UNE APPROCHE FONCTIONNELLE POUR L'ESTIMATION DES TAUX DE DÉCOMPOSITION RACINAIRE ET FLUX DE CARBONE ASSOCIÉS

Contribution à l'estimation du potentiel de séquestration du carbone dans les sols agroforestiers

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SOMMAIRE

Dans un contexte de changement climatique et d'augmentation des teneurs en gaz à effet de serre, notamment du CO_2 atmosphérique, il est crucial que les efforts de recherche soit ciblés sur les composantes encore peu connues du cycle du carbone (C). Notamment, les flux de C depuis les racines vers le sol, dont les généralisations sont encore très limitées, proviennent de 3 mécanismes : la respiration des racines, la rhizodéposition pendant la vie des racines (sécrétions racinaires, desquamation) et leur décomposition. Or les taux de décomposition des racines, et plus spécifiquement des racines fines, partie la plus active des systèmes racinaires, sont une composante encore peu connue mais importante des cycles de C terrestres. En effet, bien que la biomasse végétale souterraine représente souvent plus de la moitié de la biomasse végétale totale de l'écosystème, seulement 2% des études traitant du processus de décomposition portent sur les racines. Une meilleure compréhension des composantes racinaires des flux de C dans les sols pourrait permettre une optimisation de la séquestration de C dans les sols, notamment dans les sols agricoles, qui ont perdu environ la moitié de leur teneur en C par les changements d'utilisation des terres. L'objectif général de ce projet est de mieux appréhender les capacités de séquestration de C dans les sols agricoles, à partir d'une approche comparative utilisant les traits racinaires des espèces qui composent ces agrosystèmes. Plus précisément, cet objectif présenté dans un contexte de marché international du C, pourrait permettre de fournir une motivation économique aux cultivateurs pour planter des arbres dans leurs champs, et ainsi favoriser leur transition vers des systèmes agroforestiers (association d'arbres et de productions agricoles annuelles).

Dans un premier temps, pour un suivi dynamique et in situ des processus racinaires, une méthode non invasive et non destructive d'estimation de la biomasse du système racinaire complet d'un individu a été testée. Cette technique par mesure de capacitance électrique est prometteuse car rapide et peu coûteuse. Le peu d'études l'ayant testée utilisaient principalement des plantes poussant en milieu hydroponique ou à base de sable. L'objectif de cette étude était de déterminer

le potentiel de cette technique à estimer la biomasse racinaire de 10 espèces de plantes fourragères ou de culture poussant dans le sol et à différents stades de croissance. La relation entre capacitance et biomasse racinaire a été trouvée significative, mais trop faible pour des prédictions fiables. D'autre part, l'effet des interactions racinaires inter- ou intra-individuelles affectent la précision des mesures, et cet effet est d'autant plus marqué que les racines de l'espèces en question sont fines.

Dans un deuxième temps, cette étude rapporte les taux de décomposition in situ d'une grande variété d'espèces d'arbres et d'herbacées utilisables en agroforesterie en lien avec leurs traits biochimiques et morphologiques, dans le but de mieux décrire les flux de C des racines vers le sol en s'affranchissant des références taxonomiques. L'accès aux données de pertes de biomasses racinaires peut se faire de manière relativement rapide et sur un large spectre fonctionnel grâce aux traits racinaires initiaux (i.e. mesurés sur des racines intactes) d'une part, mais aussi à partir des taux de décomposition des fractions de C pondérés par leurs proportions respectives.

Dans un troisième temps, et pour mieux comprendre cette dynamique de décomposition du C organique racinaire, la dynamique de décomposition des fractions de C a été étudiée, mettant en évidence la décomposition préférentielle de certaines fractions de C facilement assimilables par rapport aux plus récalcitrantes. Les variations observées des taux de décomposition des fractions de C peuvent être prédites assez précisément par les traits racinaires initaux. Finalement, la combinaison des données de dynamique des fractions de C et des proportions de composés récalcitrants *vs.* facilement assimilables a permis d'obtenir un modèle prédictif précis de perte de C racinaire au cours du processus de décomposition.

Les traits racinaires initiaux facilement mesurables ont montré un potentiel très prometteur de prédiction des taux de décomposition racinaires, mais aussi des différentes fractions de C et devraient se révéler utiles dans les modèles généraux de cycle de C du sol, notamment pour leur application aux systèmes agroforestiers.

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A ma famille, que je n'ai pas beaucoup vue ces dernières années, mais qui a beaucoup changé.

TABLE DES MATIÈRES

vii

LISTE DES TABLEAUX

TABLES DES FIGURES

INTRODUCTION

A - Avant propos

La durabilité de l'agriculture productiviste actuelle est remise en question (Tilman *et al.*, 2001), d'autant qu'elle repose et reposera encore largement sur l'énergie fossile, pourtant de plus en plus rare et chère (Butler *et al.*, 2007). Les systèmes agroforestiers, alliant sur une même parcelle une production agricole annuelle (culture, pâtures) et une production ligneuse à long terme, semblent être une approche intéressante pour une agriculture associant productivité et stabilité/durabilité du système. Les sols agricoles ont perdu jusqu'à plus de la moitié de leur teneur en carbone (C) en une dizaine d'années. Or, une transition vers les cultures agroforestières pourrait permettre de raugmenter cette teneur en matière organique : il s'agit alors de séquestration de C dans les sols. Ce potentiel de séquestration de C aurait des bénéfices sur chacun des aspects clé d'une pratique durable : environnementale, économique et sociale.

La teneur en matière organique est gage de fertilité des sols (partie B). Ceci bénéficie à la fois au cultivateur par l'amélioration de la productivité et à la composante environnementale par la diminution de l'érosion, des risques d'inondation, et du lessivage des nutriments. La séquestration de C dans le sol permet de diminuer d'autant la teneur en CO_2 atmosphérique (partie C). Ceci serait une des solutions pouvant tamponner le plus rapidement les émissions anthropiques de CO_2 et toutes les conséquences socio-économiques du changement climatique sur la population.

Bien qu'elle représente un investissement à long terme et qu'elle semble être un bon candidat pour la séquestration de C (partie D), l'agroforesterie (association d'arbres et de cultures annuelles) est encore marginale à cause de son coût initial. Ainsi, dans le contexte de changement climatique, et grâce à la possibilité de délivrer des crédits de carbone, l'agroforesterie pourrait gagner en rentabilité ainsi qu'en attractivité. Pour pouvoir attribuer

un chiffre au potentiel de séquestration de C des pratiques agroforestières, il est nécessaire de pouvoir quantifier le gain net de C au sol (équivalent à la diminution de C atmosphérique). Pour cela, il est essentiel de connaître la contribution des racines à l'apport de C au sol (partie E) et de quantifier l'influence du processus de décomposition des racines sur cette séquestration (partie F). Les méthodes d'estimation de séquestration de C restent souvent dans un contexte d'application locale et sont généralement lourdes et controversées (Peichl *et al.*, 2006). Il n'est donc pas envisageable de procéder ainsi pour chaque espèce cultivée, quel que soit son type biologique, surtout si les réponses varient selon l'environnement.

Il est proposé ici d'utiliser une méthode comparative et fonctionnelle, basée sur les caractéristiques racinaires de chaque espèce (les traits racinaires), qui sont plus facilement mesurables et répandues dans la littérature. Les traits fonctionnels ne sont pas associés au hasard chez une espèce, ils répondent à la stratégie de croissance, de développement et de reproduction de l'espèce. Il existe ainsi des patrons d'association de traits, qui correspondent aux différentes stratégies adaptatives des espèces, et dont les composantes biochimiques affectent les taux de décomposition des organes (partie G). Il s'agit alors de connaître la variabilité interspécifique de ces traits racinaires et leurs éventuelles corrélations avec les traits des parties aériennes, qui permettraient une accessibilité encore plus aisée aux variables de décomposition racinaire (partie H). Finalement, étant donné que les traits foliaires ont largement montré leur potentiel quant à la prédiction des taux de décomposition de la litière foliaire, il s'agira de chercher au niveau des racines, notamment prioritairement avec les traits homologues à ceux des feuilles, quel est le potentiel des traits initiaux (i.e. mesurés sur les racines intactes) pour évaluer les taux de décomposition des racines (partie I).

B - Fertilité des sols agricoles et teneur en matière organique

Le maintien durable de la productivité des agrosystèmes est un défi majeur pour nourrir la population mondiale en croissance. Cet objectif peut seulement être atteint en augmentant la productivité des écosystèmes par unité de surface, en évitant au maximum les impacts néfastes sur l'environnement (Carpenter *et al.*, 1998; Tilman *et al.*, 2002; Tilman *et al.*, 2001; Vitousek *et al.*, 1997) ou en revalorisant les terres marginalisées pour la production agricole. Depuis plusieurs décennies, dans une grande partie des pays en développement, l'augmentation de production a été atteinte par une conversion des écosystèmes naturels en parcelles agricoles (Giardina *et al.*, 2000; Tilman *et al.*, 2002) et non par l'augmentation de la productivité. Les perturbations anthropogéniques, qui accompagnent ces changements d'utilisation des terres, par la culture continue et le labour, entraînent une perte immédiate et rapide de carbone au niveau des sols (Davidson et Ackerman, 1993; McLauchlan, 2006; Solomon *et al.*, 2007; Tilman *et al.*, 2002). Ceci est dû à la perturbation des mécanismes physiques, chimiques et biochimiques impliqués dans la stabilisation de la matière organique du sol, qui est alors exposée à la dégradation microbienne.

Plus de 50% du carbone du sol a été perdu en une dizaine d'année dans de nombreux écosystèmes (Feller et Beare, 1997; Lemenih *et al.*, 2005; McLauchlan, 2006; Rumpel *et al.*, 2006; Solomon *et al.*, 2007; Tilman *et al.*, 2002; Tittonell *et al.*, 2007). Les pertes pourraient être plus sévères sous les climats chauds et humides des régions tropicales (Spaccini *et al.*, 2002). Or la matière organique du sol est responsable de nombreuses qualités du sol (Rice *et al.*, 2007):

- L'approvisionnement en nutriments : 50 à 80% des besoins en azote peuvent être fournis par la matière organique du sol dans les systèmes de cultures. Si l'on considère seulement l'azote, le phosphore et le soufre, l'apport potentiel de la matière organique du sol est de 11-300 kg N.ha^{-1}, 40 kg P.ha^{-1}, 36 kg S.ha^{-1}. Selon les ordres de grandeur récents, ceci correspondrait à une économie de 31 à 220 \$.ha^{-1}.an^{-1}.

4

- La capacité d'échange cationique (CEC) est la capacité d'un sol à stocker les nutriments présents sous forme cationique (e.g. le potassium K^+, le calcium Ca^{2+}). Les sites d'échanges cationiques se situent principalement sur les argiles et les oxydes de fer. La matière organique du sol est responsable d'environ 20 à 80% de la CEC. Un g de C organique.kg^{-1} de sol représente environ 4.3 mmol.kg^{-1} de CEC dans des sols à argiles peu actives (Bationo et Mokwunye, 1991; Deridder et Vankeulen, 1990).

- La matière organique du sol améliore la structure et l'agrégation du sol. Or la formation d'agrégats plus stables protège le sol contre la compaction et l'encroûtement (Diaz-Zorita et Grosso, 2000), améliore l'infiltration d'eau, l'aération et la croissance racinaire. Il en résulte en une augmentation de l'activité microbienne, une activation du cycle des nutriments et une diminution de l'érosion (Benito et Diazfierros, 1992; Weesies *et al.*, 1994).

- La capacité de rétention en eau est améliorée par la teneur en matière organique du sol. L'augmentation d'un g de matière organique du sol par kg de sol permet d'augmenter de 1 à 10 g la teneur en eau biodisponible pour les plantes (Emerson, 1995).

- La matière organique du sol constitue une source de C pour les organismes du sol et permet de maintenir la biodiversité du sol (Fox, 2003).

Ainsi, les efforts fournis pour recouvrer la fertilité des sols par la simple application de nutriments sous forme biodisponible (principalement par fertilisants minéraux) sont contrebalancés par le lessivage en profondeur de ces nutriments labiles, les rendant indisponibles pour beaucoup de cultures (Giardina *et al.*, 2000; Holscher *et al.*, 1997; Renck et Lehmann, 2004). La restauration et le maintien de la teneur en matière organique des sols agricoles sont donc essentiels pour conserver une productivité à long terme.

C - Changements climatiques et séquestration de C dans les sols agricoles

L'inventaire des réservoirs de C mondiaux montre que les fonds océaniques correspondent au compartiment principal (IPCC, 2000, 2001). Cependant, notre capacité à modifier ce compartiment est limitée et présente des risques pour les écosystèmes en place (notamment en terme d'acidification du milieu). Le carbone organique du sol constitue le deuxième plus gros compartiment et le principal réservoir du cycle continental du C. Il contient environ trois fois plus de C (1500 Pg de C sur 1 m de profondeur, et 2500 Pg de C sur 2 m de profondeur; $1Pg = 1 \times 10^{15}$ g) que la végétation (650 Pg) et le double de la teneur atmosphérique actuelle de 750 Pg (Batjes, 1996; Kumar *et al.*, 2006).

Une partie de ce C du sol est hautement variable spatio-temporellement, alors qu'une partie "inerte" du stock de C pourrait entrer dans le cycle si on l'expose à de nouvelles conditions environnementales. En effet, environ 25% des terres de l'hémisphère nord sont constituées de pergélisol (i.e. sol qui se maintient gelé pendant au moins 2 ans), incluant des terres du Canada, de la Chine, de la Russie et de l'Alaska, ainsi que des zones montagneuses dans de nombreux autres pays (Zhang *et al.*, 1999). En cas de dégel, ce type de sol libèrerait du CO_2 (en condition aérobie), et du méthane (en condition anaérobie). Or les modèles de circulation générale prédisent jusqu'à plusieurs degrés d'augmentation des températures dû à un doublement de la teneur en CO_2 (Flato *et al.*, 2000), alors que plus de la moitié des pergélisols mondiaux sont maintenus à seulement quelques degrés sous 0°C. Il faut donc rester prudent quant aux impacts des changements climatiques rapides, qui pourraient ainsi convertir certains sols en sources de C (Davidson et Janssens, 2006). Le bilan net de libération de C du sol vers l'atmosphère, par la respiration de la matière organique, augmente avec la conversion d'habitats naturels en cultures, pâturages, et avec l'utilisation de pratiques telles que le labour excessif. Mais le bilan des échanges peut s'inverser, le sol peut alors fixer

du C atmosphérique. Cette capacité de séquestration de C par les sols suggère ainsi leur utilisation potentielle pour tamponner l'augmentation du CO_2 atmosphérique. Smith *et al.* (2008) ont montré que la séquestration de C dans les sols compte pour environ 90% du potentiel agricole global de tamponner l'augmentation de CO_2 atmosphérique. La synthèse d'une tonne de matière organique du sol correspond à 3,7 tonnes de CO_2 atmosphérique séquestré (Bowen et Rovira, 1999).

D'après l'estimation de Lal (2004a, b), le potentiel de séquestration du C dans le sol serait de $0,9 \pm 0,3$ Pg C.an^{-1}, soit un quart à un tiers de l'augmentation annuelle du C atmosphérique. Le potentiel d'utilisation du sol comme un puits de C existe, mais il reste une solution à court terme. En effet, après une période d'environ 50 ans, un nouvel équilibre du C organique du sol sera atteint (Lal, 2004b). Des moyens complémentaires de réduction des émissions de C doivent donc compléter les efforts de séquestration de C dans les sols et prendre la relève si l'on souhaite atteindre une stabilisation du CO_2 atmosphérique à une teneur acceptable.

Les mécanismes impliqués dans la séquestration du C dans le sol restent incertains et l'extrapolation à long terme des équilibres du C global à partir d'études empiriques à court terme reste hasardeuse (Rustad, 2006). Il est cependant incontestable qu'une optimisation de la séquestration de C dans les sols sera doublement bénéfique, à la fois pour la fertilité des sols et pour diminuer l'augmentation du CO_2 atmosphérique et tamponner les effets du changement climatique.

D - L'agroforesterie : candidat pour la séquestration de carbone

Les systèmes agroforestiers allient une production agricole annuelle (culture, pâture) et une production ligneuse à long terme. Ces systèmes encore marginaux en Amérique du Nord, à cause de leurs investissement à plus long terme que les agrosystèmes classiques, représentent pourtant un potentiel pour dynamiser la plantation de feuillus dans l'écozone des plaines à

forêts mixtes du Québec (Rivest et Olivier, 2007). Il est vrai que la compétition des cultures sur les arbres peut parfois être légèrement pénalisante (Burgess *et al.*, 2005; Powell et Bork, 2004) mais, le plus souvent, la croissance des arbres au milieu des cultures n'est pas affectée, voire est nettement améliorée (Balandier et Dupraz, 1998; Chifflot *et al.*, 2006; Mulia et Dupraz, 2006; Paris *et al.*, 2005). En revanche, la compétition des arbres sur les cultures augmente au cours de la croissance des arbres (Gillespie *et al.*, 2000; Reynolds *et al.*, 2007). Les effets de cette compétition se font le plus sentir au plus près des rangées d'arbres, lieux où la compétition pour l'eau, la lumière et les nutriments est la plus importante. Cette compétition peut être retardée en procédant au cernage des racines des arbres (Benjamin *et al.*, 2000; Miller et Pallardy, 2001). La présence des arbres permet un apport de litière qui, le plus souvent lorsqu'il s'agit de feuillus, produit un humus riche et constitue un élément de fertilisation, limitant les besoins en intrants minéraux. Les peupliers hybrides peuvent apporter l'équivalent de 7kg $N.ha^{-1}.an^{-1}$ (Thevathasan et Gordon, 2004). L'effet de l'apport en matière organique au sol a été discuté dans la partie B. La présence de racines d'arbres en profondeur sous les cultures permet une diminution du lessivage des éléments nutritifs, réduisant ainsi la contaminations des nappes souterraines (Allen *et al.*, 2004).

En terme de rentabilité, les modèles tendent à montrer que ces systèmes sont souvent plus lucratifs (Garrett *et al.*, 1991). Pourtant l'investissement de départ, les connaissances requises pour l'exploitation et l'incertitude liée à la nouveauté freinent les propriétaires à s'engager dans ces pratiques. Les cultures agroforestières semblent être une approche intéressante pour l'agriculture de demain, tentant de combiner productivité et stabilité/durabilité du système par la biodiversité. Ces systèmes semblent également utiles pour l'activation du cycle du C et ainsi avoir un effet positif dans les processus de restauration des sols par l'augmentation de la teneur en matière organique, et pour la diminution du CO_2 atmosphérique.

Les systèmes agroforestiers sont communément considérés comme des puits de C, notamment par la séquestration de C dans la composante arborée en place (Dixon, 1995; Kursten et Burschel, 1993; Montagnini et Nair, 2004; Sampson, 2001). Sur des parcelles

agroforestières avec peupliers hybrides, on observe sur 8 ans une augmentation d'environ 1% de la teneur en matière organique du sol, jusqu'à 4 m de la rangée d'arbre (de 2,3 à 3,3%) (Thevathasan et Gordon, 2004). Ceci correspond à une augmentation relative de 35%. A l'âge de 15 ans, les peupliers hybrides avaient cet effet jusqu'à 15 m de la rangée. Les principales études sur le potentiel de séquestration de C des parcelles agroforestières sont regroupées dans le tableau 1. Ces modèles s'appuient sur des chiffres absolus de flux de C mesurés sur un type de culture à la fois. Dans cette optique, le présent projet est une contribution à trouver un modèle universel permettant de trouver ces chiffres à partir des caractéristiques de chaque espèce. Cette contribution se focalisera sur l'apport de C par les racines par le processus de décomposition racinaire (partie F). Le problème des études rassemblées (deux premières lignes du tableau 1) est que la majorité sont basées sur des modèles et des estimations de biomasse, c'est à dire qu'elles ne prennent pas en compte les pertes de C lixivié ou respiré par le sol. En effet, d'après Peichl et al. (2006), la prise en compte de ces deux flux supplémentaires modère les chiffres annoncés, mais ne remet pas en cause la capacité des systèmes agroforestiers à séquestrer le C (3$^{\text{ème}}$ ligne du tableau 1).

Tableau 1. Potentiel de séquestration de carbone des systèmes agroforestiers.

Latitudes	Potentiel de séquestration de C (t C.ha^{-1})	Durée de l'étude	Références
tropicales	21 à 240	10 à 20 ans	Adesina et al. (1999); Dixon (1995); Montagnini et Nair (2004); Schroeder (1994); Swisher (1991)
tempérées	10 à 208	20 à 50 ans	Dixon (1995); Dixon et al. (1994); Kort et Turnock (1998); Montagnini et Nair (2004); Schroeder (1994); Turnock (2001)
tempérées	1.1 à 13.2	13 ans	Peichl et al. (2006)

Une autre raison pour laquelle on peut s'attendre à une meilleure séquestration de C de ces systèmes est la productivité accrue de l'association d'espèces. En effet, il existe des interactions positives entre les espèces végétales (Callaway, 1995); notamment au niveau racinaire, les cultures associées permettent de diminuer la compétition intraspécifique, le défi étant alors de limiter la compétition interspécifique (e.g. par cernage des racines pour les systèmes agroforestiers). Ces interactions de facilitation permettent une meilleure productivité d'une espèce lorsqu'elle est associée à une seconde, sans pour autant que le rendement de cette dernière ne soit affecté.

Il existe plusieurs facteurs favorisant les interactions de facilitation :

1. La distribution et l'architecture racinaire

   - Les "cluster roots", chevelu racinaire très dense, sont plus efficaces pour absorber et mobiliser les nutriments rares. Elles peuvent être bénéfiques à l'autre espèce en sol pauvre (Lambers *et al.*, 2002; Shen *et al.*, 2003).

   - Que les racines soient génétiquement semblables (de Kroon, 2007) ou non (Mulia et Dupraz, 2006), il existe un mécanisme d'évitement des racines des autres individus. Ainsi, un évitement de la compétition permet dans certains cas d'observer une facilitation apparente. Par exemple, les systèmes racinaires de noyer sont forcés en profondeur par les tapis racinaires de la culture de blé dur à proximité. Les noyers accèdent alors à la nappe phréatique ce qui leur permet de continuer leur croissance même durant les mois secs de la saison estivale (Mulia et Dupraz, 2006). Lorsque les racines sont génétiquement semblables, l'évitement permet de limiter la compétition pour l'acquisition des ressources. En effet, plus les espèces sont semblables génétiquement, plus leurs besoins en nutriments seront similaires et la compétition pour les ressources importante (de Kroon, 2007).

2. Les transferts de nutriments, particulièrement par les espèces fixatrices d'azote (Giller *et al.*, 1991; Jensen, 1996)

3. Transferts par mycorhizes (Martin *et al.*, 1982 ; Van Kessel *et al.*, 1985)

4. Mobilisation et/ou solubilisation des nutriments par les exsudats racinaires (acides aminés, enzymes extracellulaires, acidification du milieu) (Tarafdar et Jungk, 1987)

5. Formation d'un habitat moins propice aux invasions de pathogènes et ravageurs (également valable pour les parties aériennes) (Trenbath, 1993)

En plus de la séquestration de C dans le sol et la biomasse ligneuse, il faut considérer que les systèmes agroforestiers peuvent également contribuer à conserver et substituer l'utilisation du C fossile par l'utilisation du bois (Montagnini et Nair, 2004). Il existe donc un potentiel avantage double des exploitations agroforestières : la séquestration de C dans les sols et la substitution possible d'une partie de l'utilisation du C fossile.

Depuis la Conférence de Montréal concernant la Convention Cadre des Nations Unies sur les changements climatiques le 7 décembre 2005, la Bourse de Montréal a conclu une entente avec le Chicago Climate Exchange. Cette entente, appelée Marché climatique de Montréal, permet d'instaurer un système d'échange de quotas d'émission de gaz à effet de serre, notamment du CO_2. Il devient alors possible de valoriser les pratiques agricoles qui permettent de séquestrer du C, par l'intermédiaire de la vente de crédits d'émissions aux entreprises qui dépassent leurs quotas d'émission de gaz à effet de serre. Cependant, pour connaître le nombre de tonnes équivalent CO_2 créditées à l'exploitant, il est nécessaire de pouvoir connaître au cas par cas les quantités de C séquestrées. Et de son côté, l'exploitant a intérêt à connaître plus précisément les paramètres qu'il peut contrôler pour améliorer cette séquestration de C.

