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RÉSUMÉ

L'interdiction d'enfouir les boues de fosse septique municipales à partir de 2020 contraindra les municipalités du Québec à recycler ou à valoriser leurs matières résiduelles fertilisantes. Ces boues constituent une source de nutriments assimilables et de matière organique disponible pour la restauration des sols dégradés. Cette étude vise à évaluer le potentiel d'utilisation des boues de fosse septique déshydratées pour la restauration de sols forestiers dégradés notamment ceux ayant perdu leur horizon organique de surface (couverture morte) suite à des opérations forestières et présentant des conditions sous-optimales pour la croissance végétale.

Des sols soumis à l'épandage de boues sont comparés à des sols non restaurés (témoin) et à des sols restaurés par épandage de couverture morte d'origine forestière. Nous avons évalué dans quelle mesure ces traitements pouvaient (1) permettre le rétablissement des processus biogéochimiques et physiques du sol, et (2) engendrer un lessivage de nutriments et de métaux traces. Dix-huit parcelles expérimentales ont été établies en 2013 dans une plantation de peupliers hybrides âgée de 8 ans de la Forêt d'enseignement du Lac Duparquet (FERLD). Six traitements ont été appliqués, soit une combinaison de deux types de boue de fosse septique et deux épaisseurs (15 et 25 cm), l'épandage de couverture morte d'origine forestière (8 cm) ainsi qu'un traitement témoin sans épandage. Nous avons évalué la structure chimique des amendements, la minéralisation de la matière organique (boues et couverture morte), la respiration et la biomasse microbienne, les réserves d'azote, de phosphore et de cations basiques, les propriétés physiques du sol minéral ainsi que la croissance d'épinettes blanches plantées au printemps 2014. Des résines échangeuses d'ions (PRSTM probes) ont été installées dans l'horizon de surface et dans le sol minéral pour mesurer la biodisponibilité des nutriments et des métaux traces. Nos résultats montrent que l'épandage a amélioré l'apport de certains nutriments (NO_3^- , PO_4^- , SO_4^- , Ca^{2+}) dans le sol minéral sous les boues, mais n'a pas eu l'effet escompté sur les propriétés physiques du sol et les concentrations en C et en N total dans le sol minéral. Toutefois, l'augmentation de la disponibilité des nutriments a amélioré la nutrition foliaire et la croissance des semis d'épinette blanche. Par ailleurs, la biodisponibilité de certains métaux-traces (Cu, Zn, Pb) a augmenté sous les boues. Dans la mesure où l'épandage de matières résiduelles fertilisantes en milieu forestier permet d'améliorer l'apport en nutriments de sols dégradés, il constituerait un moyen écologique de détourner une quantité importante de matière résiduelle fertilisante de l'enfouissement et de l'incinération. Cette étude pourrait avoir des retombées non seulement en foresterie, mais aussi pour d'autres milieux perturbés tels les sites miniers.

MOTS CLÉS: sol forestier, restauration du sol, boues de fosse septique, *Picea glauca* (Moench) Voss, potentiel fertilisant, processus biogéochimiques.

CHAPITRE I : INTRODUCTION GÉNÉRALE

1.1 Problématique

En raison de l'augmentation des populations et des besoins en eau potable (Rizzardini and Goi, 2009) ainsi que de législations plus sévères quant à la qualité de cette eau (Garcia-Delgado et al., 2007), le nombre d'usines de traitement des eaux et la production de matières résiduelles issues de ces usines ont augmenté de façon significative au cours des 30 dernière années (Giusti, 2009). L'interdiction de disposer de matières résiduelles dans les plans d'eau et, plus récemment, les restrictions relativement à leur enfouissement et leur incinération font en sorte qu'une plus grande quantité de biosolides municipaux doit être valorisée (Lu et al., 2012).

Au Québec, une nouvelle politique sur la gestion des matières résiduelles a été mise en place en mars 2011 dans le cadre du Plan d'action sur les changements climatiques (PACC; Villeneuve and Dessureault, 2011). L'objectif de cette politique est de réduire les émissions de gaz à effet de serre (GES) associées à l'incinération et à la décomposition des matières résiduelles et de freiner l'encombrement des sites d'enfouissement en incitant au recyclage de matières résiduelles (MDDEP, 2012). La nouvelle réglementation prévoit la valorisation d'au moins 60% des matières résiduelles dites fertilisantes d'ici 2015 et le bannissement de l'enfouissement de celles-ci d'ici 2020 (Villeneuve and Dessureault, 2011).

