SR4 (four species mixtures) and SR12 (all twelve species together). Ab (*Abies balsamea*);Ar (*Acer rubrum*); As (*Acer saccharum*); Ba (*Betula alleghaniensis*); Bp (*Betula papyrifera*); Ll (*Larix laricina*); Pg (*Picea glauca*); Pru (*Picea rubens*); Pre (*Pinus resinosa*); Ps (*Pinus strobus*); Qr (*Quercus rubra*); To (*Thuja occidentalis*). In the case of monocultures, means with the same letter are not significantly different based on the Tukey-HSD post hoc test.

Treatment	**SR levels**	**FDclass levels**	**PLFA$_{tot}$**		**PLFA$_{fun}$**	**PLFA$_{bac}$**		**PLFA$_{pos}$**		**PLFA$_{neg}$**
SR1	1	0	1995		141	918		535		384
SR2	2	-	1997		140	896		521		375
SR4	4	-	2055		131	835		454		381
SR12	12	-	1730		178	846		416		430
Ab	1	0	2017	ab	159	863	ab	511	ab	353
Ar	1	0	2191	ab	199	1127	ab	591	ab	537
As	1	0	2270	ab	125	1081	ab	674	ab	407
Ba	1	0	1913	ab	157	888	ab	477	b	412
Bp	1	0	2034	ab	162	929	ab	536	ab	393
Ll	1	0	2736	a	212	1329	a	795	a	534
Pg	1	0	1673	ab	86	731	ab	452	b	279
Pre	1	0	1815	ab	118	830	ab	490	ab	340
Pru	1	0	1716	ab	115	758	ab	453	b	305
Ps	1	0	2031	ab	146	877	ab	521	ab	356
Qr	1	0	2120	ab	119	939	ab	522	ab	416
To	1	0	1430	b	95	669	b	396	b	273
PrePru	2	1	1590		86	699		414		285
LlPs	2	2	2266		211	1054		629		425
BaQr	2	3	1789		123	797		458		339
BpQr	2	4	1982		190	975		510		465
PgPs	2	5	2122		155	912		514		398

AbAr	2	6	1725	72	777	525	252
AsLl	2	7	1826	120	827	541	287
AsTo	2	8	1940	113	838	509	328
ArBa	2	2	1941	157	923	520	403
PrePs	2	2	1876	131	878	504	373
LlPg	2	4	2607	189	1128	627	501
BpPs	2	5	2004	165	916	487	430
AbAs	2	7	1765	89	763	472	291
ArTo	2	7	2531	155	1064	584	480
AbPgPrePru	4	1	1934	111	809	481	328
ArBaBpQr	4	2	2206	107	782	407	375
BaPrePruPs	4	3	2002	187	955	497	459
AbBpLlPg	4	4	2235	115	866	495	371
AbAsPruPg	4	5	1709	117	786	426	360
LlPsQrTo	4	6	1654	86	803	436	367
ArQrPsTo	4	7	1474	96	667	383	283
AsBaPgTo	4	8	3268	174	1016	502	514
PrePsPruPg	4	2	2134	141	821	478	343
BpPsAsPg	4	7	1575	126	741	412	329

Table S4. Model fit statistics and AICc index for the different functions describing the relationship between soil microbial parameters and tree species richness. Basal respiration (BR), active microbial biomass (GIR) expressed in µg C-CO_2 g^{-1}soil h^{-1} and metabolic quotient (qCO_2) based on 4 replications for each identity plot for a total of 148 plots. AICc measures the relative goodness of fit of a given model, the lower its value, the more likely it is that this model is correct. Two models with ΔAICc > 2 are substantially different. The power function can fit multiple shapes and may represent either functional or no functional redundancy depending on each particular case.

Microbial parameter	**Model**	**R^2**	***P***	**AICc**	**Delta AICc**	**Represented Model**	**Model group**
Basal respiration (BR)	Exponential[1]	0.071	0.001	-283.640	16.265		
	Linear[2]	0.080	0.004	-285.268	14.637		
	Power[3]	0.137	<0.001	-294.563	5.342		
	Logarithmic[4]	0.147	<0.001	-296.172	3.733		
	Michaelis-Menten[5]	0.168	<0.001	-299.905	0.000	✓	No redundancy
Active microbial biomass (GIR)	Exponential[1]	0.001	0.650	204.571	3.729		
	Linear[2]	0.001	0.623	204.536	3.694		
	Power[3]	0.015	0.138	202.545	1.703		
	Logarithmic[4]	0.016	0.122	202.356	1.514		
	Michaelis-Menten[5]	0.026	0.049	200.842	0.000	✓	No redundancy
Metabolic quotient (qCO_2)	Exponential[1]	0.021	0.074	-102.793	0.127		
	Linear[2]	0.021	0.074	-102.801	0.119		
	Power[3]	0.024	0.069	-102.918	0.002		
	Logarithmic[4]	0.025	0.069	-102.920	0.000		
	Michaelis-Menten[5]	0.022	0.069	-102.910	0.010	✓	No redundancy

Models

[1] $Y = a*\exp(b*X)$

[2] $Y = a*X + b$

[3] $Y = a*X\text{^}b$

[4] $Y = a + b \cdot \log(X)$

[5] $Y = a*X/(b+X)$

Table S5. Description of understory vegetation cover in the subplots of the plantation measured during the 2013 growing season (S = blade scarification, F = fertilization, V = vegetation control; 0 = no treatment, 1 = application of treatment).