11

E - Contribution des racines au potentiel de séquestration de C dans le sol

1 - Flux de C racines - sol

Le carbone organique du sol provient de matériaux dérivés des végétaux. Les 3 principales sources de C dans le sol sont :

- L'accumulation de matière organique par le mécanisme d'humification après la mort des matériaux végétaux. L'humification est le processus de transformation en humus par les organismes du sol de la matière organique apportée au système (débris végétaux, animaux et microorganismes), qui peut alors être complexée aux argiles pour former des colloïdes électronégatifs, stables et insolubles. Ceci a été résumé par Basilevich et Rodin (1971); Rodin et Basilevich (1965); Schlesinger (1977) et Titlyanova et Tesarzheva (1991).

- La rhizodéposition. Ceci concerne tout le carbone organique libéré par les racines vers la rhizosphère :
  - o La production de mucilage
  - o Les lysats cellulaires
  - o Les sécrétions enzymatiques
  - o Les exsudats racinaires transférés à la rhizosphère pendant la croissance de la plante.
  - o La desquamation des cellules des coiffes, de l'épiderme (dont les poils absorbants) et éventuellement du cortex.

- La respiration racinaire. Une étude comparative sur 83 espèces a montré que la respiration des tissus racinaires semble pouvoir être estimée à partir de la teneur en azote des racines (Reich *et al.*, 2008).

Les 2 premiers processus sont responsables du transfert de C organique au sol, qui font intervenir la litière foliaire et les racines. La contribution du C racinaire (rhizodéposition et racines mortes) à la formation de la matière organique du sol dépend de la productivité racinaire, des demi-vies racinaires, de l'exsudation, de la colonisation mycorhizienne et des caractéristiques du sol. Tous varient avec le type d'écosystème (Matamala *et al.*, 2003).

L'allocation de C vers les racines varie en fonction des espèces mais aussi en fonction du stade de développement de la plante (voir ci-dessous l'exemple des céréales). Il est possible d'en faire une estimation à un instant donné de la croissance par le ratio *Racines/Parties aériennes*. Or la litière racinaire est d'une manière générale de moindre qualité que la litière foliaire et représente donc un flux de C plus récalcitrant (Craine *et al.*, 2005; Tjoelker *et al.*, 2005). Des ratios *Racines/Parties aériennes* élevés reflèteraient un potentiel de séquestration de C plus élevé. Cette allocation aux racines peut représenter de 20 à 80% du C assimilé par photosynthèse, mais est plus généralement compris entre 30 et 50% (Kuzyakov et Domanski, 2000). Une fois le carbone dans les racines, environ 40% est respiré, 30% est alloué à la croissance, et 30% est rhizodéposé. Encore une fois, ces chiffres sont des tendances grossières et les valeurs peuvent varier selon les espèces et au cours du développement. Ces données sont encore rares pour les racines. Or il est peu envisageable que ces chiffres d'allocation de C soient rapidement disponibles au niveau racinaire pour la majorité des espèces cultivées.

a - Le cas des céréales (exemple du blé et de l'orge)

La proportion de C transféré au sol, par rapport au C net fixé, diminue au cours du développement de la plante (Keith *et al.*, 1986; Kuzyakov et Domanski, 2000; Swinnen *et al.*, 1994). En d'autres termes, l'allocation aux racines diminue au profit des parties aériennes jusqu'à la récolte. 26% et 17% des produits de photosynthèse de ces céréales (blé et orge respectivement) sont transportés au sol (Kuzyakov et Domanski, 2000). Ce flux de C vers le sol est alors réparti en proportions différentes selon les processus suivants :

- La **croissance racinaire** (7 à 13 % du C assimilé). C'est ce C qui sera ensuite rapporté au sol à la mort des racines.
- La **rhizodéposition** (1 à 2%), dont les produits sont décomposés rapidement par les microorganismes de la rhizosphère. Une partie du C reste adsorbé aux argiles, l'autre est incorporé par les microorganismes (2 à 4%).
- La **respiration racinaire** (7 à 14%), qui est résultat de la maintenance, croissance racinaire et absorption des ions.

Bien que la quantité totale de C assimilé augmente, la fertilisation azotée a pour effet de diminuer la proportion de C allouée aux racines, et donc au sol. Ainsi, en ce qui concerne les céréales en monoculture, fertilisées ou non, la quantité relative de C transloquée au sol par les racines est assez faible (environ 20 %). De plus, l'effet de la fertilisation diminue le temps de résidence du C dans le sol. Les monocultures céréalières ne semblent donc pas efficaces pour raugmenter la teneur en matière organique des sols agricoles.

b - Le cas des espèces de pâture

La translocation de C aux racines est plus élevée pour les espèces de pâture que pour les céréales. La quantité de C transloqué peut atteindre 80% du C assimilé (Dormaar et Sauerbeck, 1983; Sims et Singh, 1971; Zagal, 1994). En moyenne, la translocation relative de C au sol est 1,5 à 2 fois plus élevée qu'avec les céréales. La croissance racinaire, la rhizodéposition et la respiration correspondraient respectivement à 6-9%, 10-20% et 10-17% du C assimilé (Kuzyakov et Domanski, 2000). D'une manière générale, les espèces de pâture allouent plus de C aux racines et finalement la quantité de C apporté au sol et aux microorganismes dépasse d'environ 30% celle apportée par les céréales.

Sur toute la saison de végétation, les quantités totales de C transférées au sol par les céréales et les plantes de pâtures sont respectivement de 1500 et 2200 kg C.ha^{-1} (Bowen et Rovira, 1999). Ce transfert représente 5 à 21 % du C fixé par photosynthèse, et entre 20 et 50 % de la biomasse de la plante.

c - Le cas des arbres

Le transfert du C assimilé vers le sol varie de 40% pour *Liriodendron tulipitera* (Edwards et Harris, 1977) à 60% pour *Pinus sylvestris* (Persson, 1978). Le puits principal de C chez les arbres est plutôt la partie aérienne. Comparés aux herbacées, les nutriments sont majoritairement stockés dans la tige. Ainsi, la translocation relative de C vers les racines devrait être plus faible. Mais la quantité de C transloqué devrait être supérieure, car la productivité des écosystèmes forestiers est plus élevée que celles des prairies. De plus, le C transféré a un temps de résidence moyen plus long que celui des herbacées, car il contient

15

plus de composés récalcitrants comme la lignine ou les polyphénols. Or le taux de renouvellement des litières racinaires diminue avec la profondeur (Gill et Burke, 2002). Ainsi, une proportion de composés récalcitrants plus élevée que chez les herbacées, couplée à une profondeur d'enracinement et une translocation de C vers le sol élevée, font des arbres d'excellents candidats à la séquestration de C dans les sols.

d - Implications pour les systèmes agroforestiers

La présence des arbres dans les systèmes de cultures annuelles ou de pâturages permet un transfert de C au sol efficace en quantité et en qualité, en activant le cycle du C du sol et en apportant une matière organique dont le temps de résidence est plus long. Cependant, pour avoir accès à ces chiffres pour le plus d'espèces cultivables possible, il devient nécessaire de faire appel à une technique comparative plus générale, permettant de s'affranchir des barrières taxonomiques. Il est proposé ici d'estimer les taux de décomposition des racines à partir de caractéristiques racinaires facilement mesurables.

2 - La rhizodéposition

Les résultats de la majorité des études physiologiques sont peu transposables aux conditions de croissance sur de vrais sols. En effet, les interactions entre les racines, la matrice minérale du sol et les microorganismes entraînent des allocations et une séquestration de C différente par les racines, comparées aux cultures en solution nutritive (Meharg et Killham, 1991; Schonwitz et Ziegler, 1989) ou en sols stériles (Merbach *et al.*, 1991; Warenbourg, 1975). Les 3 principales difficultés rencontrées sont :

1. **Faible concentration** des substances organiques dérivées des racines, comparées aux autres composés organiques (matière organique du sol, résidus de plante, produits intermédiaires de décomposition de matière organique du sol, produits du métabolisme des organismes du sol).
2. **Décomposition rapide** par les microorganismes du sol
3. Les composés rhizodéposés restent dans un **volume de sol restreint**, en contact direct avec les racines.

L'utilisation d'isotopes du C (^{14}C et ^{13}C) a permis des progrès significatifs dans la compréhension du cycle du C dans la rhizosphère. La quantité de C dérivé des racines est alors estimée 3 à 7 fois supérieure à celle trouvée par les techniques classiques.

Les racines libèrent plus de 200 composés carbonés sous forme d'exsudats. Ces exsudats peuvent être classés selon plusieurs caractéristiques:

- Les composés solubles dans l'eau : acides aminés, acides organiques, sucre, et divers métabolites secondaires
- Les polymères complexes : polysaccharides, polypeptides et enzymes.
- Composés à poids moléculaire élevé : mucilage, gels, ectoenzymes
- Composés à faible poids moléculaire : acides organiques, sucres, phénols, acides aminés, phytosiderophores (chélatants du Fe et autres métaux), flavonoïdes et vitamines.

F - Séquestration de C et taux de décomposition des racines

Puisque la teneur en C organique d'un sol est liée à de nombreuses qualités du sol (capacité de rétention en eau, capacité d'échange cationique, structure du sol, richesse en nutriments), il

s'agit donc d'un paramètre primordial influant sur les processus écosystémiques du sol. Or le C organique du sol provient en quasi totalité de matériaux dérivés des végétaux (Figure 1).

Les taux de croissance élevés correspondent généralement à des flux importants et rapides, associés à des réservoirs de C du sol relativement petits. Les faibles taux de croissance, en revanche, correspondent à des flux plus faibles et à des réservoirs de C plus récalcitrants et plus persistants. La lignine, par exemple, est bénéfique à la séquestration de C dans le sol, grâce à son temps de résidence long dans le sol, dû notamment à la spécificité des enzymes impliquées dans sa dégradation et à la présence limitée des champignons capables de les produire (de Boer *et al.*, 2005; Zak *et al.*, 2006). Par la suite, l'adsorption du C par les fractions minérales, qui est souvent promue par la rhizodéposition des racines et par les processus de complexation, permet d'allonger le temps de résidence du C (De Deyn *et al.*, 2008). Cette structuration du sol par l'apport en matière organique est stimulée par les microorganismes du sol, dont l'activité sera d'autant plus élevée que le ratio C:N du C organique apporté sera bas. Ainsi, dans les systèmes tempérés, cet apport en matière organique nutritionnelle pour les microorganismes est importante pour une séquestration de C à long terme par une stabilisation des agrégats du sol (Lavelle *et al.*, 1997).

Les estimations de flux de C dans les sols de cultures font intervenir des méthodes soit controversées soit assez lourdes à mettre en oeuvre (Peichl *et al.*, 2006). Cette étude n'insistera pas sur les aspects quantitatifs absolus de séquestration de C dans les sols de cultures, mais s'appuiera sur les variations en terme de vitesse de décomposition des racines selon les espèces et les traits racinaires respectifs. Etant donné la complexité des interactions au niveau racinaire (enchevêtrement, compétition pour les ressources, mycorhizes, flore bactérienne), un moyen d'accéder de manière plus simple et rapide au taux de décomposition racinaire que par des mesures systématiques, serait de trouver des traits racinaires aisément mesurables et corrélés à ce processus. Nous définirons un trait fonctionnel comme étant un caractère morphologique, physiologique, biochimique ou phénologique, ayant un effet sur la performance d'un individu (Choler, 2002).
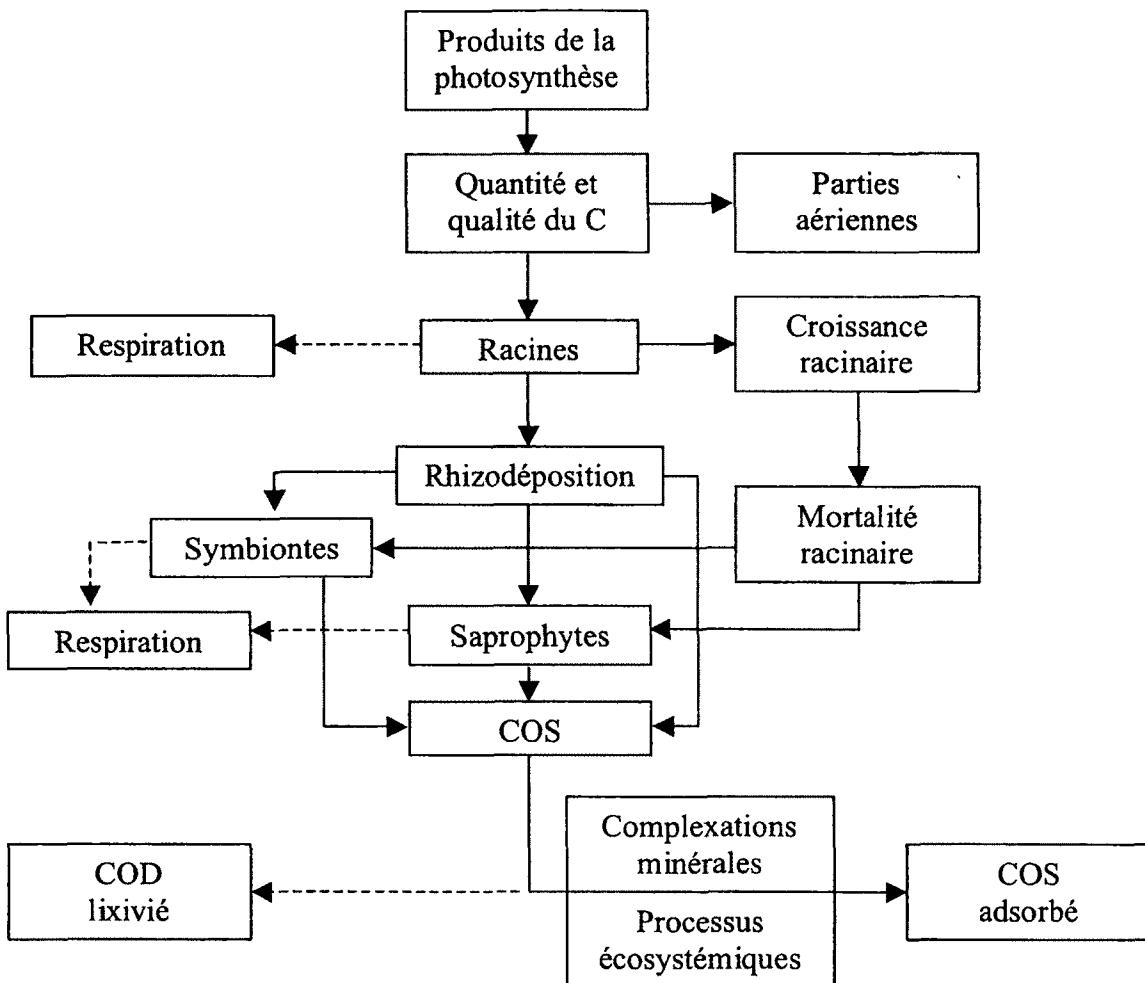
Figure 1. Flux de carbone (C) par les racines de plantes et leurs organismes associés. La séquestration nette de C au sol dépend de la quantité et de la qualité de la matière organique des plantes et des hétérotrophes. Ceci détermine l'efficience d'utilisation du C et son temps de résidence. Les lignes continues et pointillées correspondent respectivement à de l'incorporation et des pertes de C au sol. COS: Carbone organique du sol. COD: Carbone organique dissout. D'après De Deyn *et al.* (2008).

Chez les racines, la mesure des taux de décomposition devrait impliquer une définition de la mort racinaire qui est moins évidente que pour les feuilles. Bien que la mort des racines soit le plus souvent considérée comme une variable dichotomique, la mort d'une racine correspond à une mort progressive de ses cellules et tissus (Comas *et al.*, 2000). En effet, la mort d'une portion de racine n'implique pas nécessairement la perte de ses fonctions. Le cortex peut mourir alors que la stèle et le péricycle restent intactes, la fonction de conduction est donc encore assurée, ainsi que la possibilité de produire de nouvelles racines (Dubrovsky *et al.*, 1998; Lo Gullo *et al.*, 1998; Spaeth et Cortes, 1995).

G - Stratégies adaptatives et patrons d'association des traits

Depuis la classification des espèces végétales selon le niveau de stress et de perturbation par Grime (Grime, 1979), les travaux portant sur les feuilles ont permis l'acquisition de nombreux traits pour une large gamme d'espèces (GLOBNET, Wright *et al.* 2004 ; LEDA, Kleyer *et al.* 2008 ; TRY, Kattge *et al.* 2011). L'analyse des covariations entre ces traits ont révélé des patrons d'association des traits au niveau global (Reich *et al.* 1997 ; Reich *et al.* 1999 ; Wright *et al.* 2004) et ont montré que les plantes sont soumises à des compromis, notamment entre l'acquisition des ressources et les contraintes biophysiques (Monk, 1966; Reich *et al.*, 2003; Williams *et al.*, 1989). Ainsi on observe que chez les plantes, des patrons ont émergé où l'on retrouve souvent les mêmes associations de traits fonctionnels. C'est ce que l'on appelle les syndromes de traits qui interviennent dans la définition des groupes fonctionnels de plantes (Reich *et al.*, 2003). Les plantes des habitats pauvres sont plutôt associées à de faibles taux de croissance et auront tendance à avoir également des feuilles à durée de vie longue, faible SLA (surface foliaire spécifique) et faibles concentrations en N des feuilles (Reich *et al.*, 2003; Wright *et al.*, 2004). Cependant, ces relations ont été étudiées

chez les feuilles et encore peu chez les racines. Il est cependant possible de voir apparaître certains patrons au niveau souterrain (tableau 2).

Tableau 2. Corrélations positives (+) et négatives (-) entre les traits racinaires. SRL: Longueur racinaire spécifique (m g^{-1}). RDMC: Teneur en matière sèche (g.g^{-1}). Références : (1) Tjoelker *et al.* (2005). (2) Withington *et al.* (2006). (3) Miller *et al.* (1990). (4) Comas et Eissenstat (2004). (5) Comas *et al.* (2002). (6) Roumet *et al.* (2006). (7) Bloomfield *et al.* (1993).

Corrélation entre traits	N (%)	SRL (m.g^{-1})
Calcium (%)	+ (3)	
Lignine (%)	- (7)	
SRL (m g^{-1})	+ (1); ± (2)	
RDMC (g g^{-1})	- (4)	- (4,5,6)

H - Variations interspécifiques des traits racinaires et comparaisons racines - feuilles

Chez les feuilles, la relation entre les traits de feuilles sénescentes et leur vitesse de décomposition a été largement étudiée (Aber *et al.*, 1990 ; Aerts et Decaluwe, 1997 ; Melillo *et al.*, 1982 ; Taylor *et al.*, 1989). Etant donné que la littérature est beaucoup plus détaillée concernant les feuilles que les racines, la question serait de savoir à quel point les feuilles et les racines sont homologues et peuvent être comparées. Les derniers niveaux hiérarchiques racinaires ont effectivement des propriétés comparables à celles des feuilles : les deux sont éphémères, ont une croissance déterminée, ne présentent pas de croissance secondaire, et ont comme fonction principale d'acquérir des ressources (Eissenstat et Yanai, 1997; Pregitzer *et*

al., 2002; Wells et Eissenstat, 2001). Or il existe de nombreuses différences entre feuilles et racines qui pourraient empêcher la transposabilité de nos connaissances sur les feuilles aux racines, notamment le fait que les niveaux racinaires ultimes représentent la partie terminale d'un réseau et ne sont pas des entités discrètes comme les feuilles. De plus, contrairement aux feuilles, les racines acquièrent des ressources nombreuses et hétérogènes, mais surtout interagissent fortement avec leur environnement rhizosphérique. Ainsi, les processus racinaires dépendent énormément des symbiontes du sol, notamment au niveau des flux de carbone, de l'acquisition de ressources et peut-être même des défenses de la plante (Withington *et al.*, 2006).

Dans le cas où l'approche fonctionnelle se révélait efficace pour le système racinaire et dans un souci d'accéder plus rapidement et simplement à l'information concernant les taux de décomposition des racines, il serait optimal de pouvoir obtenir ces données à partir de traits aériens, qui sont plus accessibles. En effet, dans l'hypothèse que les stratégies aériennes sélectionnées se reflètent au niveau racinaire, la mesure des traits foliaires permettrait d'accéder aux informations racinaires. Bien que les travaux sur ce sujet ont effectivement trouvé des similitudes, aussi bien chez les herbacées (Tjoelker *et al.*, 2005) que chez les arbres (Withington *et al.*, 2006), les stratégies sélectionnées, et plus précisément la correspondance des traits homologues entre feuilles et racines, ne semble pas suffisamment précise pour prédire les traits racinaires à partir des traits aériens. En effet, il a même été trouvé des stratégies contraires entre parties aériennes et souterraines (Personeni et Loiseau, 2004).

Tableau 3. Variations interspécifiques des traits racinaires. C : carbone (%), N : azote (%) ; Ca : calcium (%) ; SRL : longueur spécifique racinaire.

Espèce	Diamètre	Commentaire	Lignine %	N:C	N (%)	Ca (%)	SRL (m g⁻¹)	Densité tissu (g cm⁻³)	Références
Juniperus occidentalis	< 1mm	juvéniles			0 67	1 21			
	< 1mm	petits adultes			0 73	1 15			
	1-5 mm	juvéniles			0 34	1 22			Miller et al , 1990
	1-5 mm	petits adultes			0 38	1 09			
	> 5mm	petits adultes			0.25	1.10			
Metrosideros polymorpha			26 92		4 17				
			30 38		4 23				Ostertag & Hobbie, 1999
			32 67		3 05				
Chamaecyparis thyoides	< 10 mm			0 0083	0 38				Crawford et al , 2007
Triticum aestivum			8 70	0 0222	0 91				
Medicago sativa	< 2 mm		2 77	0 0385	1 69				Jalota et al , 2006
Cenchrus ciliaris			14 80	0 0135	0 65				
Acacia aneura			19 50	0,0217	1 07				
Bidens pilosa					0 85		59 8	0 091	
Bromus catharticus					0 49		49 7	0 113	
Medicago lupulina					2 41		45 3	0 047	
Melilotus albu					1 69		28 3	0 058	
Muhlenbergia peruviana					1 09		197 8	0 044	
Tagetes minuta					1 03		44 8	0 081	
Vicia graminea					2 89		11 0	0 070	
Vulpia myuros					0 75		268 9	0 040	
Zinnia peruviana	système racinaire intégral				0 83		44 0	0 136	Roumet et al 2006
Adesmia bicolor					2 24		10 1	0 104	
Eustachys retusa					0 38		29 3	0 159	
Hypochaeris argentina					0 37		11 6	0 142	
Noticastrum marginatum					0 76		11 9	0 157	
Paspalum dilatatum					0 62		18 4	0 102	
Stipa eriostachya					0 37		19 6	0 168	
Stylosanthes guianensis					1 15		3 9	0 196	
Taraxacum officinale					0 58		3 9	0 129	
Trifolium repens					2 47		90 1	0 041	

$$N (g\,g^{-1})\text{ masse fraîche}$$

Espèce	Diamètre	Commentaire	Lignine %	N:C	N (%)	Ca (%)	SRL (m g⁻¹)	Densité tissu (g cm⁻³)	Références
Acer negundo					1 67		83 0	0 118	
Acer saccharum					1 42		50 0	0 160	
Betula lenta					1 89		117 0	0 160	
Fagus grandifolia					1 37		77 0	0 165	
Quercus rubra	non communiqué				1 34		47 0	0 165	
Quercus alba					1 42		43 0	0 155	Comas & Eissenstat, 2004
Carya ovata					1 67		83 0	0 145	
Carya glabra					1 38		52 0	0 260	
Pinus virginiana					1 36		30 0	0 295	
Pinus strobus					1 82		38 0	0 140	
Tsuga canadensis					1 51		19 0	0 272	
Acer negundo				0 0613	2 65		16 9	0 192	
Acer saccharum				0 0481	1 95		15 2	0 191	
Quercus rubra	1er 2e et 3e ordre			0 1124	4 80		16 9	0 185	
Quercus alba				0 0420	1 64		31 7	0 150	Comas et al , 2002
Pinus virginiana							32 5	0 084	
Tsuga canadensis							28 6	0 082	

23

Tableau 4. Principales études de décomposition des racines. Les types biologiques sont graminoïdes (G), conifères (C), et feuillus (F). Les classes de diamètre sont: racines fines (F, <2mm), moyennes (M, 2-5mm), larges (L, >5mm). *Ex* exponentiel, *Lin* Linéaire. Adapté de Silver et Miya (2001).