Par ailleurs, le développement économique et l'exploitation des sols y étant associée contribue souvent à la dégradation des sols (MEA, 2005). La dégradation d'un sol se manifeste par une réduction de sa qualité et de sa productivité (Ortas, 2006). Entre autres, le changement d'affectation des terres, les pratiques agricoles intensives, et la déforestation rendent les sols plus susceptibles à l'érosion (Kaiser, 2004), perturbent le cycle du carbone (C) et de nutriments essentiels (Reich and Eswaran, 2004), modifient la structure et le fonctionnement des écosystèmes, et compromettent leur résilience. Dans plusieurs pays, la désertification et la diminution de la surface arable découlant de la dégradation des sols peuvent précariser la sécurité alimentaire (Lal, 2009).

La restauration d'un sol consiste donc, entre autres, à rétablir les structures et les processus écologiques qui lui sont associés. L'équilibre hydrologique, le cycle des nutriments, la biodiversité, le stockage de l'eau et des nutriments, la séquestration de C et la résilience des écosystèmes sont restaurés (Bell, 2006). En milieu forestier, la restauration vise notamment à rétablir la capacité du sol et de l'écosystème à supporter la croissance de la végétation (Ciccarese et al., 2012).

L'épandage de matières résiduelles fertilisantes en milieu agricole ou forestier constitue un moyen de détourner une quantité importante de matériel de l'enfouissement et de l'incinération. Jusqu'à présent, au Québec, l'épandage de boues se fait surtout en milieu agricole, et son utilisation demeure marginale en foresterie (MDDEP, 2014). Le potentiel d'utilisation des boues de fosse septique municipales en sylviculture a été exploré au Québec dans les années 1990, et semble connaître récemment un regain d'intérêt (Pion and Hébert, 2011). Par ailleurs, des mesures de restauration impliquant l'utilisation de ces boues pourraient permettre de rétablir la structure, les fonctions et les processus écologiques de sols forestiers dégradés.

Les boues de fosse septique sont riches en matière organique et en nutriments et pourraient remplacer les horizons organiques de surface (Epstein, 2002a). Cependant, l'épandage comporte aussi certains risques environnementaux, notamment l'eutrophisation des eaux de surface suite à la mobilisation du phosphore (P) et de l'azote (N) contenus dans les biosolides, ainsi que la contamination des eaux de surface et souterraines, et de la végétation par les métaux lourds ou de transition (ex. cuivre (Cu), manganèse (Mn), cadmium (Cd), zinc, (Zn), etc.).

L'objectif général de cette étude est d'évaluer le potentiel des boues de fosse septique municipales à des fins de restauration et de fertilisation des sols en milieu forestier. Plus particulièrement, nous nous intéresserons aux effets des boues de fosses septiques déshydratées sur les processus pédologiques qui gouvernent la séquestration du C, la disponibilité de nutriments, la production de biomasse, et la

rétenion des contaminants. De plus, nous évaluerons si les effets des boues se comparent à ceux de la couverture végétale d'origine forestière.

1.2 État des connaissances

1.2.1 Fonctions écologiques des sols

Les sols sont indispensables au maintien des fonctions écologiques gouvernant la séquestration du C, la production végétale, le cycle des nutriments, et la régularisation du cycle et de la qualité de l'eau. En milieu forestier boréal ou tempéré, les sols comprennent des horizons organiques de surface, soit la couverture morte (*forest floor*). La couverture morte exerce de nombreuses fonctions sous-tendant les processus écologiques énumérés précédemment. Ainsi, la couverture morte constitue une banque de semences, un lit de germination, une source d'énergie pour les organismes décomposeurs et une source et un puits de nutriments et de C (Bonan and Shugart, 1989; Prescott et al., 2000). Elle contribue à l'absorption et à la rétention des eaux de pluie, à l'absorption et à la diffusion de l'énergie solaire et tamponne les fluctuations environnementales (Prescott et al., 2000). En forêt boréale, on retrouve dans la couverture morte une forte proportion de racines fines (Finér et al., 1997), confirmant son importance pour la nutrition végétale.