Species	**Type**	**S0F0V0**	**S0F0V1**	**S0F1V0**	**S0F1V1**	**S1F0V0**	**S1F0V1**	**S1F1V0**	**S1F1V1**
Acer sp	**Shrubs**	2,6	0,2	0,3	2,9	0,2	1,8	0,3	0,5
Populus tremuloides	**Shrubs**	0,1	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Betula papyrifera	**Shrubs**	1,3	0,0	0,0	0,3	0,0	0,0	0,2	0,0
Rubus idaeus	**Shrubs**	0,0	0,0	0,0	0,0	0,4	0,4	0,0	0,0
Vaccinum angustifolium	**Shrubs**	0,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Acer pensylvanicum	**Shrubs**	0,0	0,0	0,0	0,0	0,0	0,0	4,2	0,0
Acer spicatum	**Shrubs**	0,0	0,0	1,3	0,0	0,0	0,0	2,5	0,0
TOTAL	**Shrubs**	**4,1**	**0,2**	**1,6**	**3,2**	**0,6**	**2,3**	**7,2**	**0,5**
Viola sp	**Herbs**	0,1	0,4	0,2	0,9	0,3	0,3	0,3	1,0
Trilium erectum	**Herbs**	0,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Rubus pubescens	**Herbs**	0,0	0,0	0,2	0,0	0,0	0,0	0,2	0,0
Trientalis borealis	**Herbs**	1,0	0,0	0,4	0,8	1,3	0,3	2,8	0,2
Maianthemum canadense	**Herbs**	4,2	0,8	2,2	3,5	2,7	0,9	3,3	1,3
Graminee sp	**Herbs**	0,2	0,0	0,0	0,1	0,2	0,0	0,3	0,0
Linnaea borealis	**Herbs**	0,2	0,3	0,6	1,6	0,6	0,1	0,2	0,4
Diervilla lonicera	**Herbs**	0,8	0,0	1,0	0,3	4,3	0,0	2,1	0,4
Carex sp	**Herbs**	0,6	0,2	0,5	0,9	0,6	0,2	0,8	1,0
Clintonia borealis	**Herbs**	0,4	0,0	0,3	0,0	0,0	0,0	0,0	0,0
Cornus canadensis	**Herbs**	0,3	0,0	0,0	0,6	0,5	0,3	3,4	0,9
Aster macrophyllus	**Herbs**	7,8	0,3	8,5	1,3	11,1	3,3	10,7	2,1
Aralia nudicaulis	**Herbs**	3,0	4,2	32,1	2,5	11,7	6,7	6,7	5,0
TOTAL	**Herbs**	**18,6**	**6,2**	**45,8**	**12,3**	**33,2**	**12,1**	**30,6**	**12,3**
Pteridium sp	**Ferns**	0,0	0,0	0,0	0,0	0,0	0,0	1,7	0,0
Pteridium aquilinum	**Ferns**	3,3	0,0	0,0	0,0	5,0	0,0	3,3	1,3
Onoclea sensibilis	**Ferns**	0,0	0,0	0,0	0,0	0,0	0,0	2,5	0,0
Osmunda sp	**Ferns**	0,0	0,0	0,0	0,1	0,0	0,0	0,0	0,0

Ferns sp	**Ferns**	0,0	0,0	0,0	0,0	0,3	0,0	0,0	0,3
Dryopteris sp	**Ferns**	0,0	0,0	0,0	0,0	4,7	0,0	1,3	0,1
TOTAL	**Ferns**	**3,3**	**0,0**	**0,0**	**0,1**	**9,9**	**0,0**	**8,8**	**1,6**
Polytrichum sp	**Moss and others**	0,0	0,0	0,0	0,3	0,0	0,2	0,0	0,2
Lycopodium obscurum	**Moss and others**	1,3	0,0	1,7	0,0	0,0	0,0	0,0	2,5
Pleurozium schreberi	**Moss and others**	0,0	0,0	0,0	4,2	0,0	0,0	0,0	0,0
Lycopodium sp	**Moss and others**	0,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Equisetum sp	**Moss and others**	0,0	0,2	0,0	0,2	0,1	0,5	0,0	0,1
Lycopodium clavatum	**Moss and others**	0,0	0,0	0,0	0,0	0,2	0,0	0,2	0,0
TOTAL	**Moss and others**	**1,7**	**0,2**	**1,7**	**4,6**	**0,3**	**0,7**	**0,2**	**2,8**

† Vegetation cover percentage determined on three quadrats randomly distributed within each subplot.