Latitude (°N)	Type biologique	Modèle et nombre de valeurs k	Classes de diamètre	Méthodes	Maille (mm)	Références
4	G	Ex (16)	F	Pots enterrés	-	Gijsman *et al.* (1997)
8	G	Ex (6)	F,M	Sacs à litière	0,2 ; 5.0	Lehmann *et al.* (1995)
18	F	Ex (2)	F	Sacs à litière	1,5	Bloomfield *et al.* (1993)
18	F	Ex (3)	F	Tranchées	-	Silver et Vogt (1993)
19	F	Lin (14)	F	Sacs à litière	0,3	Ostertag et Hobbie (1999)
25	F	Ex (3)	F	Sacs à litière	1,0	Arunachalam *et al.* (1996)
25	G	Ex (3)	NR	Sacs à litière	1,0	Singh et Shekhar (1989)
25	G	Ex (1)	F	Sacs à litière	1,0	Tripathi et Singh (1992)
27	G, F	Ex (8)	F	Sacs à litière	0,35	Jalota *et al.* (2006)
30	C	Ex (2)	F,M	Sacs à litière	0,2 ; 1,0	Gholz *et al.* (1986)
32	G	Ex (5)	F	Sacs à litière	1,0	Mun et Whitford (1997)
32	G	Ex (3)	NR	Sacs à litière	1,2	Parker *et al.* (1984)
34	C	Ex (3)	M,L	Sacs à litière	1,0	King *et al.* (1997)
36	G, F	Lin (4)	F	Minirhizotron	-	Satomura *et al.* (2006)
37	G,F	Ex (8)	F,M	Sacs à litière	1,0	Conn et Day (1997)
39	G	Ex (2)	F	Sacs à litière	1,0 ; 3,0	Seastedt (1988)
39	G	Ex (1)	F	Sacs à litière	Pool 1,0; 3,0	Seastedt *et al.* (1992)
41	C	Ex (6)	L	Carottes successives	-	Yavitt et Fahey (1982)
42	G, F	Ex (3)	F, M	Carottes intactes	0,1	Dornbush *et al.* (2002)
42	G, F	Ex (3)	F	Sacs à litière	0,1	Dornbush *et al.* (2002)
42	F	Ex (2)	F	Minirhizotron	-	Comas *et al.* (2000)
43	C	Ex (2)	F	Sacs à litière	0,1 ; 2,0	Aber *et al.* (1990)
43	F	Ex (4)	L	« Tethered »	-	Fahey et Arthur (1994)
43	F	Ex (12)	F,L	« Tethered »	-	Fahey *et al.* (1988)
43	C,F	1er ordre (4)	F,M	Sacs à litière	0,1; 0,4; 3,0	McClaugherty *et al.* (1982)
44	F	Ex (6)	F,M	Sacs à litière	2,0	Burke et Raynal (1994)
44	C	Ex (1)	M	Sacs à litière	1,0	Fogel et Hunt (1979)
46	F	Ex (6)	F,M,L	Sacs à litière	0,1; 1,0; 3,0	Camire *et al.* (1991)
51	F	Ex (6)	F,M,L	Sacs à litière	Pool 1,0; 4,0	Scheu et Schauermann (1994)
52	G	Ex (2)	M	Sacs à litière	0,1	Vanvuuren *et al.* (1993)
54	G,F	Ex (2)	NR	Sacs à litière	1,0	Heal *et al.* (1978)
59	G	Ex (4)	NR	Sacs à litière	1,0	Andren *et al.* (1992)
59	C	Ex (2)	F	Sacs à litière	0,1	Lohmus et Ivask (1995)
60	C,F	Ex (9)	F,M,L	Sacs à litière	1,0	Berg (1984)
64	C	Ex (1)	L	Sacs à litière	1,0	Baath *et al.* (1984)
38,43,45	F	Ex (8)	F,M	Sacs à litière	0,1; 0,4; 3,0	McClaugherty *et al.* (1984)
56-66	C	Ex (28)	F,M	Sacs à litière	1,0	Berg *et al.* (1998)

Dans le cas de stratégies contrastées entre les parties aériennes et souterraines, la coexistence de deux espèces dont l'une est conservatrice au niveau des racines, l'autre au niveau des feuilles, serait facilitée (Personeni et Loiseau, 2004). Il est probable que cette opposition de stratégie feuille-racine puisse expliquer les compositions plus complexes de certaines communautés végétales. Il semble donc que les stratégies adoptées par les systèmes aériens ne soient pas suffisamment dépendantes de celles des systèmes racinaires pour pouvoir baser nos prédictions à partir des feuilles. Il semble donc nécessaire, dans un premier temps, d'utiliser les traits racinaires comme base prédictive.

Chez les feuilles, les variations interspécifiques des traits comme le taux de photosynthèse, le taux de respiration, la longévité, la teneur en N et la SLA (surface foliaire spécifique), sont très élevées et varient jusqu'à un facteur 100 pour la SLA (Reich *et al.*, 1997). Chez les racines, même si le nombre d'espèces étudiées est plus faible, on remarque également des variations allant jusqu'à un facteur 70 pour son trait homologue : la longueur spécifique racinaire, SRL (tableau 3).

I - Estimation des taux de décomposition des racines par les traits racinaires

Bien que la technique très majoritairement utilisée (tableau 4) des sacs à litières soit peu adaptée aux racines, Jalota *et al.* (2006) ont montré que la vitesse de décomposition des racines est corrélée avec la concentration en lignines des racines. La méta-analyse de Silver et Miya (2001) semble confirmer que certains paramètres biochimiques sont corrélés à la décomposition des racines, notamment la concentration des racines en calcium et le ratio

C:N, respectivement positivement et négativement corrélés. Cependant, ces corrélations sont encore controversées chez les racines car basées sur des méthodes souvent peu adaptées (Dornbush *et al.* 2002) et leurs correspondances avec les feuilles sont encore peu étudiées et ne font pas l'objet d'un consensus (Tjoelker *et al.*, 2005 ; Withington *et al.*, 2006).

J - Les objectifs du projet et organisation du manuscrit

1 - Estimation dynamique et non-destructive de la biomasse racinaire

La compréhension des processus racinaires reste encore un défi pour les années à venir, notamment pour alimenter les modèles généraux de prédiction des flux de C des racines vers le sol. Contrairement aux feuilles, les racines sont en constante interaction avec leur milieu environnant, la rhizosphère, posant à la fois un défi de compréhension de la complexité de ces interactions, mais surtout un défi méthodologique d'accès à des données dynamiques. Notamment, l'estimation dynamique et non-destructive de la biomasse individuelle racinaire serait d'un grand intérêt pour alimenter les modèles de circulation de C par des données dynamiques et in situ. Le premier chapitre de cette étude a comme objectif principal d'estimer la corrélation entre la biomasse racinaire totale d'un individu et la capacitance électrique du système racinaire complet. Plus précisément, il sera premièrement déterminé à quel point ces corrélations sont spécifiques et si ces effets spécifiques peuvent être expliqués par la variabilité interspécifique des densités de tissus racinaires, ici mesurées par le ratio de masse sèche sur masse fraîche racinaire. Le deuxième objectif est d'évaluer les imprécisions de mesure dues à l'enchevêtrement des racines d'un individu voisin. Le dernier objectif est d'estimer le sens et l'intensité des imprécisions causées par une augmentation des surfaces de

contact intra-individuelles et une diminution des contacts racines-sol. Par la diminution de la taille du pot, ces effets seraient principalement attribués à l'augmentation des contacts entre racines ainsi que entre les racines et les parois du pot.

2 - Prédiction des taux de décomposition racinaires in situ par une approche interspécifique à partir de traits chimiques et morphologiques

Pour des raisons principalement méthodologiques, les études comparatives testant les ressemblances entre la décomposition des feuilles et des racines sont controversées, et il n'est toujours pas clair jusqu'à quel point il est possible d'extrapoler aux processus racinaires la riche littérature portant sur les feuilles. Cependant, il est évident que, considérant les nombreuses différences qui les distinguent des feuilles, notamment en terme de phénologie, d'architecture et d'interaction avec leur milieu, on peut s'attendre à des processus de décomposition singuliers de la part des racines. Pour tenter de répondre à cette interrogation, il est proposé ici une approche comparative d'estimation des taux de décomposition par les traits racinaires, sur une large gamme fonctionnelle d'espèces, incluant des espèces de cultures, de fourrages et des espèces d'arbres. Ce deuxième chapitre aura comme objectif principal d'évaluer le potentiel de l'approche fonctionnelle pour la prédiction des taux de décomposition racinaires in situ. Plus précisément, il aura comme premier objectif spécifique d'évaluer la variabilité interspécifique des traits fonctionnels racinaires ainsi que des taux de décomposition mesurés in situ. Le deuxième objectif spécifique sera d'estimer la proportion de la variabilité des taux de décomposition attribuée d'une part aux différentes fractions de C et d'autre part aux différences interspécifiques des taux de décomposition de ces différentes fractions de C. Enfin, le dernier objectif spécifique sera d'évaluer la qualité des prédictions des variations interspécifiques des taux de décomposition racinaires à partir (i) des proportions initiales des différentes fractions de C, (ii) des traits initiaux des racines de toutes

27

les classes de diamètres récoltés, et (iii) des traits initiaux des racines absorbantes seulement, ce qui permet une véritable comparaison fonctionnelle interspécifique.

3 - Prédiction des dynamiques de décomposition des différentes fractions de carbone racinaires à partir des traits initiaux.

La séquestration de C dans les sols a été proposée comme une excellente candidate pour atténuer les effets des émissions anthropiques de C atmosphérique. Dans le but de quantifier le potentiel de séquestration de C dans différents sols, il paraît nécessaire de faire appel à des modèles écosystémiques de flux de C, incluant notamment le processus de décomposition des tissus végétaux. Un de ces modèles largement utilisé, le modèle Century, est basé sur la séparation de la matière organique libérée par les plantes en différentes fractions selon leur vitesse de décomposition. Dans ce troisième chapitre, il est proposé de simuler la dynamique de décomposition des fractions de C racinaires de 10 espèces d'arbres à partir de données de décomposition in situ, puis de proposer des estimateurs alternatifs pour les modèles de dynamique de C des résidus dérivés de plantes. Dans un premier temps, la possibilité de prédire les taux de décomposition de chaque fraction de C à partir de traits racinaires initiaux sera testée. Ensuite, il sera vérifié si le taux de décomposition d'une même fraction de C racinaire est constante au sein des différentes espèces. Si tel n'est pas le cas, alors la variabilité interspécifique de ces valeurs sera étudiée. Puis, pour tenter de vérifier si les préférences d'utilisation des fractions de C des litières foliaires par les microorganismes s'appliquent aux racines, il sera testé si la vitesse de perte de masse de ces différentes fractions dépendent des abondances relatives des fractions moins récalcitrantes. Finalement, l'amélioration des prédictions des taux de décomposition du C racinaire sera testée après l'intégration de ces nouvelles données des dynamiques des fractions de C racinaires.

CHAPITRE I -

ESTIMATION DYNAMIQUE ET NON-DESTRUCTIVE DE LA BIOMASSE RACINAIRE PAR LA CAPACITANCE ELECTRIQUE SUR 10 ESPECES HERBACEES.

Avant propos

Ce premier chapitre a pour objectif l'estimation dynamique et non-destructive de la biomasse individuelle racinaire. Pour cela, le potentiel de prédiction de la masse racinaire par la technique de mesure de la capacitance électrique sera testée. Plus précisément, il sera déterminé à quel point ces corrélations capacitance – masse sont spécifiques à chaque espèce et si ces effets spécifiques peuvent être expliqués par la variabilité interspécifique des densités de tissus racinaires. Les impacts des interactions racinaires sur les imprécisions de mesure seront également évalués chez un même individu ou avec son voisin.

Les résultats de ces analyses sont regroupés et discutés dans le présent article intitulé "Non-destructive and dynamic estimation of root mass using electrical capacitance on ten herbaceous species", qui sera soumis à *Plant and soil*. Les données utilisées pour réaliser ces analyses ont été mesurée par moi-même avec l'aide successive de deux stagiaires (Jessy Loranger et Philippe Grégoire). Toutes les analyses statistiques utilisées dans ce chapitre ont été effectuées par moi-même. Etant donné que l'ensemble du manuscrit a été écrit par moi-même avant d'être révisé par mon directeur de thèse, le professeur Bill Shipley, j'en suis le premier auteur.

Non-destructive and dynamic estimation of root mass using electrical capacitance on ten herbaceous species

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Abstract

A non-invasive and non-destructive method to estimate plant root biomass can be very useful for dynamic measurements, either on isolated individuals or ones growing within their communities. Electrical capacitance is a quick and affordable technique. Few studies have evaluated this method and of these, most have used plants grown in either pure hydroponic or sand-based growing media. The objective of this study was to determine if measures of electrical capacitance can accurately estimate the root biomass of 10 crop and forage species grown in soil. The relationship between electrical capacitance and root dry mass was significant but too weak (r^2 = 0.30) to accurately estimate the biomass of the root systems without prior specific calibrations. Root interactions had significant effects on the specific capacitance response, and fine-root species seemed to be more affected.

Introduction

Despite the fact that over half of the biomass of most plants resides in the soil, the vast majority of research on plant traits has concentrated on aboveground tissues. For instance, in the global TRY database (Kattge *et al.*, 2011), which contains information on 53 traits of over 61000 species, one of the few root traits (Rooting depth) is only measured on 443 species. The main reason for this lack of data is that current methods to study root growth, dynamics, and root-soil interactions are either time and labour intensive, destructive, or both (Polomski & Kuhn, 2002). In particular, dynamic non-destructive estimates of root mass for individual plants would be of great interest for root carbon models, but would require reliable measurements. A non-destructive method that could accurately estimate root mass and root growth of plants growing in the field and without destructive harvests would greatly increase our knowledge of root behaviour, rates of belowground productivity, and root-soil interactions.

Unfortunately, not many such methods exist. One important exception is the method proposed by Chloupeck (1972), because it is an easy, rapid and non-destructive method to estimate root biomass based on a correlation between root mass and the electrical capacitance of the whole root system. This method was compared with allometric root diameter equations (Kendall *et al.*, 1982), and was later found to be fairly efficient in hydroponics and sand media, but appeared less reliable under field or potted soil conditions in the small number of attempts to date (Table 1). Although the relationship between physical (including electrical) and biological properties of plant organs have been studied for decades in an agronomical (Currie *et al.*, 1987) or forestry context (Lekas *et al.*, 1990) we still do not know how well one can use these electrical properties to assess biological characteristics of plants in the field and across species and growth forms.

Root epidermal membranes, which play the role of electric insulators, separate two conductive elements, the soil solution on one side, and the root internal medium on the other side. More precisely, all biological membranes situated between the two measurement

electrodes also play this role. These membranes thus act as an imperfect electric capacitor, which consist of two conductive media separated by an insulator, and is characterised by its ability to store electric charges. The electrical capacitance method is based on the active polarization of the root membranes with an external energy source, and measures the resulting dielectric properties of the root system (Chloupek *et al.*, 1999; Dalton, 1995). Previous studies have shown the sensitivity of the method to the soil water content (Chloupeck, 1977; Dalton, 1995; van Beem *et al.*, 1998). In the case of a reduction in soil water content, the contact between roots and the soil solution is diminished, and the root surface area is then underestimated. This proportion of root surface area in contact with its surrounding soil solution has been estimated by Herkelrath *et al.* (1977) via the relative saturation of the soil. This explains why the soil moisture has to be saturated at the time of measurements. More detailed interpretations on the practical significance of these measurements in relation to the soil, root internal medium or the root-soil interface is given in Ozier-Lafontaine and Bajazet (2005).

Practically, the method requires one to place the first electrode of the LCR (Inductance –L–, Capacitance and Resistance) meter at the base of the plant shoot, and the second one to a metal rod inserted into the surrounding soil. The resulting capacitance, measured in farads, relates the amount of electric charge stored by the root membranes for a specific applied potential, and depends on the active surface area and length of roots (Dalton, 1995). As a practical matter, root mass (either fresh or dry) has more frequently been used as the variable to be predicted.

Despite the relatively good results of the previous studies, most of which were based on single species growing in hydroponic culture or on other mineral substrates, it is not clear if this method can be used in field-grown plants; only a few studies (Table 1) have been based on field measurements, and revealed poorer correlations. Preston *et al.* (2004), for example, grew 33 young hybrid poplar clones from whips in the field in a potting soil mixture made of one-third vermiculite and perlite and two-third triple mix consisting of manure, peat, and loam by weight. Seventeen of these young poplars were grown for another season on the

34

surface of a municipal solid waste with a clay loam textured landfill cover, and their root electrical capacitance were measured thereafter. The authors found good linear correlations between electrical capacitance and both root dry and fresh biomass of the potted poplar whips. However, older individuals grown on the landfill appeared to decrease the strength of the correlation but were unfortunately too few to be analysed in a separate model. Most of the encouraging studies grew the plants in either hydroponic culture or in a sand culture with nutrients added hydroponically (Table 1). Since soils are structurally and chemically much more complex and heterogeneous than hydroponic or sand-based culture, it is difficult to extrapolate from such studies to the field.

Table 1. Review of published literature on root capacitance measurements associated to root fresh or dry mass. Studies are presented in chronological order.

Plant	Species	Substrate	Growing condition	Current frequency (kHz)	Regression type	number of samples n	Fresh weight r^2	Dry weight r^2	reference
Maize	Zea movs L	sand	-	0 8	linear	24	0 74	0 73	
Sunflower	Helianthus annuus l	sand		5	linear	15	0 92	0 90	
Sunflower	Helianthus annuus I	clayish soil		5	linear	10	0 69	0.43	Chloupek (1972)
Oats	Avena sativum L	clayish soil		5	linear	15	0 57	0 46	
Onion	Allium cepa L	sand		1	linear	14	0 57	0.54	
Rape	Brassica napus L	-	-	-	linear	18	0 08	0 14	
Carrot	Daucus Carota L	loam	field	1	linear	~230	0 40		Chloupek (1977)
Red clover	Trifolium pratense	nutrient solution	hydroponics	1	linear and quadratic	21		0 67 - 0 76	Kendall et al (1982)
Alfalfa	Medicago sativa	silt loam	field	1	linear and quadratic	20		0 29 - 0.55	
Tomato	Lycopersicon esculentum Mill	nutrient solution	hydroponics	-	linear	24		0 77	Dalton (1995)
Maize	Zea mays L	vermiculite	greenhouse	1	linear	320	0 73		van Beem et al (1998)
Maize	Zea mays L	loam	field	1	linear	360	0 53		
Poplar	Populus deltoides x P nigra	potting soil mixture	pots in field	1	linear	33	0 87	0 90	Preston et al (2004)
Tomato	Lycopersicon esculentum Mill	potting soil mixture	greenhouse	spectra	linear	15		0 96	Ozier Lafontaine and Bajazet (2005)
Tomato	Lycopersicon esculentum Mill	potting soil mixture	greenhouse	1	linear	15		0 83	
Maize	Zea mays L	clay and silica	hydroponics	1	linear	30-32		0 13 - 0 72	McBride et al (2008)

The other main challenge of this method, in order to be of use in multispecies plant communities, is to give accurate root mass estimates without species specific calibrations. In this study we therefore assess the potential of electrical capacitance to estimate root biomass in controlled experiments of ten common crop or forage species grown in soil. Regardless of abiotic factors, such as soil water content, the biological reasons for interspecific differences in regression equations between capacitance and root mass remain unclear. Although Chloupeck (1972) revealed these interspecific differences, our understanding of the plant

tissue characteristics that would modify the electrical behaviour of roots is still poor. As proposed by Dvořák *et al.* (1981), a current applied to plant tissue has different pathways, apoplastic (extracellular) and symplastic (intracellular), and its proportions between these two pathways would depend on the frequency of the applied current. Capacitance properties of root tissues are only measured at frequencies where membranes are crossed by the applied current. The tissue density of roots may also be an important biological factor regulating these pathways, by changing the extracellular fibre content and density. In this study, we will test the effect of root dry matter content (dry mass:fresh mass ratio) as a factor regulating the behaviour of roots towards electrical capacitance.

Our main objective is to correlate root capacitance and root biomass in an interspecific context. More precisely, we first aim at determining the degree to which such correlations are species specific, and assess to what extent these species effects can be explained by differences in root dry matter content. Our second objective is to test for the possible inaccuracy due to intermingled roots from neighbouring individuals, which would decrease the quality of the root-soil contacts. Finally, our third objective is to assess the potential imprecision caused by increased intra-individual root contact and decreased root-soil contact, especially due to root-root and root-pot wall contacts, when reducing the pot size.

We hypothesise that whatever the kind of root contacts and interactions, i.e. intra- or inter-individual, the contact between roots and the soil solution would not be representative of the whole root system and so the root surface area estimated by root capacitance would be underestimated. However, the proportions of root-root compared to proper root-soil contacts will at least depend on species, which can be calibrated with species-specific calibrations, but also on substrate quality and volume, plant size, and other environmental conditions.

36

Materials and methods

Three different controlled growth experiments were performed, with exactly the same controlled conditions (Table 2). These three experiments varied either the total number of species individually tested, the number of individuals per pot or the pot size. In Experiment 1, 10 species were individually tested with 1.5L pots in a randomized block design with 15 blocks and one individual per pot. In Experiment 2 and 3, the same experimental design was used. In Experiment 2, two individuals were grown per pot, which allowed to test for inter-individual interactions when compared to Experiment 1. In Experiment 3, the pot size was 4.5L, which allowed to test for intra-individual interactions when compared to Experiment 1. In all experiments, the plants were grown in a controlled temperature room with day/night temperatures of 22°C and 18°C respectively, with 16 hours of light daily. The humidity level was kept at 70% and the photon flux density (PAR) was 116.6 ± 16.2 $\mu mol.m^{-2}.s^{-1}$. The potting soil mixture was composed of humus, sheep manure compost and mineral sieved agricultural soil (proportion 1-1-1). No fertilizer was added. Pots were arranged in a randomized block design, with 15 blocks, each block containing one sample of each species. Twice a week, a block was randomly chosen and electrical capacitance, expressed in nanofarads (nF), was measured with a BK Precision 879 meter (B&K Precision Corp., Yorba Linda, CA) at 1 kHz. The pots were watered to soil field capacity one hour before the measurements. The electrical contact with the plant was established by connecting the positive electrode attached (via an alligator clamp) to a small stainless steel pin, embedded into the stem 1 cm above the soil surface. The negative electrode was connected, via an alligator clamp, to a copper rod (2 mm diameter, 13.1 cm long) inserted in the potting soil. Great care was given to the precision of the position of the stem electrode. After measuring the electrical capacitance the plant roots were separated from the soil and washed with care, so that even detached roots were collected. The fresh root systems were weighed in experiments 1 and 3, then oven-dried at 50°C until constant weight was reached (about 48

hours) and dry mass was measured (for all experiments). The root dry matter content was calculated as the ratio of root dry mass on root fresh mass.

We used linear mixed models, with block number being the random factor, to assess the effect of our treatments (species, pot size, planting density) and covariate (root dry matter content), on the dependent variable (either capacitance or the ratio of capacitance to root dry mass).

Table 2. List of the species grown in our three experiments.

	Experiment 1	Experiment 2	Experiment 3
Species	*Avena sativa* L. *Brassica napus* L. *Bromus inermis* Leyss. *Festuca rubra* L. *Phleum pratense* L. *Secale cereale* L. *Trifolium pratense* L. *Triticum aestivum* L. *Vicia faba* L. *Zea mays* L.	*Avena sativa* L. *Brassica napus* L. *Bromus inermis* Leyss. *Vicia faba* L. *Zea mays* L.	*Bromus inermis* Leyss. *Triticum aestivum* L. *Vicia faba* L. *Zea mays* L.
Individuals per pot	1	2	1
Number of blocks	15	15	15
Pot size	1.5L	1.5L	4.5L

Results

We first present our results related to the potential of the electrical capacitance method to predict root dry mass from an intact herbaceous plant. Second, we describe the effect of inter-individual root interactions on measurement accuracy. Third, we assess the effect of intra-individual root interactions on our root mass predictions. Finally, we show our results estimating the effect of root tissue density on the capacitance-mass relationships.

Global relationship between root dry mass and electrical capacitance.