1.2.1.1 Séquestration du carbone

Les sols constituent le réservoir le plus important de C des écosystèmes terrestres (FAO and ITPOS, 2015), avec le tiers stocké dans les sols forestiers (Janzen, 2004). La séquestration de C dans la végétation et les sols forestiers constitue un moyen efficace de séquestrer davantage de C afin de freiner son accumulation dans l'atmosphère (Chazdon, 2008; Jandl et al., 2007), et pourrait être un outil important dans la lutte contre les changements climatiques (Lal, 2010; Mondini and Sequi, 2008).

La séquestration de C dans le sol peut aussi être bénéfique pour la fertilité et la biodiversité des sols (Smith, 2004). Le C organique du sol (COS) contribue à la

formation d'agrégats, à la protection contre les changements rapides de pH (effet tampon), à l'accroissement de charges pour l'échange d'ions, et à l'augmentation de la capacité d'infiltration et de rétention de l'eau (Lal, 2004). Le COS est aussi un régulateur de la biomasse microbienne qui, à son tour, contribue à la fertilité et au maintien de la structure du sol (Barrios, 2007). De plus, la disponibilité de N dans le sol est liée à la minéralisation de la matière organique et à l'immobilisation de N par la biomasse microbienne. La disponibilité de N est donc indirectement liée au contenu de COS.

1.2.1.2 Productivité végétale et cycle des nutriments

La fertilité du sol est liée à sa capacité de fournir en quantité suffisante et équilibrée les nutriments et l'eau nécessaire à la croissance végétale. En forêt boréale, la couverture morte du sol constitue une réserve importante de nutriments (Prescott et al., 2000). Brais et al. (1995, 2002) rapportent des réserves équivalentes à 1080 kg N total ha⁻¹ et 990 kg Ca échangeable ha⁻¹ dans la couverture morte de sols argileux mésiques de l'Abitibi comparativement à 2930 kg N total ha⁻¹ et 4218 kg N ha⁻¹ pour le sol minéral entre 0 – 10 cm de profondeur. Pour des sols sableux de la même région, les réserves en N total et en Ca échangeable de la couverture morte et du sol minéral (0 – 20 cm) s'élèvent respectivement à 1364 et 1260 kg N ha⁻¹ et 306 et 405 kg Ca ha⁻¹ (Brais et al., 2000).

Par ailleurs, la composition chimique de la couverture morte influence le taux de dégradation et l'apport en nutriments dans le sol, et a donc une incidence sur les cycles biogéochimiques des nutriments essentiels à la croissance végétale (Thiffault et al., 2008). Certaines caractéristiques de la couverture morte et des sols dont la température, le taux d'humidité, le pH et la disponibilité de nutriments contrôlent aussi la décomposition de la matière organique, ce qui donne lieu à une distribution hétérogène des taux de nitrification en milieu forestier (Ste-Marie and Paré, 1999).

1.2.1.3 Régularisation du cycle et de la qualité de l'eau

Les sols régularisent la circulation de l'eau dans les écosystèmes en conditionnant son interception, son infiltration et son mouvement à la surface terrestre. Ils constituent un réservoir d'eau disponible pour la nutrition et la croissance végétale. Ils contribuent aussi au cycle de l'eau indirectement par le biais de la croissance des plantes qui favorisent l'interception et l'échange d'eau avec l'atmosphère par évapotranspiration (Power, 2010). Les interactions complexes entre les sols et la végétation en forêt permettent de régulariser une gamme de processus écologiques essentiels au cycle de l'eau. Les sols participent aussi à la filtration et la purification de l'eau par la transformation et la rétention de nutriments et de contaminants dans le sol (Brauman et al., 2007).