When combining the results from our three experiments (Figure 1), there was a significant $(F_{1,291} = 225.4$; $p < 2 e^{-16})$, but weak $(R^2 = 0.30)$ positive relationship between root dry mass and electrical capacitance. However, there were also significant differences between species $(F_{9,240} = 16.8$; $p < 0.0001)$ and between the different treatments $(F_{1,240} = 168.2$; $p < 0.0001)$. Ten species are represented, with two different pot sizes, and two planting densities. The linear regression is highly significant. The experiment and the species effects are also highly significant.

Inter-individual root interactions and electrical capacitance measurements accuracy

Figure 2 presents the comparative Capacitance:Root dry mass ratios for the five herb species grown in experiments 1 and 2. There were clear differences among species in the amount of capacitance per gram of root dry mass $(F_{4,118} = 17.2$; $p < 0.0001)$. Although the effect of planting density was found significant overall $(F_{1,118} = 9.2$; $p = 0.0029)$, and the effect of planting density on the ratio also differed among species $(F_{4,118} = 18.9$; $p < 0.0001)$, the Capacitance:Root Mass ratios of Canola were about 7 times higher at doubled density and probably played a dominant role in these findings. This means that canola root masses would have been highly overestimated if this effect had been neglected. For all other species, the effect of planting density was not significant.
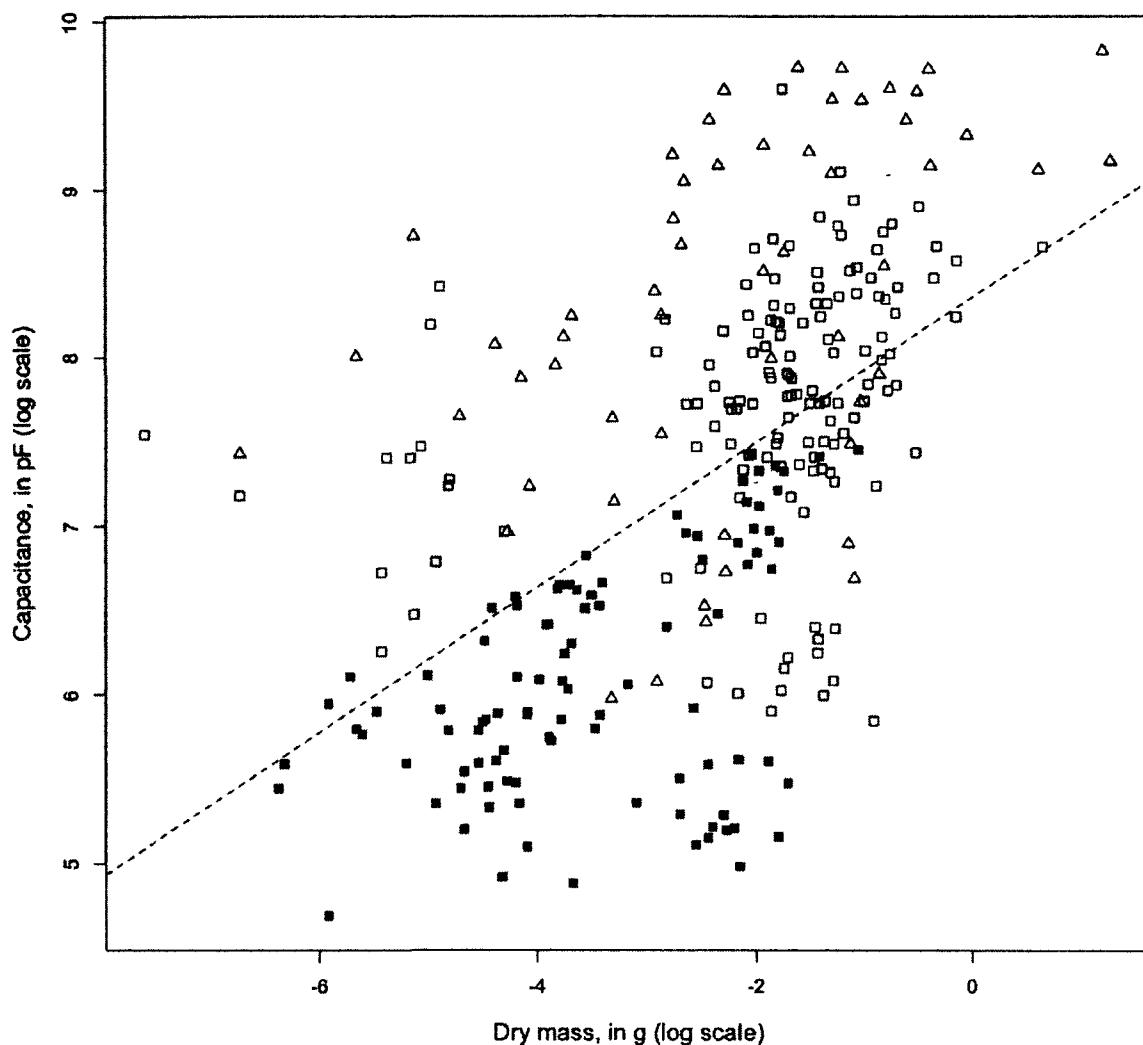
Figure 1. Root capacitance vs. dry mass relationship of ten herbaceous species. The dashed line corresponds to the linear regression model (R^2 = 0.30). Squares represent 1.5L pots with 1 and 2 individuals per pot represented in empty and full squares respectively. Empty triangles correspond to 4.5L pots with 1 individual per pot.

Intra-individual root interactions effects and root mass predictions

The Capacitance:Root Mass ratios were consistently higher in the 4.5L pots compared to 1.5L pots (Figure 3, $F_{1,91}$ = 12.7 ; p = 0.0006). The ratio differed among species ($F_{3,91}$ = 6.6 ; p = 0.0004) and the effect of pot size on the ratio also differed among species ($F_{3,91}$ = 7.4 ; p = 0.0002). Individually tested with a post-hoc Tukey test, the capacitance to dry mass ratio was found to be significantly different only in the 4.5 L pots of Brom Grass, compared to all other species and treatments. Indeed, the brome grass capacitance was diminished by a factor of 24 in the smaller pot size, whereas that of the other three species only decreased by a factor of about 4.

Effect of root dry matter content on the capacitance-mass relationship

The effect of root dry matter content was found positively correlated to the Capacitance: Root dry mass ratios ($F_{1,169}$ = 16.9 ; p = 0.0001). The higher the tissue density, the higher the capacitance for a similar root mass. However, only 14% of the variation in the ratios was explained by the root dry matter content.
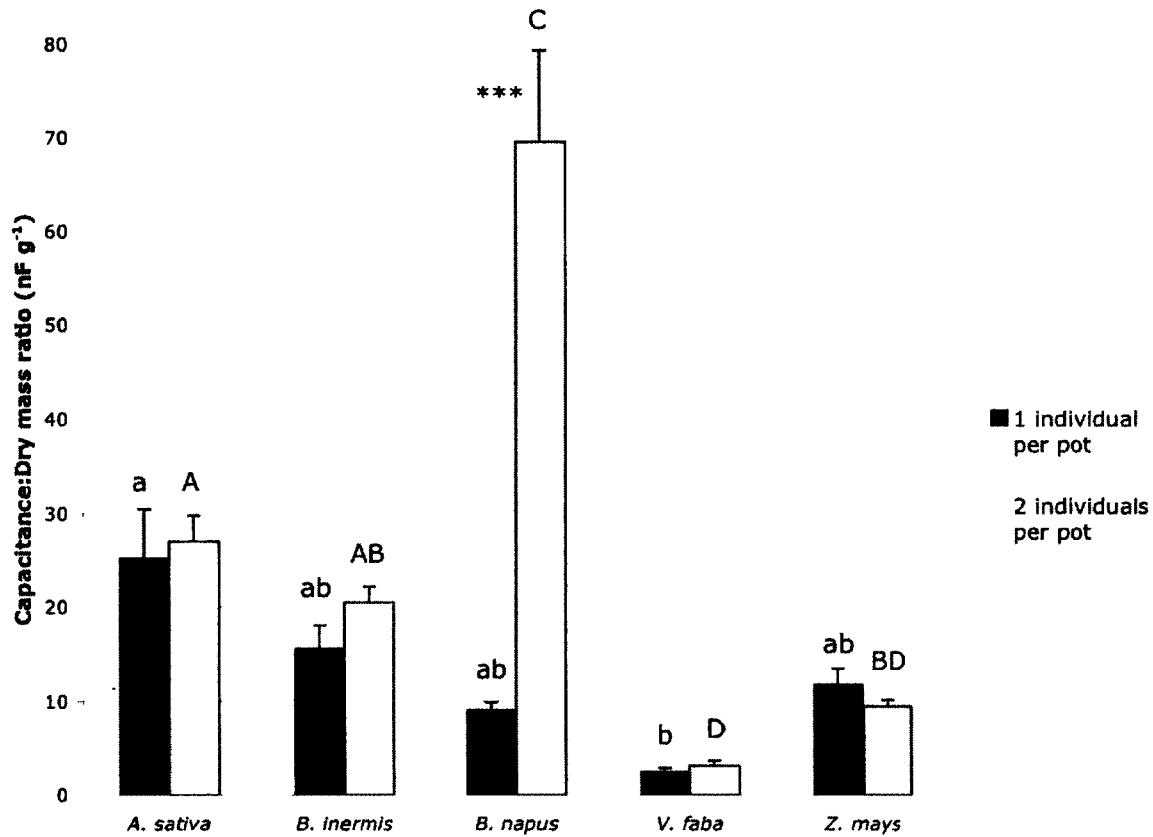
Figure 2. Planting density effect on Capacitance: Root dry mass ratios on five herbaceous species. Values (+SE) correspond to one or two individual per pot, in dark and white respectively. Different letters correspond to significant differences between species within the same treatment using Tukey's honestly significant difference test ($p < 0.05$). Asterisks represent significant differences between treatments (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$).
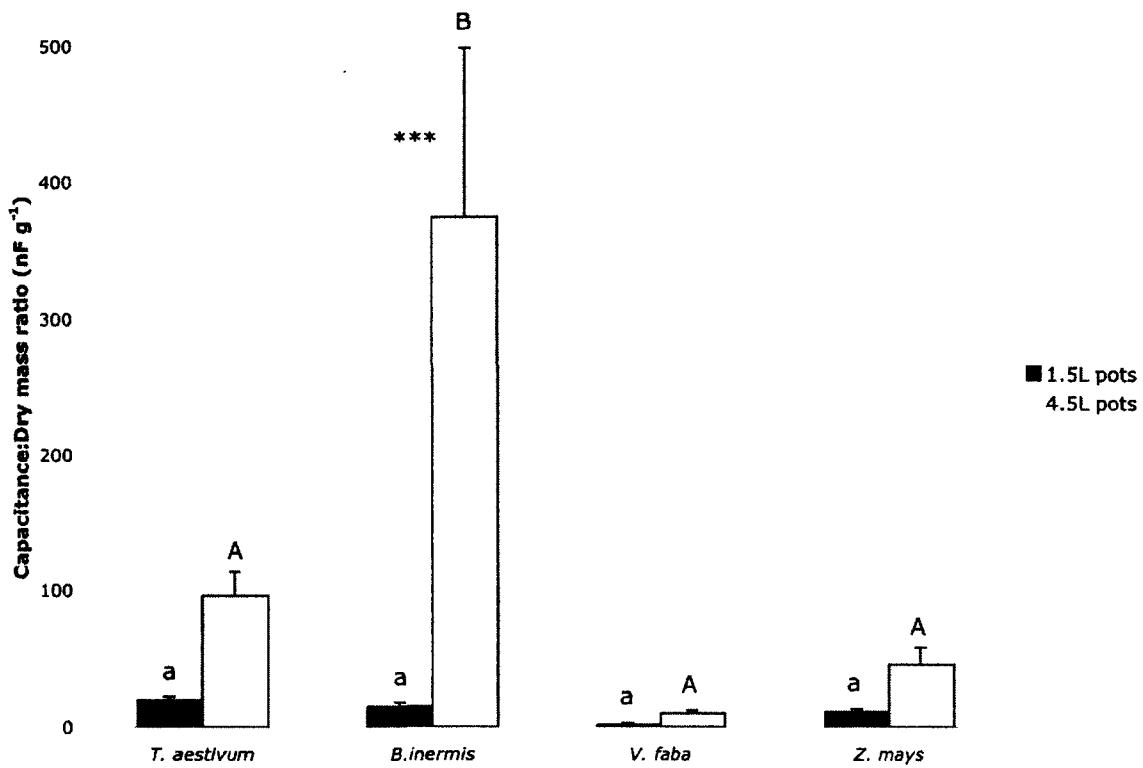
Figure 3. Pot size effect on Capacitance:Root dry mass ratios on five herbaceous species. Values (+SE) correspond to 1.5L or 4.5L pot volume, in dark and white respectively. Different letters correspond to significant differences between species within the same treatment using Tukey's honestly significant difference test ($p < 0.05$). Asterisks represent significant differences between treatments (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$).

Discussion

Although the global linear regression between root capacitance and root dry mass was found to be highly significant, this method does not appear reliable enough, without specific calibrations, to predict accurately root dry mass of herbaceous species grown in soil. Indeed, the regression was not as strong as those reported in previous hydroponics or sand-based

experiments. As first reported by (Chloupeck, 1972), based on six species of crops (but only three grown on the same sand medium), and as found recently with different maize genotypes (McBride et al., 2008) having genotype-specific predicting equations, we confirmed that the species effect on the capacitance-mass relationship is very strong. This species effect is even confirmed when root interactions are minimized, with large pot sizes and a single individual per pot. This means that, in pot experiments, the species effect can not be attributed only to increased inter- or intra-specific root contacts. Other factors may also play a significant role. As found in this study, one of the biological factors affecting the capacitance – mass relationship is the root tissue density, which may change the capacitance response of the root by affecting the current preferential pathways. Experimental factors may also modify the specific regression coefficients. Our slope estimate for maize was indeed quite different from that reported by van Beem et al. (1998) ; 0.55 nF g^{-1} vs. 6.7 nF g^{-1} (fresh weight) respectively. This large difference is likely caused by two differences. First, the pin was inserted at only 1 cm above the soil in our experiment whereas it was inserted at a height of 6 cm from the soil in that of van Beem et al. (1998). As mentioned by Ozier-Lafontaine & Bajazet (2005), the position of the electrode in the stem has a large impact on the results obtained. Second, van Beem et al. (1998) grew their plants in vermiculite and fertilized with a slow-release fertilizer (Osmocote, 17-6-10 N-P-K). Although other experimental conditions were quite similar to ours, ionic properties of the medium were certainly very different in their fertilized inorganic matrix. Chloupeck (1972) obtained a slope of 0.59 nF g^{-1}, using the root dry mass and the electrical capacitance of maize, whereas we obtained a result of 5.4 nF g^{-1} using similar parameters to theirs but the substrate used, which was quartz sand in Chloupeck (1972).

Surprisingly, increased planting density of canola ended up with higher capacitances. Because the root system of Canola is typically organized with a strong tap root connected to a very fine and dense root network, these fine roots from two individuals within a single pot may be in close interaction in the soil. Thus, the electric connections with the other root system may become a strong source of bias.

We also found that increased root density of the same individual in a decreased soil volume affected the root capacitance response. Intra-individual root density effects also existed. As expected, root agglomeration consistently decreased the capacitance for a given root mass, by decreasing the root-soil contact, but this significance was mostly due to Brome Grass. Since Brome Grass, like Canola, had particularly fine roots, this may explain this high effect of pot size for these species.

Another possible biological characteristic of the root system affecting the capacitance response might be the propensity of a particular species to concentrate fine roots in small areas of the soil volume, such as root clusters in fertile soil microsites (Shen et al., 2003), which would increase root intra-individual interactions and decrease proper root-soil electrical contact. As mentioned above, the measurement frequency applied to the root system affects the proportion between the two current pathways, i.e. apoplastic and symplastic. The optimal frequency, corresponding to the greater tendency of the root system to behave as a capacitor but also to the minimized soil response (Rajkai et al., 2005), was found to vary slightly depending on the age of tomato plants (Ozier-Lafontaine & Bajazet, 2005). Species specific calibrations of these optimal frequencies would probably be profitable to improve our capacitance-mass relationships, though it requires a more specialised impedance analyser. Another potential improvement of our models would be to take into account the cell ionic characteristics, e.g. by measuring root tissue osmolarity, which probably greatly influences the current pathways and behaviours in plant tissues.

In conclusion, we found that the relationship between electrical capacitance and root dry mass was highly significant, but not accurate enough to fulfill the current needs for dynamic and non-destructive root mass estimates. Root interactions had significant effects on the specific capacitance response, and fine-root species seemed to be more affected. Although we found that root dry matter content is one biological factor that influences these capacitance-mass relationships, biological explanations of these capacitor behaviours of plant

tissues need further investigation. We might then take into account other targeted root traits as covariables and gain accuracy in our models to be able to use this rapid method in the field.

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PRÉDICTION DES TAUX DE DÉCOMPOSITION RACINAIRES IN SITU PAR UNE APPROCHE COMPARATIVE À PARTIR DE TRAITS CHIMIQUES ET MORPHOLOGIQUES

Avant propos

Dans le premier chapitre, dans le but d'estimer les processus racinaires à l'échelle individuelle, il a été tenté d'estimer la biomasse racinaire totale d'individus d'espèces herbacées à différents stades de développement. Dans ce deuxième chapitre, une approche comparative d'estimation des taux de décomposition par les traits racinaires sera utilisée sur une large gamme fonctionnelle d'espèces, incluant des espèces de cultures, de fourrages et des espèces d'arbres. Ce deuxième chapitre aura comme objectif d'évaluer le potentiel de l'approche fonctionnelle pour la prédiction des taux de décomposition racinaires in situ.

Une première version de cet article intitulé "Prediction of in situ root decomposition rates in an interspecific context from chemical and morphological traits" a été soumise à *New Phytologist*. Les dispositifs experimentaux et les données utilisés pour réaliser ces analyses ont été élaborés par moi-même. Il en est de même pour toutes les analyses statistiques utilisées dans ce chapitre. Etant donné que l'ensemble du manuscrit a été écrit par moi-même puis été révisé par mes directeurs de thèse, les professeur Bill Shipley, et Robert Bradley, je suis le premier auteur du manuscrit qui en découle.

Prediction of in situ root decomposition rates in an interspecific context from chemical and morphological traits

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51

Abstract

Aims and scope. We quantitatively relate *in situ* root decomposition rates of a wide range of trees and herbs used in temperate agroforestry to root chemical and morphological traits in order to better describe carbon fluxes from roots to the soil carbon pool without reference to taxonomic identity.

Methods. In situ root decomposition rates were measured over an entire year by twin intact coring methods on 10 tree and seven herb species typical of temperate agroforestry systems and quantified using decay constants (k-values) from Olson's single exponential model. Decay constants were related to root chemical (total carbon, nitrogen, soluble carbon, cellulose, hemicellulose, lignin) and morphological (specific root length, specific root length) traits. Traits were measured for both absorbing and non-absorbing roots.

Key results. From 61% to 77% of the variation in the different root traits and 63 % of that in root decomposition rates was interspecific. N was positively correlated, but total carbon and lignin were negatively correlated with k-values. Initial root traits accounted for 75% of variation in interspecific decomposition rates using partial least squares regressions; partial slopes were consistent with functional expectations.

Conclusion. Easily measured initial root traits can be used to predict rates of root decomposition in soils in an interspecific context.

Introduction

The global carbon cycle has been intensely studied for a long time because of its importance in climate regulation and ecosystem functioning. A key part of this cycle is the link between the carbon pools in the vegetation and in the soil. This link can be further divided into aboveground and belowground components of the vegetation pool. Unfortunately, the decomposition of aboveground litter and its subsequent incorporation into the soil carbon pool is much better studied than the decomposition of belowground plant tissues, despite the fact that root biomass represents a substantial, often dominant, proportion of plant biomass. In fact, about 98 % of decomposition experiments are related to leaves, and the remaining 2% to roots (Zhang *et al.*, 2008). Roots therefore play an important role in the carbon cycle but one that is hidden, relatively poorly understood, and that presents substantial methodological challenges that put into question much of the existing research concerning root decomposition rates in natural soils. In order to incorporate knowledge of root decomposition into more general models of carbon cycling we need to be able to estimate in situ rates of mass loss during root decomposition in natural soils and across many different species and environments. As a first step towards this general goal we therefore use a method of measuring in situ root decomposition that better reflects natural conditions, we use initial root chemical and morphological traits to predict specific decomposition rates across a diverse group of plant species, and we allow the specific decomposition rates to vary over time as a function of changes in the trait values during the decomposition process.

The methodological challenge

Studies of root decomposition are not only scarce in comparison to those using leaf litter, but they are often poorly adapted to field conditions. Among root decomposition studies, about 90% use buried litterbags of detached roots (Silver & Miya, 2001), the other popular method

being mini-rhizotrons, but both suffer from some important limitations. Furthermore, estimates of fine root decay rates differ greatly depending on the technique used; litterbag studies report that 50% to more than 80% of the initial fine root biomass remains after one year (Fahey *et al.*, 1988; McClaugherty *et al.*, 1984; Vanvuuren *et al.*, 1993; Whitford *et al.*, 1988), whereas mini-rhizotron studies document fine root disappearance within a few weeks (Dubach & Russelle, 1995; Hendrick & Pregitzer, 1992; Pregitzer *et al.*, 1993). The slow decomposition rates observed with litterbags may be due to the process of detaching roots from their mycorrhizosphere (Fahey & Hughes, 1994) and then washing and drying them before placing them in litterbags; it is also likely that very fine roots (below 0,5 mm Ø) are underrepresented in the sampled roots.

Besides technical challenges, mini-rhizotrons have drawbacks with respect to estimating rates of root decomposition. First, it is often difficult to know from visual inspection when root death occurs. Second, assuming that one can know when the root dies, one can only measure rates of root disappearance, not the rate at which biomass is decomposing. On the other hand, an advantage of this method is that the root remains relatively undisturbed in the soil. Unlike mini-rhizotrons, buried litterbags do allow one to measure the rate of mass loss of roots during decomposition. Although litterbags are useful to study leaf litter decomposition they are not well designed to assess root decomposition for at least three reasons. First, unlike dead leaves, naturally decomposing roots are constantly and intimately interacting with their surrounding rhizosphere at a very fine spatial scale. The act of removing the root from the soil, washing and drying it, and then placing it inside a litterbag largely destroys this root-rhizosphere interaction. While living, most roots are associated with mycorrhizal fungi and other symbionts. Not only do these symbionts strongly influence resource acquisition (Clark & Zeto, 2000; Marschner & Dell, 1994) of living roots but they also influence the carbon budgets of both living and dying roots (Rygiewicz & Andersen, 1994; Wright *et al.*, 1998), including decomposition rates of dead roots (Langley *et al.*, 2006) and protection against soil toxicity (Van Tichelen *et al.*, 2001). Second, unlike dead leaves, dead roots from the same plant vary greatly in morphology, especially with respect to diameter, which is another

potential source of bias in root decomposition studies (Pritchard & Strand, 2008) unless the roots placed in the litterbag have the same diameter distribution as the natural root system. Most studies define fine roots as an absolute diameter class (e.g. below 2mm in diameter). However, if we focus on a particular function of roots (e.g. water absorption, sap conduction, physical and biological protection), diameter classes can be extremely variable between species for this particular function. Morpho-anatomical studies are very informative for such comparisons (Valenzuela-Estrada et al., 2008). In this work, we chose the absorbing function to functionally compare our tree species, and will test whether it improves the predictions of our models. Finally, we know that roots behave differently from leaves in terms of phenology. Unlike leaves, the process of root death is not abrupt. A dying root will undergo a progressive death of its cells and tissues (Comas et al., 2000) while remaining attached to still-living roots, and some root functions of the dying root can persist while others are already lost (Dubrovsky et al., 1998; Lo Gullo et al., 1998; Spaeth & Cortes, 1995). The act of excising a root and placing it in the litterbag changes this natural phenology of root death and decomposition.

For all these methodological reasons, comparative studies testing similarities between leaf and root decomposition (Hobbie et al., 2010; Wang et al., 2010) are controversial and we do not really know to what extent one can extrapolate from the rich literature on leaf litter decomposition to the processes of root decomposition. In this study we instead use a twin-core method adapted from Dornbush et al. (2002) that maintains the decomposing roots in their natural rhizosphere environment while allowing us to follow and quantify the process of mass loss during decomposition. We use this method with 17 species of crops, forage herbs and trees, including fast growing poplar hybrids and highly conservative conifers.