1.2.2 Mécanismes de dégradation des sols

La dégradation des sols se manifeste par une réduction de la qualité et de la capacité du sol à remplir ses fonctions écologiques (Lal, 2009, 2010, 2012). Pour les sols forestiers, la dégradation découle de processus physiques (ex. compactage du sol par la machinerie et érosion par l'eau et le vent), chimiques (ex. appauvrissement en nutriments par la récolte, acidification par les dépôts atmosphériques acides), et biologiques (ex. perte de couverture morte par la préparation de terrain, perte de biodiversité; Lal, 2009; Lal et al., 1989). L'exploration et l'extraction minière sont aussi responsables de la dégradation de superficies importantes (Rowland et al., 2009; Turcotte et al., 2009).

1.2.2.1 Perte de couverture morte

La préparation de terrain (par déblaiement, débroussaillage mécanique, hersage sur billons, étalage) en vue du reboisement est une méthode employée pour augmenter la productivité des arbres plantés en éliminant la compétition et en facilitant l'accès aux nutriments (Fleming et al., 2006). Cependant, dans des cas extrêmes, la perte de couverture morte et les faibles concentrations de matière organique et de nutriments

qui l'accompagne ralentissent la croissance de la végétation (Ballard, 1978; Nyland et al., 1979). Une diminution de substrats disponibles peut alors entraîner un ralentissement de la croissance et du métabolisme des microorganismes, et par conséquent ralentir la minéralisation des nutriments dans le sol (Tan et al., 2005). Dans ces conditions, le rétablissement de la couverture morte et du COS par l'apport des litières aériennes et souterraines devient restreinte, limitant encore davantage la capacité du système à supporter la croissance de la végétation par l'établissement des propagules végétaux (Tan et al., 2005) et la séquestration de carbone (Lal, 2005).

1.2.2.2 Compactage du sol

Le compactage du sol occasionne la détérioration des agrégats du sol, une diminution de la macroporosité et de l'aération du sol, une réduction de la conductivité hydraulique et une augmentation de la porosité capillaire et de la rétention en eau, et de la résistance à l'enracinement, particulièrement dans les sols fins (Brais, 2001; Demir et al., 2007; Fleming et al., 2006). Le compactage nuit aussi au développement des racines en réduisant l'accès à l'eau et aux nutriments. Il peut aussi restreindre la formation d'agrégats dans le sol par les racines, les animaux et les microorganismes (Batey, 2009; Hamza and Anderson, 2005).

Dans des cas extrêmes, le compactage peut mener à la formation d'une croûte dure à la surface du sol (battance) et l'accumulation d'eau, ce qui peut réduire le taux de germination des semences de façon significative. La minéralisation de N dans le sol peut diminuer suite à un compactage parce que la biomasse microbienne est normalement affectée négativement par la réduction de substrat et d'oxygène (O) disponible (Jordan et al., 2003).

L'impact du compactage diffère selon la texture du sol. Il peut s'avérer bénéfique pour la croissance végétale dans des sols grossiers à faible rétention d'eau et peut réduire la compétition végétale (Brais, 2001). Dans les sols fins et humides, le

compactage peut nuire à la productivité végétale et à la séquestration du COS, et entraver la circulation et la rétention de l'eau (Ballard, 2000).

Plusieurs études (ex. Gomez et al., 2002; Page-Dumroese et al., 2006; Ponder, 2008) ont évalué les effets de la perturbations du sol par le compactage et par la perte de couverture morte en milieu forestier. Ces études démontrent que l'effet du compactage sur la croissance de semis et sur la productivité du sol est complexe. La croissance des semis serait liée aux interactions entre la porosité et l'humidité initiale du sol, et la concentration de COS. En effet, lorsque la porosité naturelle du sol et la concentration de COS sont élevées, surtout dans certains sols loameux et sableux, la rétention d'eau et de nutriments dans le sol peut être suffisante pour permettre la croissance des arbres suite au compactage et à la perte de couverture morte (Gomez et al., 2002). En revanche, dans les sols argileux, une diminution importante de la macroporosité accompagnée d'une hausse importante de la masse volumique apparente et de la résistance du sol peut influencer négativement la respiration des racines et l'approvisionnement en eau et en nutriments (Tan and Chang, 2007), surtout en milieu plus sec et faible en COS (Zhang et al., 2005). Ces facteurs contribuent à réduire la croissance des arbres de façon significative (Gomez et al., 2002). Ainsi, dans les sols argileux très perturbés dont les valeurs de COS sont faibles, l'effet du compactage pourrait être plus prononcé et même exacerbé par la perte de couverture morte.

1.2.3 Pratique de restauration des sols

La restauration vise à rétablir les processus écologiques (ex. les apports en litières, les processus de décomposition, d'immobilisation de C et du cycle des nutriments, la rétention et la circulation des eaux de surface) de façon à recouvrer autant que possible les conditions biologiques, physiques et chimiques d'origine du milieu dégradé (Ciccarese et al., 2012). Au Québec, les réglementations concernant les sites miniers obligent entre autres les compagnies à extraire et entreposer temporairement

la couche superficielle du sol, et subséquemment à la remettre en place pour réhabiliter le site une fois les travaux d'exploitation terminés (Ministère des ressources naturelles, 1997). Dans d'autres milieux fortement dégradés, une toute nouvelle couche superficielle de sol prélevée d'autres milieux tels des champs agricoles ou des sites de construction peut être mise en place pour restaurer le sol (Wang et al., 2008).

En milieu forestier, certaines techniques de labourage (ex. herse à disques, herse rotative) peuvent atténuer les effets du compactage (Godefroid et al., 2007). Toutefois, ces pratiques peuvent endommager la structure des sols forestiers, surtout les sols à texture fine (Bulmer, 2000; McNabb, 1994). Il faut donc proscrire ce type d'intervention pour ameublir les sols forestiers compactés argileux (Ampoorter et al., 2011). En effet, le labourage doit être fait dans des conditions optimales d'humidité du sol pour être efficace. Il doit aussi être fait en présence de suffisamment de matière organique pour maintenir les agrégats du sol, ce qui assurera le maintien de conditions favorables à la croissance végétale à long terme (Bulmer, 2000).

L'ajout d'amendements riches en matière organique est aussi couramment utilisé à des fins de restauration de sols dégradés forestiers (ex. Ampoorter et al., 2011; Sanborn et al., 2004; Wang et al., 2003), miniers (ex. Brown et al., 2003; Haering et al., 2000; Rowland et al., 2009) ou de sites d'enfouissement (ex. Bolan et al., 2013; Gregory and Vickers, 2003; Lamb et al., 2012). L'épandage de boues de fosse septique municipales riches en matière organique constitue donc une alternative intéressante pour rétablir les services écologiques du système (Bommarco et al., 2013) puisqu'elle permet d'agir comme couverture physique du sol minéral (i.e. un proxy de l'effet tampon de la couverture morte), de recouvrer les réserves de C et de nutriments typiques aux sols forestiers (ex. horizons LFH) et de réenclencher les processus pédogénétiques (Garcia et al., 1996; Larney and Angers, 2012; Pascual et al., 1997).

1.2.4 Caractérisation des boues de fosse septique

Les boues de fosse septique municipales constituent des matières résiduelles fertilisantes (MRF) puisqu'ils sont riches en N et P sous formes organique et inorganique. Elles contiennent aussi d'autres nutriments quoi qu'en plus petite quantité (Table 1.1; Epstein, 2002b). Les MRF doivent répondre à certains critères de qualité qui déterminent leur potentiel d'utilisation. La classification C-P-O-E évalue la qualité des MRF selon la concentration de contaminants chimiques, d'éléments essentiels et non essentiels (C), la présence de pathogènes (P), l'odeur (O), ainsi que la présence de corps étrangers (E) (MDDEP, 2012). Certains procédés de stabilisation tel que le compostage contribuent à générer des MRF de qualité supérieure, notamment en diminuant les concentrations de pathogènes (Edmonds, 2005; Selivanovskaya and Latypova, 2006). Les concentrations des différents composés azotés organiques ou inorganiques varient largement selon l'origine et le traitement des boues et affectent la disponibilité de nutriments à long terme. Le compostage et la méthanisation contribuent à augmenter la stabilité des nutriments dans les boues (Sanchez-Monedero et al., 2004), et à diminuer la disponibilité et l'effet phytotoxique des métaux traces (Fuentes, 2004; Fuentes et al., 2006; Oleszczuk, 2008). Cependant, certains procédés tels la déshydratation des boues pourraient augmenter les concentrations et la disponibilité de certains métaux comparativement au compostage (Walter et al., 2006). L'utilisation de boues déshydratées permet toutefois de limiter les coûts associés au transport (réduction du volume) et de réduire les odeurs.