The Olson exponential decomposition model

Classical decomposition kinetics (expressed in total mass or carbon mass remaining) describe an exponential loss of mass through time, $M(t) = M(0)\,e^{-kt}$, in which $M(0)$ is the initial mass and k is a constant measuring the proportional mass loss per unit time (Olson, 1963). This model has at least two drawbacks in the context of root decomposition. First, the k value is unique to each species and environmental condition. Plant communities in both natural and agro-systems encompass many species and their composition varies between locations. Given the difficulty of studying roots it is impossible to estimate root decomposition rates for every species and environment. We therefore use a comparative and functional approach, described below, in order to generalise beyond taxonomic barriers. Second, some studies involving leaf (Castro *et al.*, 2010) or root litter (Fogel & Hunt, 1979; McClaugherty *et al.*, 1984) have reported that k values vary during decomposition; to account for this, authors either use a two-stage model or calculate sequential k values corresponding to each sampling date. In order to address this possibility we will express the k values as a function of root chemical and morphological traits affecting decomposition. In this way, interspecific differences in k values will be predicted by these traits, and k values can also change over time according to temporal changes in the proportion of different carbon fractions during decomposition. Finally, since temperature is an environmental factor regulating root decomposition, at least for initial stages (Berg *et al.*, 1998; Cusack *et al.*, 2009), we express time (t in the exponential model) in degree-days.

A comparative approach

Because our general goal is to incorporate knowledge of root decomposition into more general models of carbon cycling, it is necessary to link the k values of the Olson model to easily measured traits of roots before decomposition begins. Comparative statistical analyses across a broad range of species consistently show rates of leaf litter decomposition to

increase with increasing litter nitrogen concentration and decrease with increasing concentrations of cellulose and lignin as well as with increasing dry matter contents (Cornelissen & Thompson, 1997; Kazakou *et al.*, 2009; Melillo *et al.*, 1982). Such chemical and morphological characteristics related to individual performance are called functional traits, more precisely defined in Lavorel & Garnier (2002). As with leaves, root traits such as lignin contents, C:N ratios and calcium concentrations seem to influence root decomposition rates (Jalota *et al.*, 2006; Silver & Miya, 2001). Similarly, as found for leaf litter (Minderman, 1968), Larsson & Steen (1988) noticed that decomposition rates of soluble carbohydrates, cellulose and lignin in roots decrease in that order. It is, however, important to interpret these correlational patterns with care. As reported in Prescott (2005), the effect of N on litter decomposition rate is controversial.

In this study we quantify initial root traits and follow the mass loss dynamics of roots from initially intact roots of ten tree and seven herbaceous species typically used in temperate agroforestry systems over 401 days of in situ and root-adapted decomposition. We ask three specific questions:

1) How variable are the initial functional root traits, and the *in situ* measured root decomposition rates, among species?

2) What proportion of the variation in root decomposition rates is due to different proportions of carbon fractions among species versus interspecific differences in the decomposition rates of theses different carbon fractions?

3) How well can we predict interspecific variation in root decomposition rates as a function of (i) the initial proportions of the different carbon fractions, (ii) initial traits of entire root networks, and (iii) initial traits of absorbing roots, which allows for a functional comparison among species.

57

Material and methods

The measurement of root decomposition rates consisted of three steps: (i) obtaining soil cores containing newly dead roots of a single species at a time, (ii) placement of these cores in situ during decomposition while preventing invasion by new roots, and (iii) sampling and extraction of decomposing roots. Each of these steps was performed on 10 species of trees and seven herbaceous species (Tables 1 and 2).

Table 1. List of the ten sampled tree species at the Saint-Nicolas site.

Scientific name	Family	Abreviation	Year of plantation	Density (trees ha^{-1})
Betula alleghaniensis Britton	Betulaceae	Ba	1992	700
Fraxinus americana L.	Oleaceae	Fa	2003	1200
Juglans cinerea L.	Juglandaceae	Jc	1992	700
Juglans nigra L.	Juglandaceae	Jn	1992	700
Larix x marschlinsii Coaz (*L. decidua x L. kaempferi*)	Pinaceae	Lx	1997	1200
Picea abies L.	Pinaceae	Pa	1997	1800
Pinus strobus L.	Pinaceae	Ps	1995	1200
Populus x canadensis Moench (*P. deltoides x P. nigra*)	Salicaceae	Px	1998	700
Quercus macrocarpa Michaux	Fagaceae	Qm	1992	700
Quercus rubra L.	Fagaceae	Qr	1992	700

Table 2. List of the seven herbaceous species grown in an open field on the Sherbrooke University campus.

Scientific name	Family	Abreviation
Agropyron cristatum L.	Poaceae	Ac
Bromus inermis Leyss.	Poaceae	Bi
Festuca rubra L.	Poaceae	Fr
Lolium multiflorum Lam.	Poaceae	Lm
Poa pratensis L.	Poaceae	Po
Phleum pratense L.	Poaceae	Ph
Trifolium pratense L.	Fabaceae	Tp

The soil cores containing the tree roots came from a study site situated in Saint-Nicolas (Quebec, Canada – 46°41'21'' N, 71°27'55'' W). This site is organized as a mosaic of 13 small monospecific tree plantations of approximately 2000 m² on marine deposits within a 6 ha area. Drainage, which is performed by a network of pipes and underground agricultural drains, and soil properties, are similar among the plantations.

We randomly chose five trees within each of the 10 monospecific stands (Table 1). We established a 50cm X 50cm sampling square at each of the four cardinal directions and 1 m from the trunk of each tree in early June 2008. Each square was trenched to a depth of 30cm and a geotextile was placed in the trench in order to both start the senescence of living roots within the square and to prevent new roots from growing into it. This was performed to maximize the proportion of newly senescent roots at harvest. At the end of September 2008 we took twinned soil cores (15cm long, 5cm diameter and taken at 5cm below soil surface) from the centre of each square. One of the twinned soil cores was returned to the laboratory in order to get an estimate of the initial amount of roots in the other twin. The second twinned core was placed in a polyethylene terephtalate glycol (PETG) tube whose ends were covered by a 50µm mesh plastic cloth. The mesh allowed water, gases and the microflora and fauna in the surrounding soil and atmosphere to interact naturally with the soil core while preventing roots from growing into the cores. In total we used 4 pairs of cores per tree, 5 trees per species and 10 species, i.e. 200 cores in situ and their 200 twinned cores.

In order to determine the likely level of error in our estimates of initial root mass per core, we also performed a preliminary sampling to test how mass and length variability were spread among our different hierarchical levels (i.e. among species, among individual trees of the same species, among paired cores of the same individual, and between twin cores of the same pair. We performed linear mixed models (maximum likelihood estimation) using either root mass or root length as fixed effect, and the intercept as random effect, in order to estimate the variance components at each hierarchical level of our experimental design.

The tubes containing the soil cores for in situ measurements were brought to the University of Sherbrooke campus (Quebec, Canada – 45°22'48'' N, 71°55'34'' W, 260m asl) with mean annual temperatures of 4.1°C and precipitations of 1144 mm. Each tube containing the soil core was placed vertically into the ground in a common garden that had previously been a lawn while respecting a randomized block design. There were 20 blocks of 10 cores (one from each species). Five blocks were harvested at each of days 228, 282, 341, 401 (i.e. 724, 1490, 2548, 3050 degree-days) respectively.

Soil cores containing roots of each of the seven herbaceous species were obtained as follows. Each species was grown from seed as a monoculture in plastic containers (40 x 30 x 35cm high). Seeds were sawn on June 19, 2008. Soil was a commercial homogenized top soil. We planted 5 replicate containers per species and the containers were placed in a full randomized block design in an open field on the University campus. Sufficient seeds were sown to produce a continuous canopy by mid-August. As with the tree species, four twinned cores were harvested in early September 2008 from each container. The root mass in one of the randomly chosen twins was immediately extracted, while the other twin was placed in the same PETG tube as described above. All the in situ tubes were then placed into holes in a same common garden site on the University of Sherbrooke campus, in a randomized block design: 20 blocks of 7 cores (one from each species). Five blocks were harvested at each of day 228, 282, 341, 401 (i.e. 724, 1490, 2548, 3050 degree-days) respectively.

To assess the level of variability in initial root mass for the herbaceous species we used the values of the initial twin. This allowed us to determine how mass and length variability was spread among our different hierarchical levels (i.e. among species, among individuals of the same species, among cores of the same container). Because of the time-demanding work of a preliminary study, and because samples within a container were about the same distance between them as between twin cores, we assumed that the variability between these two last hierarchical levels would be the same. We performed similar linear mixed models as described for trees.

Upon removal from the common garden the sampled cores were stored at 3°C in a cold room for up to 30 days. Roots from a core were then gently hand washed and separated from soil particles in a 0.85 mm sieve. Washed roots were spread in a water tray and digitized for morphological measurements with WinRHIZO software (Regent Instruments Inc., Québec, Canada). Because the cores containing the tree roots came from monocultures with a geotextile on the ground to prevent growth of herbs there were few herbaceous roots found and these were easily detected and removed. Total root length inside each sampled core was measured to calculate specific root length values (root length per tissue dry mass). Roots were then dried at 50°C until constant dry weight is reached, and then weighed. Samples were ground to a 1mm particle size in a ball mill, and then used for the measurement of biochemical traits.

Root biochemical traits measurements

The following traits were measured on each soil core: total carbon and nitrogen (g g^{-1}), neutral detergent extractables (simple sugars, amino-acids, peptides, water-soluble phenolics), acid detergent extractables (here considered mostly hemicelluloses), acid-hydrolyzable carbohydrates (here considered mostly cellulose) and acid-unhydrolyzable residues (mostly lignin, and less soluble condensed tannins). These were measured using a Fourier Transformed Near InfraRed (FT-NIRS) spectrophotometer (Antaris II, FT-NIR Analyzer, Thermo Fisher Scientific Inc., MA, USA) after obtaining calibration curves of each chemical trait from proximate analyses. FT-NIRS calibrations were performed because of the time efficiency of the subsequent FT-NIRS measurments, and second, for the ability to measure smaller samples than with proximate analyses.

Proximate analyses for the calibration curves of the FT-NIRS were performed on 96 randomly chosen root samples from intact roots (neighbour cores). Carbon and nitrogen concentrations were analyzed with an Elementar Vario Macro Analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). We had sufficient dry mass to directly measure

C and N for all but about 10% of samples, so we constructed separate calibration curves for each harvest date. However, this was not possible for fibre analyses since larger sample masses were needed, and so we constructed a single calibration curve by sampling equally across both species and harvest dates. The various fibre fractions were determined according to the van Soest extraction protocol (Van Soest, 1963) using a fibre analyser (Fibersac 24; Ankom, Macedon, NJ, USA). Four complementary fractions are sequentially separated with this method: (i) neutral detergent extractables (later called "water solubles"). They include simple sugars, amino-acids, peptides, water-soluble phenolics, but also some cell wall components, such as ß-glucans and pectins, mucilage and some storage polysacchalids (Van Soest *et al.*, 1991). The remaining fraction called Neutral Detergent Fibre (NDF) is separated into the three following fractions. (ii) acid detergent extractables (later called "hemicellulose"), (iii) acid hydrolysable carbohydrates (later called "cellulose") and (iv) acid-unhydrolyzable residues (later called "lignin").

Near InfraRed Spectrophotometry calibrations

The Near Infrared Radiation (NIR) spectrum (12000-3800 cm^{-1}, equivalent to 833-2630 nm) over each 0.6 nm, averaged over 32 individual scans, was acquired for each sample using the Omnic software (Thermo Fisher Scientific Inc., Waltham, USA). Calibration curves for each biochemical trait were constructed using the TQ-Analyst software (Thermo Fisher Scientific Inc., Waltham, USA) using the samples which had also been analysed using proximate analyses. Partial least squares (PLS) regression was used for these multivariate calibrations. The PLS method is a multivariate linear calibration technique that reduces large sets of raw data into coordinates within a n-dimensional space constituted of a small number of orthogonal (non-correlated) factors so that they minimize the error sum of squares among the values to be predicted. Cross validation was used to estimate the optimal number of factors used in the calibration. Two thirds of the samples in each calibration set were randomly chosen to construct the model, while the remaining third was used for validation. No outliers

were detected using Mahalanobis distances. The number of factors to be used for the model was chosen using the Root Mean Square Error (below) calculated over the validation set; $RMSE = \sqrt{\frac{\sum(predicted - measured)^2}{n}}$, with n the number of sample spectra in the validation set. The number of terms used should be the highest, but the validation RMSE should not be higher than that of the calibration, to avoid over-fitting.

Determination of root functional diameter classes

As explained earlier, we chose to functionally compare our species, instead of only comparing them with absolute diameter classes. Here we chose the absorbing function for interspecific comparisons, as it is the main function of fine roots.

Fresh fine roots of each tree species were obtained from additional soil cores taken one meter from the trunk of three randomly chosen trees per species. In the lab, 10 root fragments 10 mm in length, and from 0.1mm to 3mm in diameter with a 0.3mm increment, were collected from each soil core, quickly dipped in Formalin-Aceto-Alcohol (FAA) 4% fixative solution, embedded in Agar and sliced to a 100μm thickness with a McIlwain microtome (Stoelting Co., IL, USA). This gave a total of 900 slides: 3 transversal cuts of each diameter, 3 sampled trees per species, 10 diameter classes per species, and 10 species. The root on each slide was digitized with a camera-equipped binocular microscope (Olympus Co., Japan). Functional classes were defined depending on the absorbing function, absorbing roots being defined as still having a living cortical layer. The remaining fresh roots from each of these additional soil cores were analysed for the same chemical and morphological traits as described above.

Statistical methods

To build well-fitting and parsimonious models linking root decomposition rates and the different carbon fractions decomposition rates, we used backward multiple linear regressions. Given the problem of multicollinearity between the initial root traits that are used as

independent variables to predict root decomposition rates, we first performed principle component analyses, followed by partial least squares regressions (PLSR), allowing more quantitative predictions. PLSR is particularly well-suited to avoid over-fitting and unstable slope estimates in such situations (Höskuldsson, 1988).

Results

General Observations

The advantage of using intact soil cores is that root decomposition occurs under more natural conditions. The disadvantage is that it is not possible to get a direct estimate of initial root mass and so one must rely on an indirect estimate via the twin core. To estimate the error involved in such indirect estimates we calculated the variance components in initial root mass and length at each hierarchical level of the experimental design (Table 3). We found that 16.6% (mass) and 11.7% (length) of the total variation of initial values for the tree cores existed between twinned cores and this level of error was therefore incorporated into the estimation of decomposition kinetics. The twinned cores for the herbaceous species provided more precise estimates of initial mass since only 2.5% of the total variance existed between twinned cores but 23% of the initial variance in root length of the herbaceous roots existed at this level.

Table 3. Variability of root mass and length in the intact soil cores, distributed among the different hierarchical levels of the experimental design.

	Variable	hierarchical level	standard deviation	standard error due to intial root mass estimates	% variability
Trees	Mass	interspecific	86.3	17.3	17.3
		intraspecific	116.3	23.3	23.3
		individual	213.9	42.8	42.8
		between twins	83.2	16.6	16.6
	Length	interspecific	456.0	91.2	39.3
		intraspecific	292.8	58.6	25.2
		individual	275.5	55.1	23.7
		between twins	135.8	27.2	11.7
Herbaceous	Mass	interspecific	33.9	6.8	54.0
		between containers	27.3	5.5	43.5
		within a container	1.6	0.3	2.5
	Length	interspecific	678.5	135.7	61.6
		between containers	169.5	33.9	15.4
		within a container	252.9	50.6	23.0

Table 4 presents the mean values and coefficients of variation of the initial root traits of both trees and herbs. These initial mean values differed significantly between trees and herbs for all traits except for cellulose and lignin. Tree roots had less nitrogen per mass and more carbon per mass than did the herbs. The types of carbon also differed between tree and herb roots. Tree roots tended to have higher lignin per mass than those of herbs, but lower hemicellulose and carbon in the water soluble fraction. Carbon per root mass was the least variable among species for both trees and herbs, but the interspecific variation of this trait relative to the intraspecific variation was the largest of all chemical traits (i.e. 77.4 % of total variation). The percentages of interspecific, interindividual and individual variation of chemical traits for all species range respectively from 61.4 to 77.4%, 14.1 to 25.4%, 6.8 to 13.2%. Sixty three percent of total variation in root mass loss rate was interspecific.

Table 4. Specific mean initial morphological and chemical traits of total roots and their corresponding decomposition rates.

Species	SRL (m g^{-1})	N (mg g^{-1})	C (mg g^{-1})	Water solubles (mg g^{-1})	Hemicellulose (mg g^{-1})	Cellulose (mg g^{-1})	Lignin (mg g^{-1})	k global (10^{-4} mg g^{-1})
Betula alleghaniensis	22 7	9 6	463 1	280 2	164 2	227 1	328 4	2 24
Fraxinus americana	18 6	13 0	436 4	351 7	229 6	232 8	185 9	5 25
Juglans cinerea	27 7	16 1	447 7	475 4	186 5	182 0	156 1	4 06
Juglans nigra	23 5	13 9	444 6	464 0	210.2	210 5	115 3	4 8
Larix x marschlinsii	15 5	10 3	466 9	270 8	143 6	263 4	322 3	3 58
Picea abies	12 7	8 1	464 5	247.2	178 3	269 1	305 4	2 51
Pinus strobus	4 4	11 5	436 7	282 7	153 4	242 5	321 4	3 17
Populus x canadensis	110 2	6 3	455 2	238 1	191 8	322 7	247 4	1 82
Quercus macrocarpa	59 1	8 1	453 2	380 3	182 9	232 7	204 1	1 54
Quercus rubra	28 8	7 1	459 8	291 4	214 0	289 0	205 6	2 59
Tree species average ± SD	32 3 ± 30 9	10 4 ± 3 2	452 8 ± 11 2	328 ± 86	185 ± 27	247 ± 40	239 ± 77	3 15 ± 1 2
Trees CV	0 96	0 31	0 02	0 26	0 15	0 16	0 32	0 40
Agropyron cristatum	88 6	19 3	380 6	330 3	295 1	224 4	143 9	5 27
Bromus inermis	107 6	19 5	378 2	366 8	266 5	209 0	152 8	4 23
Festuca rubra	102 5	18 6	380 9	323 1	252 9	210 8	186 6	2 98
Lolium multiflorum	99 4	20 2	381 6	314 0	319 3	227 8	112 5	8 59
Poa pratensis	122 2	20 0	370 1	356.3	282 7	208 9	142 8	0 91
Phleum pratense	91 5	18 4	372 9	349 9	253 6	210 7	170 9	7 59
Trifolium pratense	100 9	23 6	365 2	414 8	225 3	190 9	186 2	5 92
Herb species average ± SD	101 8 ± 11 1	19 9 ± 1 7	375 7 ± 6 4	351 ± 34	271 ± 31	212 ± 12	157 ± 27	5 07 ± 2 6
Herb species CV	0 11	0 09	0 02	0 10	0 11	0 06	0 17	0 52
All species average ± SD	60 9 ± 42 8	14 3 ± 5 5	421 0 ± 40 2	337 ± 69	221 ± 52	233 ± 36	205 ± 73	3 94 ± 2 1
All species CV	0 70	0 38	0 10	0 20	0 23	0 15	0 36	4 17
Trees vs herbs comparison	$F_{1,51}=5$ 64 p=0 021	$F_{1,51}=6$ 78 p=0 012	$\Gamma_{1,51}=131$ 5 p<0 0001	$F_{1,51}=24$ 7 p<0 0001	$F_{1,51}=14$ 9 p=3e^{-4}	$F_{1,51}=1$ 58 p=0 21	$F_{1,51}=3$ 31 p=0 075	$F_{1,45}=13$ 01 p=3e-4

Table 5. Mean initial morphological and chemical traits of absorbing tree roots.

Species	SRL (m g^{-1})	N (mg g^{-1})	C (mg g^{-1})	Water solubles (mg g^{-1})	Hemicellulose (mg g^{-1})	Cellulose (mg g^{-1})	Lignin (mg g^{-1})
Betula alleghaniensis	36 1	20 1	416 2	350 8	180 5	137 9	288 9
Fraxinus americana	21 9	24 0	399 4	365 4	238 9	173 0	204 5
Juglans cinerea	34 0	22 1	399 2	494 3	198 1	125 5	168 5
Juglans nigra	23 6	20 7	394 0	512 5	184 1	137 4	175 0
Larix x marschlinsii	27 7	16 8	417 0	305 6	176 5	159 7	318 9
Picea abies	24 0	16 4	413 6	308 9	176 4	159 3	293 2
Pinus strobus	13 6	17 8	406 5	319 7	200 5	157 8	252 9
Populus x canadensis	59 1	17 6	414 3	316 5	159 1	189 3	287 3
Quercus macrocarpa	34 9	15 0	421 3	395 5	136 2	133 0	297 8
Quercus rubra	31 8	12 9	428 7	400 0	109 2	123 4	334 2
Tree species average ± SD	30 7 ± 12 2	18 3 ± 3 4	411 0 ± 11 0	377 ± 75	176 ± 36	150 ± 22	262 ± 59
Tree species CV	0 43	0 19	0 03	0 21	0 22	0 15	0 24
Absorbing vs. all root comparison	$F_{1,31}=10$ 1 p=0 003	$F_{1,31}=370$ 1 p=0 0001	$F_{1,31}=326$ 9 p<0 0001	$F_{1,31}=83$ 2 p<0 0001	$F_{1,31}=3$ 4 p=0 076	$F_{1,31}=204$ 0 p<0 0001	$F_{1,31}=4$ 7 p=0 037

66

The most variable initial root trait among the ten tree species is specific root length (Table 4). Root traits of trees appear more variable than those of herbaceous species. The specific root length of the tree roots was also much more variable among species but this was primarily due to *Populus canadensis*. Among tree species, root traits of absorbing roots were all significantly different from total roots but hemicellulose content (Table 5).

Prediction of root decomposition rates

The decomposition rate of total roots differed by a factor of five among the tree species (Table 4), with *Fraxinus americana* decomposing the most quickly and *Quercus macrocarpa* most slowly. Among herb species, these interspecific differences reached a factor of almost ten. Surprisingly, the roots of *Populus canadensis* decomposed almost as slowly as *Q. macrocarpa*. Rates of decomposition of single carbon fractions also differed among species in every case. We could not estimate the decomposition rates of the carbon types for the herbaceous species because the remaining root masses were not sufficient to perform either proximate analyses, or FT-NIRS sample predictions.

Given this interspecific variation in decomposition rates and in initial types of carbon in the tree roots, we first determined the degree to which the interspecific variation in the overall decomposition rates (k_{mass}) of root mass could be predicted from the decomposition rates and initial proportions of each carbon type (k_{type}). The simplest hypothesis is that k_{mass} is an additive function of the k value of each carbon type weighted by the initial proportion of that carbon type in the root. This model is formulated as follows where $[X]_0$ is the % of the initial mass represented by carbon type X :

$$k_i(mass) \sim [\text{water solubles}]_{i0}\, k_i(\text{water solubles}) + [\text{hemicellulose}]_{i0}\, k_i(\text{hemicellulose})$$
$$+ [\text{cellulose}]_{i0}\, k_i(\text{cellulose}) + [\text{lignin}]_{i0}\, k_i(\text{lignin})$$

The predicted and observed k_{mass} values were closely correlated ($R^2 = 0.97$; RSE = $2.26e^{-5}$; p = $2.1e^{-7}$). The slope and intercepts between observed and predicted values was 1.05 and -

$1.7e^{-5}$ (in degree-day^{-1}) respectively and these values were not significantly different from 1 ($t_8 = 0.74$, p= 0.48) and 0 (t_8= 0.79, p= 0.45), respectively. Thus, the decomposition rate of total root mass is a simple linear function of the decomposition rates of each carbon component.

Because we could not measure the actual initial root mass per core and had to rely on the value estimated from its twin, we next attempted to predict the actual loss of root dry mass during decomposition using this initial estimate and a simple exponential decay function: $M(dd)_i = M(0)_i e^{-k_i dd}$ where M(dd)$_i$ is the remaining root mass of species i at a given degree-day (dd), M(0)$_i$ is the average initial root dry mass in the soil core of species i and k$_i$ is the observed specific k value. The observed and predicted dry masses were highly correlated (fig. 1, $R^2 = 0.95$, RSE = 0.39), with a slight overestimation of the highest values. The probability that the slope of the linear regression is different from 1 is p = 0.13 (t = 1.529, DF = 65) and the probability that the intercept is different from 0 is p = 0.73 (t = 0.35, DF = 65). Thus, interspecific root mass loss over time could be accurately predicted from the initial root masses and the species-specific root decomposition rates. Furthermore, the species-specific decomposition rates could be accurately predicted from the masses of the initial carbon fractions and the species-specific decomposition rates of these fractions.