Table 1.1 Propriétés agronomiques de biosolides municipaux issus de deux types de stations d'épuration des eaux usées au Québec pour la période 2000-2006. Tiré de MDDEP (2007).

Type de stations	Mécanisées (n = 35)		Étangs (n = 68)	
<i>Paramètres</i>	Moyenne	c.v. (%)	Moyenne	c.v. (%)
Siccité (% b.h.)	21	52	5	43
M.O. (% b.s.)	66	22	42	25
C/N	9,0	52	9,8	22
N-NTK (% b.s.)	4,4	38	2,2	41
N-NH ₄ /N-NTK	0,09	69	0,13	70
N/P ₂ O ₅	1,45	57	0,74	94
P ₂ O ₅ (% b.s.)	3,9	53	4,5	61
K ₂ O (% b.s.)	0,3	68	0,2	74
Ca (% b.s.)	1,5	45	2,7	52
Mg (% b.s.)	0,4	42	0,6	71
pH	7,0	16	7,1	6

Les boues peuvent aussi contenir des contaminants ou métaux traces (Garcia-Delgado et al., 2007; Wang et al., 2008). Certains éléments sont essentiels ou bénéfiques en petite quantité à la croissance des plantes (As, Co, Cr, Cu, Mo, Ni, Se, Zn), alors que d'autres sont des contaminants stricts (Cd, Hg, Pb, dioxines/furanes) dont la concentration doit absolument être minimisée. Certains éléments dont le Cd, Zn et Cu sont mobiles à faible pH (Chuan et al., 1996; Sauvé et al., 2000). Comme les sols forestiers boréaux sont typiquement acides, l'application de boues comporte des risques associés à la mobilisation de métaux dans le sol minéral et possiblement à la contamination des horizons B et C du sol, des eaux souterraines et de surface et de la végétation.

Les pathogènes les plus communément présents dans les boues et comportant des risques de contamination pour les humains et les animaux sont les salmonelles (*Salmonella spp.*) et la bactérie *Escherichia coli* (*E. coli*). Bien que les processus de stabilisation tel le compostage permettent de réduire la concentration de pathogènes et de bactéries à un niveau non-détectable dans les boues, la recolonisation (ex. par les excréments d'animaux), surtout dans des conditions humides et de température optimales, peut mener à la contamination de la matière organique après épandage (Zaleski et al., 2005a, 2005b).

1.2.5 Effet de l'épandage sur les propriétés du sol

1.2.5.1 Effets sur le cycle des nutriments

Le N et le P contenus dans les boues sont rendus disponibles dans les horizons de surface via leur décomposition et leur minéralisation après l'épandage. Un apport élevé de N et P stable sous forme organique dans les boues compostées pourrait permettre de maintenir des concentrations suffisantes de ces nutriments sous forme minérale à plus long terme, tout en prévenant un lessivage excessif vers les cours d'eau (Nieminen and Raisanen, 2013). Au contraire, un relargage important d'ions nitrate (NO_3^-), d'ammonium (NH_4^+) et d'ions phosphate (PO_4^{3-}) dans les eaux de

surface pourrait mener à des problèmes d'eutrophisation (Robinson and Polglase, 2005). Cependant, McLaren et al. (2007) ont noté que la concentration de N inorganique dans la litière forestière est demeurée élevée pendant deux ans suite à l'épandage de boues méthanisés, et qu'il n'y a pas eu de lessivage important de N inorganique. Comme le P est moins limitant que le N dans les écosystèmes boréaux, la possibilité qu'il migre jusqu'aux cours d'eau est plus élevée. Toutefois, l'utilisation de boues stabilisées comportant du P sous forme organique pourrait limiter le lessivage de P.

Les cations basiques (Ca, Mg, K), une fois minéralisés, peuvent se fixer sur les sites d'échanges cationiques du sol (Kasongo et al., 2011), alors que l'ajout de matière organique augmente la capacité d'échange cationique (CEC) du sol (Epstein, 2002b; Thangarajan et al., 2013). Cependant, la capacité de rétention des cations basiques varie largement d'un sol à un autre. Les pertes de cations basiques par lessivage sont généralement plus élevées dans les sols plus grossiers (Epstein, 2002c; McLaren et al., 2003). Les sols argileux compactés et faibles en matière organique pourraient bénéficier de la forte teneur en C, N et P des boues, mais l'effet de l'apport en cation basiques serait minimale. En effet, les sols argileux sont souvent riches en cations basiques (Brais et al., 1995) alors que la disponibilité en N des sols forestiers boréaux est normalement très faible (Vitousek and Howarth, 1991).

1.2.5.2 Effets sur le cycle du carbone

L'ajout de C labile par les boues augmente généralement la biomasse microbienne du sol et la faune bénéfique à la détritosphère (Larney and Angers, 2012; Sciubba et al., 2012). La flore microbienne et la pédofaune contribuent au recyclage des nutriments ainsi qu'au développement de la structure du sol par l'incorporation de la matière organique aux horizons minéraux et la formation des complexes organo-minéraux stables. Plusieurs auteurs (ex. Gibbs et al., 2006; Kao et al., 2006; Khan and Scullion, 2002) ont observé une augmentation rapide à court terme de la minéralisation de C

dans les sols amendés. Le taux de minéralisation de C est un indicateur du niveau d'activité microbienne du sol, et constitue donc une mesure de la qualité du sol suite à l'épandage de boues (Haney et al., 2001; Haney and Franzluebbbers, 2009; Jin et al., 2011).

1.2.5.3 Effets sur la réaction (pH) du sol

L'effet de l'application des boues sur le pH du sol est plutôt variable et dépend, entre autres, du pH initial des boues et des propriétés physicochimiques du sol amendé. Le pH des boues est normalement entre 7 et 8, bien qu'il puisse varier si par exemple de la chaux est ajoutée (Epstein, 2002a). Suite à l'épandage de boues, Morera et al. (2002) ont noté une augmentation de pH des sols acides, et aucun changement dans des sols légèrement alcalins (pH = 7,1 et 7,9) et alcalins (pH = 8,1). McLaren et al. (2007) ont observé une forte hausse du pH dans des sols acides (pH = 5). Zhang et al. (2006) ont aussi noté une hausse du pH qu'ils attribuent à la présence de métaux basiques (Cu, Pb, Zn) dans les boues compostées. À l'inverse, certains auteurs (Harrison et al., 1996; McLaren et al., 2004; Richards et al., 2000) ont observé une baisse de pH, laquelle pourrait s'expliquer par l'acidification de la solutions de sol suite à la minéralisation des boues, principalement la nitrification (une source de H^+) et à l'oxydation du soufre qu'elles contiennent (une autre source de H^+) par la biomasse microbienne (Qureshi et al., 2003). L'effet des boues sur la réaction d'un sol amendé dépend aussi de sa capacité tampon, c'est-à-dire sa capacité à limiter les changements de pH par sa rétention des cations échangeables (capacité d'échange cationique).

1.2.5.4 Effets sur la concentration de métaux traces

Plusieurs chercheurs ont étudié la distribution et le mouvement de métaux traces dans des sols amendés avec des boues (ex. Garcia-Delgado et al., 2007; Gray et al., 2003; Morera et al., 2002; Smith, 2009; Su et al., 2003). La disponibilité et le taux de lessivage des métaux dépend largement de leur concentration initiale dans les boues

et de leur stabilité (Morera et al., 2002; Smith, 2009), mais ils dépendent aussi du pH, des teneurs en C et du potentiel d'oxydo-réduction du sol puisque ces caractéristiques déterminent la spéciation, et donc la mobilité des métaux dans le sol.