Initial root traits and decomposition rates.

Figures 2a-d show the results of PCAs involving the initial root traits and the global decomposition rates based on (i) all tree and herb roots (fig. 2a), (ii) all tree roots (fig. 2b), (iii) all herb roots (fig. 2c) and (iv) absorbing tree roots (fig. 2d). In these four PCAs the first two principle components accounted for about 80% of the total variance.
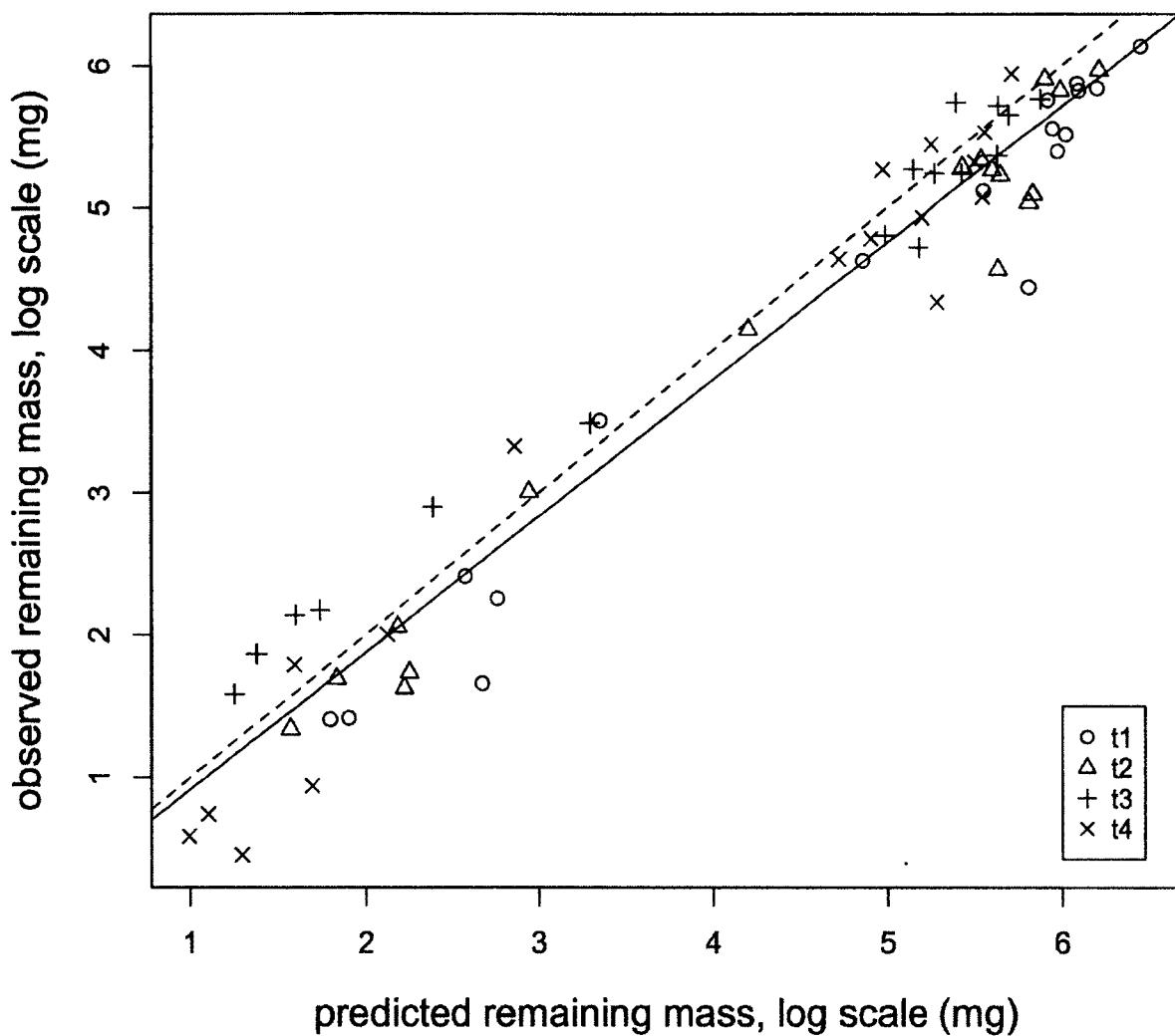
Figure 1. Relationship between the predicted specific root remaining mass and the observed specific root remaining mass of trees and herbs (log-log scale). Each species is represented by four points corresponding to the four sampling dates. The four symbols correspond to the four sampling dates (t1, 724; t2, 1490; t3, 2548 and t4, 3050 degree-days). The bottom left cluster is formed of the seven herbaceous species, the upper right one contains the ten tree species.
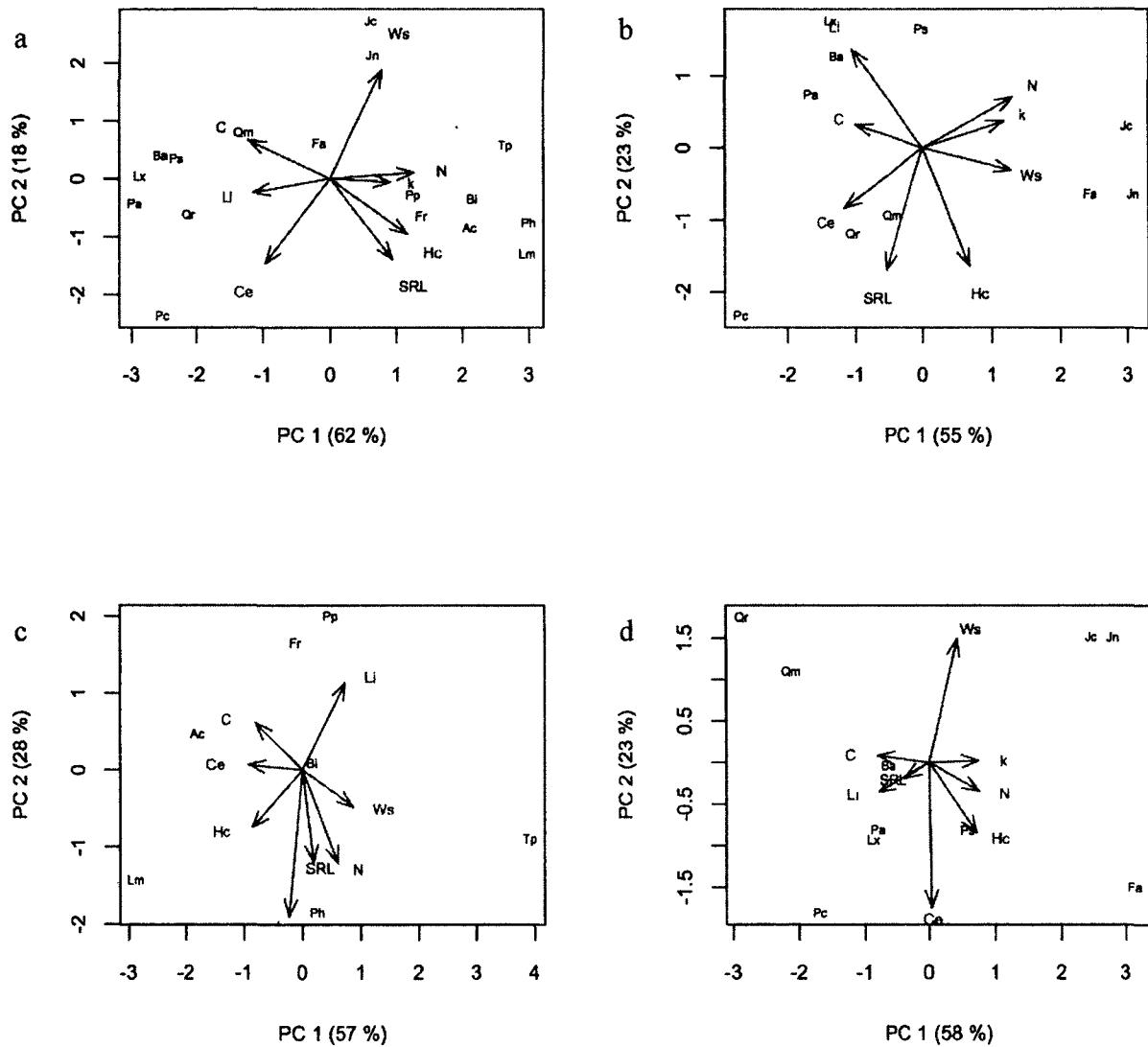
Figure 2. Principal component analyses of specific decomposition rates and initial root traits of (a) trees and herbs roots; (b) tree roots from all diameter classes; (c) herbaceous roots; and (d) absorbing tree roots. C carbon; N azote; Ws water solubles; Hc hemicellulose; Ce cellulose; Li lignin; SRL specific root length. Species abbreviations, see Tables 1 and 2.

Root decomposition rates (k values in degree days) were positively correlated with N content, and negatively with C and lignin content in the first two axes of all PCA plots. Hemicellulose and water soluble fractions were either independent of, or slightly positively correlated with, k values. Cellulose content ranged from not correlated (in herbaceous species and in absorbing tree roots) to negatively (in tree and herb roots together) and highly negatively (in total tree roots) correlated with k values. Specific root length was positively correlated with k values in the PCA of herbaceous species but negatively correlated in the PCA of tree species, regardless of the diameter class (all roots or absorbing roots).

To get a more quantitative measure of the relative importance of the initial morphological and chemical root traits in determining root mass decomposition rates we performed a series of simple regressions of k as a function of each trait. As proposed by Burnham & Anderson (2002) we used the change in the Akaike information criterion (AIC) to compare models; the $\Delta_{AIC} = AIC_i - AIC_{min}$ was calculated as an index of support of each regression model with the corresponding trait. Although initial root traits that are best correlated with root decomposition rates depend on the category of roots considered (Table 6), nitrogen, hemicellulose, total carbon (except for herbs) and lignin contents appear as good predictors regardless of the root category. Let us consider a gradient of root coarseness as follows : herbs<absorbing tree roots<total tree roots. With respect to this gradient, water soluble fraction and cellulose appear better correlated to k values for coarser than finer roots. The opposite trend is observed for hemicellulose and lignin.

The multiple regressions (PLS and ML) used to predict root decomposition rates given initial root traits are summarized in Table 7. These regressions had good predictive ability. R^2, AIC and residual standard errors gave the impression that ML models prevail over PLS for the same two datasets (total tree roots). However, the biological interpretation of the signs of the

71

partial regression coefficients associated with the initial root traits was more consistent with expectations using the PLS regression.

Table 6. Pearson product-moment correlation coefficients table between root decomposition rate (in degree-days) and the initial root traits. Independent variables were classified into three categories, depending on the level of support of the model (in bold, underligned and standard body in decreasing order of significance), based on their respective Δ_{AIC} values.

Independant variables	Tree roots – all roots			Tree roots – absorbing roots			Herbaceous roots – all roots			Tree and herbaceous roots – all roots		
	r	AIC	Δ_{AIC}	r	AIC	Δ_{AIC}	r	AIC	Δ_{AIC}	r	AIC	Δ_{AIC}
SRL	-0.53	-149.8	7.9	-0.56	-149.8	7.0	0.43	-92.0	2.8	0.30	-236.3	7.1
Nitrogen	0.82	-157.7	0	0.73	-154.2	2.6	0.49	-92.4	2.4	0.63	-243.4	0.0
Carbon	-0.55	-150.2	7.5	-0.80	-156.8	0.0	-0.03	-90.5	4.3	-0.50	-239.6	3.8
Water solubles	0.54	-149.9	7.8	0.43	-148.6	8.2	-0.03	-90.6	4.2	0.29	-236.2	7.2
Hemicellulose	0.38	-148.1	9.6	0.73	-154.1	2.7	0.57	-93.3	1.5	0.61	-242.7	0.7
Cellulose	-0.52	-149.8	7.9	0.04	-146.6	10.2	0.22	-90.9	3.9	-0.39	-237.5	5.9
Lignin	-0.46	-148.9	8.8	-0.75	-154.8	2.0	-0.68	-94.8	0.0	-0.54	-240.5	2.9

Table 7. Coefficients and characteristics of Multiple Linear Regressions (MLR) and Partial Least Squares Regressions (PLSR) using initial tree root traits from either all roots, or absorbing roots to predict the specific root decomposition rates.

Model	Root category	k_0	$[N]_0$	$[C]_0$	SRL	[water solubles]$_0$	[hemicellulose]$_0$	[cellulose]$_0$	[lignin]$_0$	R^2	RSE	p value	AIC
MLR	All roots	-9.12e-4	4.65e-5		-1.41e-6		1.44e-5	2.13e-5		0.93	4.50e-5	0.0048	-166.7
MLR	Absorbing roots	-6.97e-4			-6.82e-6	1.48e-5		4.44e-5		0.79	6.98e-5	0.0190	-158.1
PLSR	All roots	-2.79e-4	2.91e-5					1.59e-5		0.75	7.02e-5	0.0020	-160.3
PLSR	Absorbing roots	1.50e-3		-3.14e-6		2.48e-6	8.75e-6		-5.42e-6	0.66	7.63e-5	0.0041	-157.5

Discussion

The main motivation of our study was to assess the potential of a comparative approach to predict in situ plant root decomposition rates. If successful, this would be a quicker, more efficient and generalizable way of assessing root turnover and would be an important tool to better appraise carbon fluxes from plant roots to the soil organic carbon pool during the decomposition process. We specifically wanted to assess this potential in the context of sylviculture and crop production. However, this will require one to quantify carbon fluxes from roots to the soil, especially that from root decomposition process. Since many species and life forms can be cultivated in such agrosystems, it is necessary to generalize beyond taxonomic barriers. The comparative approach we used is based on morphological and chemical root traits, as the use of aboveground traits has been shown to give consistent predictions of leaf litter decomposition rates (Cornelissen & Thompson, 1997; Kazakou *et al.*, 2009; Melillo *et al.*, 1982).

Since the choice of herbaceous species was based on their use in North American agrosystems, our herbaceous species probably underrepresent the natural range of root traits found in this group of plants. Indeed, variability in N content and specific root length in our species was lower than in the grassland species described in Roumet *et al.* (2006). As expected, the root nitrogen contents followed a pattern of herbaceous species>broadleaf trees>conifers. Within tree species, nitrogen content in absorbing roots was significantly higher than in all roots.

As found by Silver & Miya (2001), lignin concentrations were lower in herbs than in trees. Contrary to Silver & Miya (2001) however, who found the following lignin content pattern broadleaf trees>conifers>graminoids for roots from all size classes, we found that lignin was always higher in conifer than in broadleaf trees, for every diameter class. The difference in lignin content between broadleaf and conifer trees seems to increase with root diameter and

is significant for the finest size classes (i.e. absorbing roots), which had a maximum mean diameter of 0.6 mm. However, since our study only considers three conifer species, we do not know to what degree this difference with Silver & Miya (2001) is real. Lignin control of decomposition rates is not totally unanimous (Alexander, 1977; Taylor *et al.*, 1991). It has been reported that lignin concentrations would not be high enough to significantly inhibit decomposition (Taylor *et al.*, 1991), or that correlations are not significant at a global scale (Silver & Miya, 2001). In this study, the correlations between lignin content and root decomposition rates follow the pattern of absorbing tree roots>herbaceous species>total tree roots. It seems that the coarser the diameter classes, the weaker the correlation. It would probably require long term studies to detect a strong effect of lignin on larger diameter classes of tree roots (Berg, 1984).

Our results support the fact that interspecific variation in global root decomposition rates are predictable given the decomposition rates of each carbon fraction, weighted by their initial proportions. Unfortunately, although this model fits well, we also detected interspecific variation in the decomposition rates of the different carbon fractions. This means that interspecific variation in the total root mass decomposition rates are not only due to differences in the initial proportions of these carbon fractions between species but also to species-specific differences in the decomposition rates of the different carbon fractions themselves, which limits the generality of the result. What might cause such interspecific differences in decomposition rates of a single carbon type? One possibility is that the fibre analysis results in our carbon types are too heterogeneous; for instance, different types of lignin, which proportions vary among species, could have different decomposition rates. Another possibility is that decomposition rates of a given carbon fraction might nonlinearly depend on the proportions of other fractions. Such non additive effects might arise, for instance, if the access of microorganisms to the carbon fraction they are able to mineralize can be altered by other forms of carbon (e.g. cellulose fibres included in the lignin matrix of secondary cell walls).

Although the interspecific variation in k values for a given carbon type reduces the generality of our results, they still show that root mass during decomposition can be accurately predicted given the initial dead root mass and the measured k value of the corresponding species and points to the importance of finding those functional traits of roots that allow for interspecific generalization. This last predictive model appeared helpful as a test of the intact soil core sampling method. The standard error found at the lowest hierarchical level (i.e. between twin cores) is still high enough to be a significant source of error when estimating initial root mass and yet good predictive ability was observed using only the average initial root mass estimates rather than the actual initial root masses. The residual errors in the predicted remaining mass values of fig 1 reflect the cumulated errors of initial root mass estimates and imperfect fits of mass loss kinetics of each species with the Olson exponential model. Nevertheless, these predictions remained very satisfactory ($R^2 = 0.95$, RSE = 0.39), regardless of the method setup (i.e. in the controlled experiment for herbaceous species or in the field for trees). Although our method of estimating initial root biomass first appeared as a potentially substantial drawback, this sampling method seems promising considering the many advantages.

An unexpected result probably arose from this *in situ* decomposition technique. In our PCA plots showing tree root decomposition rates and their initial traits, specific root length is weighted negatively (i.e. the finer the roots, the longer their decomposition). Although specific root length is not significantly correlated to tree root decomposition rates when taken separately, this unexpected result might be influenced by greater mycorrhizal colonization of fine roots, also meaning slower decomposition of these colonized roots (Langley *et al.*, 2006). Indeed, mycorrhizae, and especially ectomycorrhizal sheaths around their host root, are efficient in protecting the root from external physico-chemical attack. However, little is known about this phenomenon and the crucial factor is probably the survival of the fungi. Ectomycorrhizal enzymes can remain active for several weeks after the fungus is cut off from its root host (Garbaye, personal communication), and this is probably proportional to the remaining carbon reserves in the excised root network. It is perhaps for this reason that

Langley *et al.* (2006) found that mycorrhizal colonization slowed root decomposition, since they only harvested fine mycorrhizal roots that were dried before being put in litterbags. However, in the field, mycorrhizae probably live weeks or months after root death, and thus mineralize root organic carbon. As mycorrhizal colonization of roots occurs in the fine roots, it probably plays a dominant role in fine root mineralization. This may be a reason why specific root length was not correlated to tree root decomposition rates.

Our initial motivation of proposing general equations predicting total root mass decomposition rates with initial root traits was compromised by the relatively small number of species. This was mostly due to the issue of multicollinearity between initial traits, revealed in the signs of four out of seven of the partial regression coefficients associated with the independent variables of the multiple linear regression (MLR) equations, which were contrary to global plant economic expectations. When independent variables are strongly collinear, or when the number of observations is modest relative to the number of variables, partial least squares regression (PLSR) is expected to perform better than MLR (Höskuldsson, 1988) and this was the case in our study. Although MLR accounted for more of the variance ($R^2 = 0.93$ for total tree roots, and $R^2 = 0.79$ for absorbing tree roots) than PLSR ($R^2 = 0.75$ for total tree roots, and $R^2 = 0.66$ for absorbing tree roots) the coefficients of the independent variables from the PLSR analyses were consistent with typical expectations from functional trade-offs with decomposition rates decreasing with increasing amounts of lignin and cellulose and increasing with increasing specific root length and amounts of soluble carbon and nitrogen. This issue of collinearity also comes out in the meta-analysis of Silver & Miya (2001), were lignin, lignin:N and C:N ratios have been used in the same multiple regression model. Their regression model had a positive lignin:N coefficient, where it is expected to be negative. Since functional ecology is based on plant strategies defined by selected traits which are strongly correlated to each other, this partial least squares method seems promising for these fields of biology where explanatory variables are not truly independent.

In interpreting the partial regression coefficients of our final regression model it is important to note that the effect of N on litter decomposition rate is controversial (Prescott, 2005). To better understand the underlying N-controlled mechanisms, one must distinguish between two factors: (i) the intrinsic tissue N content and the extrinsic soil inorganic nutrient availability, and (ii) early opposed to later stages of decomposition. Unlike the external soil inorganic N, the intrinsic litter N is highly correlated with C chemistry and levels of polyphenols. However, artificial external N additions to the soil are more prone to stimulate the decomposition of lignin-poor litters and suppress that of lignin-rich ones (Carreiro et al., 2000) because N addition inhibits fungal lignolytic activity (Waldrop & Zak, 2006). Contrary to lignolytic activity, cellulase activity would be stimulated by increased soil N availability (Carreiro et al., 2000). Early stages of decomposition mostly driven by low-recalcitrance compounds would be stimulated, whereas it would be suppressed at later stages. In this study, we consider N root content as a specific chemical trait, used as an integrating index of root biochemistry.

There now exists large trait databases covering many thousands of plant species. Because our root traits are static measurements made on easily obtained roots and using standard chemical methods it should be possible to measure them on many species and incorporate the results in these pre-existing trait databases. If the results reported here can be replicated in a larger set of species and environmental conditions then it should be possible to use the resulting regressions in more general ecosystem models of carbon cycling. Besides determining the generality of our results, it will also be important to decompose the mass-loss kinetics reported here into the mass-loss kinetics of different carbon fractions since the temporal dynamics of carbon cycling in the soil is also profoundly affected by the recalcitrance of different carbon fractions in the root tissue.

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81

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PREDICTION INTERSPECIFIQUE DES DYNAMIQUES DE DECOMPOSITION IN SITU DES FRACTIONS DE CARBONE RACINAIRE PAR LES TRAITS RACINAIRES INITIAUX

Avant propos

Dans le chapitre précédant, le potentiel de prédiction des taux de décomposition racinaire par l'approche fonctionnelle a été testé. Les taux de décompositions de chaque fraction de C des racines, pondérés par les proportions relatives de ces fractions de C ont montré un potentiel prometteur en terme d'estimation des dynamiques de pertes de masse au cours de la décomposition. Dans ce troisième chapitre, il est donc proposé de simuler la dynamique de décomposition de ces fractions de C racinaires de 10 espèces d'arbres à partir de données de décomposition in situ, puis de proposer des estimateurs alternatifs pour les modèles de dynamique de C des résidus dérivés de plantes.

Cet article intitulé "Interspecific prediction of in situ decomposition dynamics of different root carbon fractions from initial root traits" sera prochainement soumis à *Journal of Experimental Botany*. Les dispositifs experimentaux et les données utilisées pour réaliser ces analyses ont été élaborés et obtenus par moi-même. Il en est de même pour toutes les analyses statistiques utilisées dans ce chapitre. Etant donné que l'ensemble du manuscrit a été écrit par moi-même puis a été révisé par mes directeurs de thèse, les professeurs Bill Shipley, et Robert Bradley, je suis le premier auteur du manuscrit qui en découle.

Interspecific prediction of in situ decomposition dynamics of different root carbon fractions from initial root traits

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Keywords: Soluble carbon, cellulose, hemicellulose, lignin, fibres, root carbon loss, absorbing roots, functional diameter class, root chemistry.

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Abstract

Aims and scopes. We quantitatively measured the dynamics of root carbon fractions from 10 tree species from *in situ* root decomposition and assess their preferential degradation according to their recalcitrance and propose alternatives of predictors to be used in C dynamics models of root derived residues.

Methods. Dynamics of four root carbon fractions (water-solubles, hemicellulose, cellulose, lignin) were measured by an intact twin coring method on 10 tree species typical of north-eastern North American agroforestry systems and quantified separately using decay constants (k-values) from Olson's single exponential model. Decay constants were related to initial root chemical composition but not morphological (specific root length, SRL) traits. Traits were measured separately for absorbing and non-absorbing roots.

Key results. Between 56 and 76% of the variation in the decomposition rates of the various root carbon fractions could be explained using initial root traits, and up to 79% considering only absorbing roots. Total root carbon loss rates were well predicted (R^2 = 0.94) using a combination of the specific decomposition rate of the "lignin" fraction plus the ratio of the mass of initial lignin to the initial mass of the less recalcitrant fractions. Trait-predictable lignin recalcitrance combined to initial lignin on less recalcitrant fractions ratios revealed promising predicting abilities of root C loss rates.

Conclusion. Easily measured static initial root traits can predict the decomposition dynamics of various carbon fractions and should prove useful in ecosystem models of carbon cycling in soils.

Introduction

The three main sources of soil organic carbon (C) in the soil are (i) the above and below ground plant tissues derived from the humification process after plant death, (ii) rhizodeposition (mucilage production, secretion of enzymes, root exudation and cell fall off), and (iii) root respiration. Because most of soil organic carbon is plant derived, and since agricultural soil organic content has been decreasing in the last decades, Smith *et al.* (2008) proposed that C sequestration in soils would be a good candidate to buffer human C emissions to the atmosphere. Quantifying this potential in different soils would necessarily require ecosystem models that include the process of plant tissue decomposition.

The Century model (Parton, 1996) is one such influential model that attempts to simulate and predict C dynamics across different ecosystems and which includes a soil organic matter decomposition sub-model. This sub-model defines five fractions of soil organic matter, covering the biochemical continuum from cellular fractions of higher plants and of microorganisms to humus compounds, and is based on the respective turnover times of these soil carbon fraction pools. As a practical matter, it would be very useful if the decomposition dynamics, and thus turnover times, of the carbon fractions coming from newly dead leaves and roots could be predicted based on easily-measured initial traits of these tissues.

This can be done for leaf litter. The proportional mass-loss (the decomposition rate constant k) of decomposing leaves can be predicted across species based on initial litter characteristics (Cornelissen & Thompson, 1997; Kazakou *et al.*, 2009) such as C:N (Taylor *et al.*, 1989) or lignin:N ratio (Melillo *et al.*, 1982). Unfortunately, the large literature on leaf litter decomposition cannot be confidently extrapolated to roots. Only about 2% of all decomposition studies use roots and, within this remaining 2%, approximately 90% of these use buried litterbags of detached roots (Silver & Miya, 2001). This method is not suited to studying in situ root decomposition, as explained in Aulen, Shipley & Bradley (in prep). Therefore, we do not yet know to what degree results based on leaf litter can be extrapolated to roots. However, the results of Aulen, Shipley & Bradley (in prep), based on 17 species of

trees and herbs and using an in situ method of measuring decomposition rates (mass loss) of roots in their natural undisturbed soil, show that 75% of the interspecific variation in the decomposition rate of mass loss could be predicted from two initial traits (nitrogen and hemicellulose content).

In this paper we move beyond the basic mass-loss kinetics of decaying roots to predict the dynamics of each of the four root C fractions from initial traits using the same improved twin-cores method as developed in Aulen, Shipley & Bradley (in prep). In the Century model, lignin content in plant residues is used as the best predictor of plant derived soil C dynamics. However, since decomposition rates vary across the different carbon fractions based on their recalcitrance, soil microorganisms should preferentially degrade these different C fractions in increasing order of recalcitrance. Since the proportions of these different C fractions differ across species, one would expect that lignin would start to degrade earlier in lignin-rich species if the specific decomposition rate of lignin varies little across species, since soil microorganisms would more quickly consume the less recalcitrant compounds. If so, then we can expect that the decomposition dynamics of different C fractions will depend on the initial proportions of the other C fractions in the newly dead roots; this hypothesis has not yet been tested in a broad comparative context. On the other hand, if the specific decomposition rates of a given C fraction vary greatly across species, then the initial proportion of the different C fractions would be less important. Little is known about the range of interspecific variation is such specific decomposition rates in roots although both Rutigliano *et al.* (1996) and Tian *et al.* (2000) reported that conifer leaf litter lignin was more recalcitrant than the lignin of a single broadleaf species. Even less information exists about the relative importance of interspecific variation in the proportions of different C fractions versus the interspecific variation in the specific decomposition rates of a given C fraction, in determining the subsequent dynamics of these C fractions during decomposition. We therefore test this hypothesis in a more comparative context using 10 tree species with very different ecologies, and propose new initial root trait combinations as predictors to be used in C dynamics models of root derived residues.

Initial root traits

Comparative statistical analyses across a broad range of species consistently show rates of leaf litter decomposition (i.e. total mass loss) to increase with increasing nitrogen concentration and decrease with increasing concentrations of cellulose and lignin as well as with increasing dry matter contents (Cornelissen & Thompson, 1997; Kazakou *et al.*, 2009; Melillo *et al.*, 1982). However, the causal explanation of these statistical patterns are controversial (Prescott, 1995). As with leaves, root biochemistry seems to influence overall decomposition rates of root mass (Silver & Miya, 2001; Aulen, Shipley & Bradley in prep). Jalota *et al.* (2006) found that root decomposition rates were inversely correlated with root lignin concentrations. More precisely, and as found for leaf litter (Minderman, 1968), Larsson & Steen (1988) noticed that decomposition rates of soluble carbohydrates, cellulose and lignin in roots decrease in that order. Indeed, assuming that one can extrapolate from leaf litter, simple compounds should be responsible for short-term C mass loss and recalcitrant compounds should be responsible for long-term mineralization. Only a precise follow up of root biochemistry during decomposition would allow us to estimate the contribution of each carbon fraction to total carbon mass during decomposition.

A first step in following the changing proportions of carbon fractions during decomposition was taken by Berg *et al.* (1982), who followed Scots pine chemistry during the decomposition process and found that the ratio of lignin to less recalcitrant solid residues reached a plateau after about 2 years of decomposition, suggesting that the "lignin" fraction regulates the rate of decomposition of the whole litter in the late stages. The changes in the regulating role of lignin and cellulose during the decomposition stages has been studied in different ecosystems (Berg *et al.*, 1982; Coûteaux *et al.*, 1998; Mcclaugherty & Berg, 1987), but remains controversial. Indeed, Rutigliano *et al.* (1996) found that lignin loss rates was positively correlated to total litter mass loss rate even during the early stages of decomposition. But as lignin may prevent access to different proportions of less recalcitrant

fractions depending on the species studied (Cooke & Whipps, 1993), interspecific variation of lignin control on litter decomposition certainly occurs. To assess and predict such interspecific and temporal variations of the C fractions control on litter decay rates with initial root characteristics, a multi-species comparison involving a simultaneous study of root traits and mass loss of different carbon fractions during sequential stages of decomposition was needed; we report such a study here. Since temperature is the main environmental factor regulating the speed of decomposition of root litter during the year, at least for initial stages (Berg *et al.*, 1998; Cusack *et al.*, 2009), we express the root decomposition dynamics using degree-days.

Assuming that roots behave similarly to leaves during the in situ decomposition process, we used traits homologous to those commonly used for leaves. In this study we therefore measured six biochemical traits and one morphological trait of roots from ten tree species with strongly contrasting ecology, and typical of sylviculture in northeastern North America. We then followed these traits over 401 days of in situ decomposition beginning with initially intact roots. We asked four specific questions:

1) Can we predict the specific decomposition rate of each C fraction using initial root traits?

2) Does the specific decomposition rate (recalcitrance) of a given C fraction vary across species and, if so, how variable are these interspecific values?

3) Does the speed at which each C fraction loses mass depend on the relative abundance of the less recalcitrant C fractions in the root?

4) Can we improve our predictions of the specific decomposition rate of total root C by including both interspecific variation in specific decomposition rates (recalcitrance) of the "lignin" fraction and its initial relative abundance?

Material and methods

We used 10 species of trees (Table 1), each growing in a study site situated in Saint-Nicolas (Quebec, Canada – 46°41'21" N, 71°27'55" W). This site is organized as a mosaic of 13 small monospecific tree plantations of approximately 2000 m^2 on marine deposits within a 6 ha area. The drainage, which is performed by a network of pipes and underground agricultural drains, and the soil properties are similar among the plantations. Details of the site, the experiment and measurements are given in Aulen, Shipley & Bradley (in prep) and are only briefly outlined here.

Table 1. Summary of the ten tree species and the Saint-Nicolas site used in the experiment.

Scientific name	Family	Abreviation	Year of plantation	Density (trees ha^{-1})
Betula alleghaniensis Britton	Betulaceae	Ba	1992	700
Fraxinus americana L.	Oleaceae	Fa	2003	1200
Juglans cinerea L.	Juglandaceae	Jc	1992	700
Juglans nigra L.	Juglandaceae	Jn	1992	700
Larix x marschlinsii Coaz (*L. decidua x L. kaempferi*)	Pinaceae	Lx	1997	1200
Picea abies L.	Pinaceae	Pa	1997	1800
Pinus strobus L.	Pinaceae	Ps	1995	1200
Populus x canadensis Moench (*P. deltoides x P. nigra*)	Salicaceae	Px	1998	700
Quercus macrocarpa Michaux	Fagaceae	Qm	1992	700
Quercus rubra L.	Fagaceae	Qr	1992	700

The measurement of root mass loss during decomposition used a twin-core method consisting of four steps: (i) obtaining twinned soil cores containing newly dead roots of a single species at a time, (ii) roots were immediately removed from one of the twin cores to provide initial estimates of root biomass and root traits (iii) placement of the other twin core in a common garden in such a way as to allow the movement of water, gases and some micro-organisms between the cores and the surrounding soil while preventing invasion by

new roots; there were 20 blocks in total of 10 cores (one block of 10 cores representing the 10 species, and five replicates times four harvests), and (iv) harvesting of the cores and extraction of decomposing roots at each of days 228, 282, 341, 401 (i.e. 724, 1490, 2548, 3050 degree-days) respectively.

Upon removal from the common garden the sampled cores were stored at 3°C for up to 30 days. Roots from each core were then gently hand washed and separated from soil particles in a 0.85mm sieve. Washed roots were spread in a water tray and digitized for morphological measurements with WinRHIZO software (Regent Instruments Inc., Quebec, Canada). Herbaceous roots were easily detected and removed. Total root length inside each sampled core was collected to calculate specific root length (SRL) values; i.e. root length per tissue dry mass. Roots were dried at 50°C until constant dry weight was reached, and then weighed. Samples were ground to a 1mm particle size in a ball mill and then used for the measurement of biochemical traits.

Root biochemical trait measurements

The following biochemical traits were measured on the extracted and ground roots from each soil core: total carbon, total nitrogen (g g^{-1}), and four fractions from fibre analysis. Carbon and nitrogen concentrations were analyzed with an Elementar Vario Macro Analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). The various fibre fractions were determined according to the van Soest extraction protocol (Van Soest, 1963) using a fibre analyser (Fibersac 24; Ankom, Macedon, NJ, USA). Four complementary fractions are sequentially separated with this method: (i) neutral detergent solubles (NDS). They include simple sugars, amino-acids, peptides, water-soluble phenolics, but also some cell wall components, such as ß-glucans and pectins, mucilage and some storage polysaccharids (Van Soest et al., 1991); for simplicity we will call this the "water soluble" fraction. The remaining fraction, called neutral detergent fibre, is separated into the three following fractions: (ii) acid detergent fibres (ADF, mostly hemicelluloses, we will call this the "hemicellulose" fraction),

(iii) acid hydrolysable carbohydrates (AHC, mostly cellulose, which we will call the "cellulose" fraction) and (iv) acid-unhydrolyzable residues (AUR, mostly lignin, which we will call the "lignin" fraction). This chemical description of the various fractions is only approximate (Van Soest *et al.*, 1991) and our use of quotes is meant to remind the reader of this.

Near InfraRed Spectrophotometry calibrations

The Near Infrared Radiation (NIR) spectrum (12000-3800 cm^{-1}, equivalent to 833-2630 nm) over each 0.6 nm, averaged over 32 individual scans, was acquired for each sample using the Omnic software (Thermo Fisher Scientific Inc., Waltham, USA). Further details are given in Aulen, Shipley & Bradley (in prep). FT-NIRS calibrations were performed because of the time efficiency of the subsequent FT-NIRS measurments, and second, for the ability to measure smaller samples than with proximate analyses.

Determination of root functional diameter classes

Functional diameter classes corresponding to absorbing roots were measured for each species. Absorbing roots were defined as still having a living cortex. This method is more detailed in Aulen, Shipley & Bradley (in prep).

Statistical analyses

We used backward multiple linear regressions to obtain well-fitting and parsimonious models predicting mass loss of different carbon compounds as a function of the independent variables. Given the issue of multicollinearity among the initial root traits that are used as independent variables to predict root decomposition rates, we first performed multiple linear regressions using the lm function of R (R.app GUI, R Fundation for Statistial Computing),

followed by partial least squares regressions (PLSR), which is less sensitive to multicollinearity in the set of independent variables. PLSR is particularly well-suited to avoid over-fitting and unstable slope estimates in such situations (Höskuldsson, 1988). PLSR regressions were performed using the plsRglm function of R. We used AIC values to compare the competing models (Burnham & Anderson, 2001).

Results

We first present the relationships between the dynamics of each carbon fraction and initial root traits. Then we report the specific recalcitrance of each carbon fraction. Last, we assess the relative importance of initial "lignin" proportion and specific recalcitrance of the "lignin" fraction, which will lead to alternative predictors to be used in C dynamics models of root derived residues.

Prediction of the decomposition rates of the carbon fractions from initial root traits

Table 2 summarizes our attempt to predict decomposition rates of each root carbon fraction from initial root traits, using both multiple linear regression (MLR) and partial least squares regression (PLSR). Most initial chemical traits participated significantly in our prediction models with the exception of specific root length,which did not prove very informative. With indices such as the R^2, the residual standard errors, and AIC values, MLR appears slightly more powerful than PLSR to predict the mass loss kinetics of the different carbon compounds; this was especially true with respect to the absorbing roots. However, looking more precisely at the coefficients of the multiple regressions assigned to initial traits, nine out of 19 of these coefficients are inconsistent with broadly accepted functional hypotheses. In contrast, PLSR models were developed using cross-validation against values not included in

the model, and the coefficients are in agreement with our expectations: biomass loss of the carbon fractions are positively correlated to initial root N content, the initial "water soluble" fraction and the "hemicellulose" fraction, and negatively to the initial "cellulose" and "lignin" fractions. Only the "lignin" fraction degradation is positively correlated to root initial "lignin" content.

Specific decomposition rates of C fractions.

Decomposition rates of single carbon fractions differed between species in every case (Table 3); the degree of recalcitrance of every carbon fraction, not only the "lignin" fraction, are species dependent. The decay rates of the "cellulose" and "hemicellulose" fractions were very similar and about twice as high as that of the "lignin" fraction. The k value of "water soluble" fraction was low, but is not easily interpretable, as microbial products progressively replace root soluble compounds. Fig. 1 shows the percentage of remaining mass of the "lignin" fraction during the five stages of decomposition. Compared to the deciduous species, the rate of mass loss of this fraction seemed more rapid in the conifers although the difference was not significantly different ($F_{1,8} = 3.10$; $p = 0.12$). Interestingly, the mass loss kinetics also revealed differences between the two types: decomposition of the conifer "lignin" fraction began more quickly and was a one step exponential process, whereas deciduous "lignin" fraction showed a slower two-step pattern of mass loss. Both types initially lost some of the "lignin" fraction but the conifers lost about 60% within 1550 degree-days, after which this fraction appeared to stabilize. On the other hand the deciduous species lost only about 30% after 1550 degree-days, there was then a period of stability, and then a second period of loss of "lignin" after about 2500 degree-days.

Table 2. Results of the multiple linear (MLR) and partial least squares (PLSR) regression models used for predicting the different carbon fractions decomposition rates from initial root traits. Results are presented using traits measured on all roots or absorbing roots. Values are the partial slopes corresponding to each initial trait. Empty cells correspond to non-significant terms, removed from the model. The p-value refers to the F-ratio associated with the regression. Also shown are the coefficient of determination between observed and predicted values (R^2), the residual mean squared error (RME) and Akaike's information criterion (AIC). NDS : Neutral detergent solubles, "water soluble" fraction. ADF : Acid detergent fibres, "hemicellulose" fraction. AHC : Acid-hydrolysable carbohydrates, "cellulose" fraction. AUR : Acid unhydrolyzable residues, "lignin" fraction.

Compound	Type of roots	Method	k_a	$[N]_a$	$[C]_a$	SRL	$[NDS]_a$	$[ADF]_a$	$[AHC]_a$	$[AUR]_a$	R^2	RSE	p value	AIC
NDS	all roots	MLR	-2.11e-4	4.21e-5							0.80	7.16e-5	0.0005	-158.7
ADF	all roots	MLR	-4.21e-4	1.66e-5				3.59e-5			0.86	5.45e-5	0.0009	-160.6
AHC	all roots	MLR	-6.68e-5			-2.04e-6		2.98e-5			0.67	7.41e-5	0.0208	-156.9
AUR	all roots	MLR	1.62e-4	6.53e-5			-1.87e-5				0.81	7.22e-5	0.0030	-158.8
NDS	absorbing roots	MLR	-5.60e-3	-6.30e-5			6.23e-5	1.33e-4	5.43e-5	5.67e-5	0.99	2.07e-5	0.0003	-176.7
ADF	absorbing roots	MLR	-1.37e-2	4.45e-5	2.61e-5	-9.20e-6	4.08e-5		9.00e-5		0.94	4.91e-5	0.0164	-165.1
AHC	absorbing roots	MLR	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
AUR	absorbing roots	MLR	-2.75e-4	-3.28e-5				6.28e-5			0.87	9.09e-5	0.0009	-157.6
NDS	all roots	PLSR	1.37e-4	1.63e-5			4.98e-6		-9.89e-6		0.70	8.85e-5	0.0027	-154.5
ADF	all roots	PLSR	-1.99e-5				3.15e-6	2.38e-5		-4.73e-6	0.76	6.86e-5	0.0011	-157.6
AHC	all roots	PLSR	-1.23e-4	2.18e-5					1.72e-5	-1.90e-7	0.61	7.55e-5	0.0073	-155.7
AUR	all roots	PLSR	-3.88e-4	3.40e-5					-1.03e-6	1.19e-5	0.56	1.01e-4	0.0127	-149.9
NDS	absorbing roots	PLSR	-1.40e-4	6.94e-6			6.96e-6	1.02e-5		-7.78e-6	0.72	8.50e-5	0.0019	-153.3
ADF	absorbing roots	PLSR	-4.09e-5	4.30e-6			1.13e-5			-2.00e-6	0.58	8.97e-5	0.0100	-152.2
AHC	absorbing roots	PLSR	1.17e-4	8.39e-6			5.50e-6			-2.28e-6	0.37	9.63e-5	0.0610	-150.8
AUR	absorbing roots	PLSR	-8.57e-4	3.68e-6				4.26e-5		1.01e-5	0.79	6.87e-5	0.0005	-157.6

Table 3. Decomposition rates (k in g.g^{-1}.degree-day^{-1}) of total root mass, and of four carbon fractions for the 10 tree species under study. NDS : Neutral detergent solubles, "water soluble" fraction. ADF : Acid detergent fibres, "hemicellulose" fraction. AHC : Acid-hydrolysable carbohydrates, "cellulose" fraction. AUR : Acid unhydrolyzable residues, "lignin" fraction. Standard deviation on mean ratio is calculated for each root trait, called coefficient of variation (CV).

| Species	k NDS (10^{-4} g g^{-1} degree-day^{-1})	k ADF (10^{-4} g g^{-1} degree-day^{-1})	k AHC (10^{-4} g g^{-1} degree-day^{-1})	k AUR (10^{-4} g g^{-1} degree-day^{-1})
Betula alleghaniensis	0.87	3.85	3.57	1.73
Fraxinus americana	4.39	5.95	5.96	4.59
Juglans cinerea	3.79	4.95	4.38	2.49
Juglans nigra	4.45	6.03	5.95	2.29
Larix x marschlinsii	2.75	3.28	4.24	3.74
Picea abies	1.20	2.65	3.26	2.56
Pinus strobus	2.20	2.64	3.78	3.42
Populus x canadensis	0.39	3.20	3.16	0.97
Quercus macrocarpa	1.38	3.41	2.60	0.12
Quercus rubra	1.09	5.28	4.94	0.56
Tree species average ± SD	2.25 ± 1.5	4.12 ± 1.3	4.18 ± 1.1	2.25 ± 1.4
Tree species CV	0.67	0.32	0.27	0.64
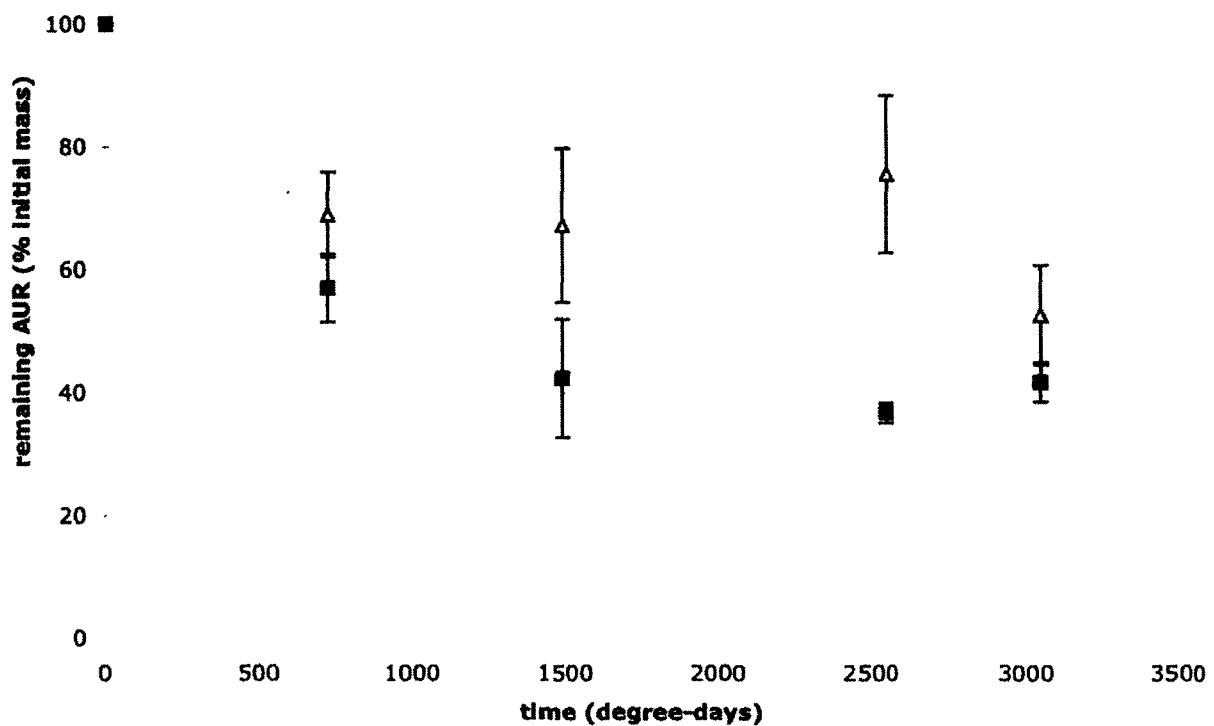
Figure 1. Root mass loss (decomposition) of acid unhydrolyzable residues (AUR, "lignin" fraction) throughout the in situ decomposition process. Values are average remaining AUR (± SE, in % of initial AUR mass) from seven deciduous species (open triangles) and three conifers (closed squares).

Alternative predictors for the fate of root derived residues in C dynamics models.

Fig. 2 shows the example of the contribution of the "lignin" fraction to C mass loss during the process of root decomposition; this figure suggests that the lower the initial mass of the "lignin" fraction in the root the higher the C mass loss, and vice versa. Initial "lignin" content appears as a good candidate and will be tested here as a predictor of C mass loss.

The dynamics of the root carbon fractions throughout the decomposition process, shown separately for the two types of tree (deciduous or conifer), are shown in Figure 3. In this figure we plot the ratio of the dry mass of "cellulose" and "lignin" fraction to the dry mass of all of the less recalcitrant ones (Figures 3a,b) or after excluding the "water soluble" fraction, since this fraction will be increasingly influenced by microbial products during decomposition (Figures 3c,d). As expected, the "lignin" fraction ratios increased for the deciduous species and reached a plateau value of 0.4 (Figure 3a) and 1.0 (Figure 3c) at 1500 degree-days. Therefore, there is a preferential decomposition of less recalcitrant compounds. Contrary to this, the "lignin" fraction ratios of the conifers remain very similar during the decomposition process (about 0.4 in Figure 3a and 0.8 in Figure 3c), slowly decreasing and eventually converging to the same value as found for the deciduous species in Figure 3a. Thus, the "lignin" fraction is being decomposed from the very first stages of decomposition in the conifers but only later in the deciduous species. Similarly, the "cellulose" fraction ratios of both deciduous and conifer roots decreased to a value of 0.3 and 0.4 respectively (Figure 3b), by 1500 degree-days. This means that the "cellulose" fraction does not seem to be degraded earlier than the "hemicellulose" one. This is confirmed in Figure 3d, where the "cellulose" fraction ratios (excluding the "water soluble" fraction) for the deciduous species remain constant while those of the conifers decrease and stabilize around 1500 degree-days. There does not appear to be a preferential degradation of the "cellulose" versus the

"hemicellulose" fractions in the deciduous roots but in the conifer species the "hemicellulose" fraction appears to be more recalcitrant than "cellulose".
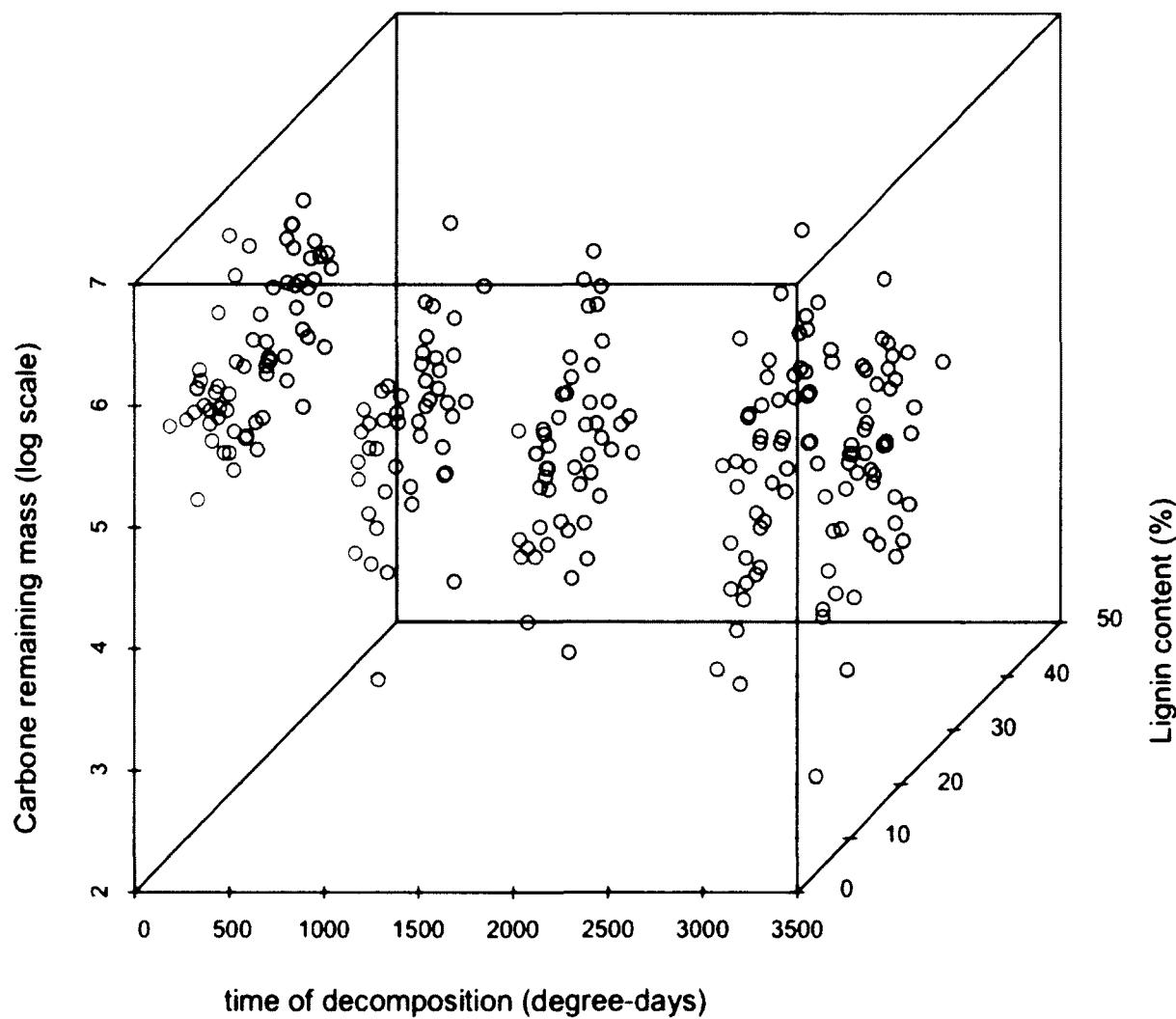
Figure 2. Root carbon mass loss rates along an AUR content gradient ("lignin" fraction). The closer to the back face, the darker the points. Each species has five replicates per sampling date; 50 points are represented for each sampling date, 250 in total.
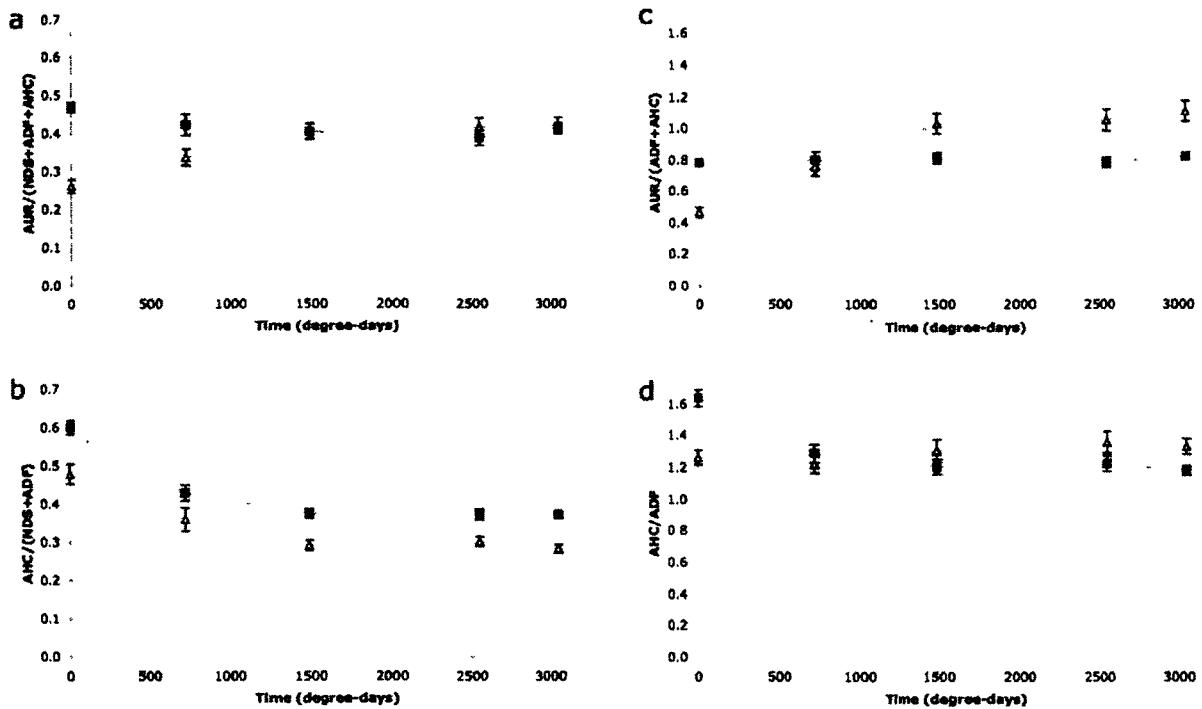
Figure 3 a-d. Root carbon fractions dynamics throughout the in situ decomposition process. Values are average mass ratios (± SE) of recalcitrant fractions on supposedly more easily degraded fractions from seven deciduous species (open triangles) and three conifers (closed squares). NDS : Neutral detergent solubles, "water soluble" fraction. ADF : Acid detergent fibres, "hemicellulose" fraction. AHC : Acid-hydrolysable carbohydrates, "cellulose" fraction. AUR : Acid unhydrolyzable residues, "lignin" fraction.

The resulting prediction equations of carbon mass loss rate are shown in Table 4 using sequentially (i) the initial "lignin" fraction only (model 1), (ii) the ratios of the "lignin" to the less recalcitrant fractions (models 2 and 3), (iii) the specific decomposition rate of the "lignin" fraction (k_{AUR}) (model 4) and (iv) the "lignin" fraction ratios plus k_{AUR} (models 5 and 6). Using only the initial "lignin" fraction or using only the initial "lignin" fraction ratios (models 1-3) gave relatively poor models. The model involving k_{AUR} only (model 4)

101

explained 63% of the C mass loss rate variability. The best models were those combining the "lignin" fraction ratio plus k_{AUR} (models 5 and 6).

Table 4. Results of the multiple linear (MLR) regression models used for predicting the total carbon mass loss during root decomposition. Values represent the partial slopes corresponding to each factor. Empty cells correspond to unused factors in the corresponding model. The p-value refers to the F-ratio associated with the regression. Also shown are the coefficient of determination between observed and predicted values (R^2), the residual standard error (RSE) and Akaike's information criterion (AIC). NDS : Neutral detergent solubles, "lignin" fraction. ADF : Acid detergent fibres, "hemicellulose" fraction. AHC : Acid-hydrolysable carbohydrates, "cellulose" fraction. AUR : Acid unhydrolyzable residues, "lignin" fraction. k (AUR) : measured decomposition rate of the AUR fraction (g g^{-1} degree-day^{-1}).

Model	k_0	[AUR]$_0$	AUR/(NDS+ADF+AHC)$_0$	AUR/(ADF+AHC)$_0$	k (AUR)	R^2	RSE	p value	AIC
1	5.45e-4	-8.01e-6				0.18	1.38e-4	0.2160	-145.6
2	4.92e-4		-4.21e-4			0.16	1.40e-4	0.2595	-145.3
3	4.95e-4			-2.48e-4		0.12	1.43e-4	0.3172	-144.9
4	1.74e-4				7.97e-1	0.63	9.27e-5	0.0060	-153.6
5	3.49e-4		-6.04e-4		9.05e-1	0.94	4.00e-5	0.0001	-169.7
6	3.64e-4			-3.74e-4	9.05e-1	0.91	5.03e-5	0.0002	-165.1

Discussion

Predicting root C mass loss rates during decomposition is a key step in modelling the soil carbon cycle and yet little effort has been provided to simulate realistic in situ root decomposition in comparison to the large literature that exists for leaf litter. Given this need to quantify root C mass loss rates in C dynamic models, and more specifically the need to better understand how each carbon fraction sequentially contributes to it, we used a comparative approach based on root traits. This approach has been shown to give consistent

predictions of litter decomposition rates for leaves (Cornelissen & Thompson, 1997; Kazakou *et al.*, 2009; Melillo *et al.*, 1982). Because it is relatively easy to measure initial root traits of many species this approach, if successful, would be a quicker, more efficient and generalizable way of assessing root derived C fluxes in C dynamic models. We first discuss the potential of this functional approach to predict the C fractions dynamics and the total root C loss through time, with the potential use of initial root traits as complementary predictors in general C model; finally, we speculate about the possibility of generating general prediction equations for both roots and leaves, and the potential problems that may arise.

Prediction of carbon fractions dynamics with initial root traits

Although leaf litter decomposition is much more intensely studied than root decomposition, relatively few of these leaf decay studies have followed the dynamics of the different leaf carbon fractions during decomposition (Berg *et al.*, 1982; Fioretto *et al.*, 2005; Rutigliano *et al.*, 1996). The authors of those leaf litter studies who followed C fractions dynamics found, as we report here for the decaying roots, that decomposition of the "lignin" fraction was slower than that of either the "cellulose" or the "water soluble" fraction. However, we are not aware of any studies, using either leaves or roots, that have attempted to predict the dynamics of these carbon fractions during the decomposition process using initial traits; yet this is an important step if one wishes to include such dynamics in soil carbon models.

Our results support the idea that initial root traits are informative and powerful predicting variables to assess the decomposition rates of the different root C fractions. Our initial morphological root trait (SRL) was not a very informative predictor in our models. This result surprised us because specific leaf area (SLA), the homologous leaf trait, proved very informative in predicting leaf decomposition rates (Cornelissen *et al.*, 1999). A few reasons may mitigate such a potential for specific root length. First, it is not clear which environmental conditions select for high specific root length (Ryser, 2006), since a resource limiting environment may select for finer and longer prospecting roots, whereas a productive

environment would also require an efficient below-ground resource acquisition, also meaning high specific root length. Other stresses may also occur and counteract selection for higher nutrient acquisition, e.g. water limitation tends to decrease with soil depth. Even mycorrhizal activity (Heinemeyer & Fitter, 2004) was found to influence specific root length. It seems likely that specific root length integrates many contrasting environmental and biotic factors, and the functional relationships between it and other root traits are more complex than that of above-ground SLA.

Interspecific variability in the decomposition rates of carbon fractions and their relative dynamics during decomposition

As found in tree leaf litter (Berg *et al.*, 1982; Preston *et al.*, 2009), the root "lignin" fraction was the most recalcitrant fraction. However, not only did the dynamics of mass loss of this fraction differ between the conifers and deciduous species as reported by Rutigliano *et al.* (1996) and Tian *et al.* (2000) for leaf lignin, but the temporal dynamics of mass loss in each of the carbon fractions were also species-specific and variable. This means that the recalcitrance of the same C fraction, as defined from fibre analysis, depends on the unique chemistry of the species. Surprisingly however, and contrary to the results Rutigliano *et al.* (1996) and Tian *et al.* (2000) reported for leaf litter, root conifer "lignin" decomposed as fast as the most rapidly decomposing "lignin" of deciduous species.

In contrast to deciduous species, the C fractions of conifers did not seem to undergo a strong preferential decay according to their recalcitrance. The faster depletion of the less recalcitrant compounds than "lignin" would not explain this faster conifer "lignin" mass loss. Roots probably differ significantly enough from leaves to reveal such differences. What is noticeably different to leaves is that these conifer roots were covered by an ectomycorrhizal sheath. This would go counter to what Langley *et al.* (2006) found, i.e. that mycorrhizal colonization resulted in a slower root decomposition. It has been suggested that mycorrhizae, and especially ectomycorrhizal sheaths around their host root, are efficient in protecting the

root from external physico-chemical aggressions (Van Tichelen *et al.*, 2001). However, little is known about this phenomenon and the crucial factor is probably the survival of the fungi. Ectomycorrhizal enzymes can remain active for several weeks after the fungus is cut off from its root host (Jean Garbaye, personal communication), and the amount of time during which the fungus remains active is probably proportional to the remaining carbon reserves in the excised root network. It is perhaps for this reason that root decomposition was slower in Langley *et al.* (2006) since they only harvested fine mycorrhizal roots that were dried before being put in litterbags. However, in the field and in our experiment, mycorrhizae probably live weeks or months after root death, and thus continue to consume root carbon compounds. It was clear from our anatomical slides that mycorrhizal colonization was much higher in conifer fine roots and, since decomposition was closer to in situ conditions, this may be a reason why conifer root "lignin" was found to decompose faster than that of deciduous species; in other words, the mycorrhizal fungus would begin to function as a saprophyte and decompose the root once it had died. Clearly, this question must be further studied but it points out the importance of simulating natural conditions, which is not the case in buried litterbag studies, when investigating root decomposition.

Although conifer root "lignin" decomposition was faster than expected, the functional approach performed very well in predicting the decomposition rate of each carbon fraction. In other words, even if biotic factors such as mycorrhization and the presence of an adapted microflora to specific root chemistry in the rhizosphere may be responsible for mitigating the results of the functional approach, this method performed efficiently. Considering PLSR models, which gave consistent results, "lignin" decomposition was better predicted with absorbing root traits, whereas "cellulose" and "hemicellulose" were better predicted with all root traits. This too might be explained by the fact that short to mid-term decomposition of recalcitrant fibres occurs mainly in fine roots, and is probably assisted by the mycorrhizae that occur in fine roots.

Predictions of root C losses and the potential use of initial root traits in general C models

Initial root traits were able to predict the mass loss of the different root C fractions during decomposition. Contrary to our original expectation, the initial amount of the "lignin" fraction was not the best predictor of the rate of total C mass loss. The best predictive model included both the ratio of the initial amount of the "lignin" fraction to the sum of the initial amounts of the less recalcitrant fractions and the specific decomposition constant (k) of the "lignin" fraction. In other words, one must know both how much of the most recalcitrant carbon type is present in the root as well as what proportion of the total amount of carbon this carbon type represents. As we also showed that the specific "lignin" fraction decomposition rate could be estimated through initial traits, this last total C mass loss prediction model ends up being very promising, while using easily measured initial root traits.

In order to further increase our ability to predict the in situ dynamics of mass loss of the different carbon fractions in the decomposing root, we would probably have to take other biotic variables into account. Two such variables are the degree of mycorrhizal inoculation, and the quality of the rhizospheric microflora, which is adapted to specific rhizospheric conditions and root chemistry (Maul & Drinkwater, 2010; Westover et al., 1997). If one can quantify the typical degree of mycorrhizal root infection of different species (perhaps if only qualitatively) then this could be another useful initial root trait. It is also likely that obvious abiotic variables like temperature (degree-days) and soil pH will be important as well as soil N concentrations. Indeed, inherent soil quality, and particularly soil N content has been found to restrain the synthesis of lignolytic enzymes (Waldrop & Zak, 2006). The two biotic factors described above are also probably linked to each other, since arbuscular mycorrhizae symbiosis was found to increase root exudation (Graham et al., 1981) and influence rhizosphere microbial communities (Marschner & Timonen, 2005).

Possibility of general predictions for both roots and leaves

Root and leaf carbon fractions dynamics seem to behave similarly, but not identically, with the "lignin" fraction being the most recalcitrant form of carbon. It might therefore be possible to produce even more general prediction equations that include both leaf and root litter. Our results for roots show that the temporal dynamics of mass loss of a more recalcitrant fraction depends also on the initial relative abundance of the less recalcitrant fractions. This might also be true for leaf litter since Rutigliano *et al.* (1996) found that conifer leaf lignin decomposed more slowly than that of a broadleaf species having leaf litter with more simple carbohydrates. Whether more labile compounds facilitate the degradation of lignin in leaf litter would require a comparative approach involving more than just a few species at a time. Another important issue, when attempting to predict root decomposition, is the possible non-additive effects of the roots of different species decomposing together. We did not investigate this question but about two thirds of experiments on leaf litter report non-additive interspecific effects (reviewed in Gartner & Cardon, 2004). Certainly, Robinson *et al.* (1999) also revealed synergic mixed-species effects involving decomposing roots. In natural, and even in agro-systems, roots from different species can be closely intermingled. The technical difficulties of studying mixed-species root systems are formidable but Roumet *et al.* (2006) showed how Near Infrared Spectrophotometry can be used to determine the relative abundance of roots of different species; it should therefore be possible to study root decomposition in multispecies communities. Besides further testing the generality of the results reported here to new species, the extension to such multispecies systems will be an important next step in obtaining empirical predictive models of root decomposition.

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CONCLUSIONS GÉNÉRALES

Cette étude a adressé trois objectifs distincts. Dans un premier chapitre, il a été souligné le besoin d'une technique de suivi dynamique et in situ des processus racinaires. En effet, contrairement aux feuilles, les racines interagissent constamment et de façon complexe avec leur mileu, le sol et leurs microorganismes associés. Une des méthodes prometteuses qui permettrait de répondre à la fois à ce défi méthodologique et à une partie de ces besoins de compréhension est la technique d'estimation non-destructive de la biomasse racinaire complète d'un individu par la capacitance électrique. Cette technique a été testée sur 10 espèces herbacées, et la corrélation entre la capacitance électrique et la biomasse racinaire complète de l'individu a été trouvée significative mais trop faible pour établir des équations prédictives générales sans calibrations spécifiques préalables. La littérature insiste sur un des facteurs abiotiques les plus importants pour la précision des estimations qui est l'humidité du sol lors des mesures pour maximiser la surface de contact entre la solution du sol et les racines. Cependant, les facteurs biotiques comme les interactions racinaires se sont révélées être d'importantes sources de variabilité dans les mesures de capacitance. Les espèces aux racines les plus fines ont été les plus affectées. Bien qu'il ait été trouvé que la densité des tissus racinaires, estimée par le contenu en matière sèche (root dry matter content), est un des facteurs biotiques affectant ces relations capacitance-masse, les explications biologiques de ces systèmes de condensateurs au sein des tissus racinaires restent à approfondir. Il serait intéressant par la suite de prendre en compte d'autres traits racinaires ciblés comme l'osmolarité des tissus, en lien avec les propriétés ioniques et donc les comportement du courant électrique au sein des tissus. Il est possible que ces traits ciblés, considérés comme covariables, permettent d'améliorer la précision de nos prédictions et éventuellement d'utiliser cette méthode sur le terrain.

Les nombreuses différences qui distinguent les feuilles des racines, notamment en terme de phénologie, d'architecture et d'interaction avec leur milieu, nécessitent que l'on distingue les racines des feuilles au niveau de l'approche méthodologique. Dans le cas contraire, les

résultats ne reflèteraient que très peu le caractère potentiellement très singulier des racines par rapport aux feuilles. C'est le risque que prend la majorité des études jusqu'à présent et qui pourrait mener à extrapoler trop vite la riche littérature portant sur les feuilles. Dans le deuxième chapitre, l'approche fonctionnelle pour estimer les variations interspécifiques des flux de C des racines vers le sol au cours de la décomposition a donc été testée par une technique de décomposition des racines intactes, la plus proche des conditions in situ. Il a été trouvé qu'entre deux tiers et trois quarts de la variabilité au sein des traits racinaires et des taux de décomposition est interspécifique. Les résultats de cette étude confirment que variabilité interspécifique des taux de décomposition est très fidèlement expliquée par les taux de décomposition des différentes fractions de C pondérés par leurs proportions initiales respectives. Cependant, les taux de décomposition d'un même type de fibre présentent également de la variabilité interspécifique, limitant la généralisation de ces derniers résultats. L'utilisation de la variabilité des traits racinaires initiaux, ainsi que leurs corrélations avec les taux de décomposition s'est avérée utile. En effet, la teneur en azote racinaire d'une part, et la lignine et le carbone total d'autre part, étaient respectivement positivement et négativement corrélés aux taux de décomposition racinaires. Finalement, la variabilité de ces traits racinaires initiaux explique environ les trois quarts de celle des taux de perte de biomasse racinaire. Si les résultats rapportés ici pouvaient être répétés pour de plus nombreuses espèces et conditions environnementales, il serait alors envisageable d'utiliser ces régressions dans des modèles écosystémiques plus généraux de flux de C.

Etant donné que la séquestration de C dans les sols est un excellent candidat à court et moyen terme pour atténuer les émissions anthropiques de C dans l'atmosphère, il paraît important de mieux appréhender la capacité de séquestration des différentes pratiques culturales dans des sols variés. Pour cela, les modèles couramment utilisés, comme le modèle Century, séparent la matière organique en différentes classes de récalcitrance, c'est à dire en terme de taux de décomposition dans le sol. Dans le troisième chapitre de cette étude, il a été proposé de

simuler la dynamique de décomposition des fractions de C racinaires de 10 espèces d'arbres à partir de données de décomposition in situ. Ces résultats montrent que jusqu'à environ trois quarts de la variation observée dans les taux de décomposition des différentes fractions de C a pu être expliquée par les traits racinaires initiaux, et jusqu'à quatre cinquième en considérant les racines absorbantes seulement. Les taux de décomposition de chacune des fractions de C se sont révélés variables interspécifiquement. La fraction correspondant à la lignine a notamment montré une décomposition plus rapide chez les espèces résineuses que chez les espèces décidues, contrairement à ce qui était attendu par comparaison avec les études antérieures basées sur les feuilles. Ces résultats pourraient être dus au fait que les racines sont soumises à des facteurs biotiques substantiels au sein de leur rhizosphère, tels que les interactions avec les mycorhizes et la présence de microorganismes adaptés à la chimie des racines. Cependant, malgré ces effets biotiques, l'approche fonctionnelle s'est révélée efficace pour prédire les taux de perte de C racinaire. En effet, la combinaison des données de dynamique des fractions de C à celles des proportions de composés récalcitrants a permis d'obtenir un modèle prédictif précis de perte de C racinaire au cours du processus de décomposition.

L'approche fonctionnelle a montré un potentiel prometteur de prédiction des taux de décomposition racinaires, mais aussi des différentes fractions de C de ces mêmes racines. Son utilisation future pourrait être encouragée d'une part pour alimenter les modèles généraux de cycle de C et d'autre part, grâce à son potentiel de généralisation, pour améliorer les connaissances sur les processus racinaires.

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