Une fois dans le sol, les métaux sont adsorbés sur les colloïdes du sol (ex. silicates, carbonates, phosphates et (hydro)-oxides, particules d'argile, matière organique) (McLaren et al., 2003), ce qui contribue à leur rétention dans la couverture morte ou en surface du sol minéral (McBride et al., 1997). De plus, certains auteurs ont observé un lien entre l'augmentation de la teneur en matière organique et l'adsorption de certains métaux dans le sol (Eriksson, 1988; Lo et al., 1992). Toutefois, les complexes formés des métaux et des colloïdes peuvent aussi devenir mobiles en se dissociant des agrégats du sol. Ils peuvent alors migrer dans la solution de sol vers les horizons B et C ou les eaux souterraines (de Jonge et al., 2004). L'infiltration d'eau dans le sol peut faciliter la mobilisation de colloïdes en induisant une diminution de la force ionique et des changements dans la chimie des solutions du sol (ex. hausse du pH; Ryan and Elimelech, 1996).

Certains métaux traces peuvent aussi être adsorbés sur le C organique dissout dans la solution de sol (COD), et ainsi être mobilisés (McGechan and Lewis, 2002). Cependant, l'affinité des métaux pour le COD varie selon l'élément étudié (McBride et al., 1997). Par exemple, la complexion avec le COD serait plus importante pour le Cu et Pb que pour le Zn, Cd et Ni (Weng et al., 2002). Une forte macroporosité favoriserait aussi le lessivage des colloïdes mobiles, du COD ou des ions libres par les canaux préférentiels d'écoulement (McLaren et al., 2004). Le pH du sol contrôle aussi la complexion des métaux avec la matière organique (Bradl, 2004). À faible pH, les métaux seraient plutôt solubilisés et pourrait être exportés hors de la portée des racines dans les horizons B et C.

La dégradation de la qualité des eaux souterraines et de surface peut survenir si les contaminants contenus dans les boues ne sont pas stabilisés par les constituantes du sol ou absorbés par la végétation. De plus, la présence de métaux ou d'autres

contaminants pourrait inhiber la croissance de la biomasse microbienne (Fernandes et al., 2005), ou encore limiter la croissance végétale dû à leur effet phytotoxique (Walter et al., 2006). Conséquemment, les métaux pourraient perturber le cycle des nutriments et la productivité du sol.

1.2.5.5 Effets sur les propriétés physiques du sol

Plusieurs études ont documenté une amélioration générale des propriétés physiques de sols dégradés suite à l'application de boues (ex. Aggelides and Londra, 2000; Salazar et al., 2012; Tsadilas et al., 2005). La forte teneur en matière organique des boues contribue à améliorer la structure des sols en augmentant la stabilité des agrégats par la création de composés organo-minéraux, et en augmentant la capacité de rétention d'eau dans le sol (Singh and Agrawal, 2008). L'addition de matière organique peut aussi contrer les effets néfastes associés au compactage du sol en diminuant la masse volumique apparente et en augmentant la macro- et microporosité du sol (Larney and Angers, 2012), rétablissant ainsi la capacité d'infiltration de l'eau et de drainage du sol. L'amélioration de la structure du sol pourrait être plus prononcée dans des sols argileux que dans des limons ou autres sols plus grossiers (Aggelides and Londra, 2000). Par ailleurs, l'augmentation de la croissance de racines dans un sol amendé pourrait favoriser la régénération de sols dégradés (García-Orenes et al., 2005) en augmentant l'apport en C par les litières souterraines et en contribuant à ameublir les sols.

1.2.5.6 Effets sur la production de biomasse végétale

L'application de boues et l'augmentation subséquente de la disponibilité des éléments nutritifs du sol favorise la croissance végétale (Gaulke et al., 2006; Holm and Heinsoo, 2013; Selivanovskaya and Latypova, 2006). Cavaleri et al. (2004) ont observé, dans des plantations juvéniles de peupliers hybrides (1 an), une augmentation de la biomasse au moins aussi importante pour les arbres amendés avec des boues que pour les arbres amendés avec un fertilisant chimique azoté, et

beaucoup plus importante que pour les arbres n'ayant reçu aucun amendement. Cependant, la quantité de N était moindre dans les feuilles des arbres amendés avec des boues comparativement aux arbres ayant reçu du N inorganique, possiblement à cause du processus plus lent de libération du N par les boues que le fertilisant chimique.

L'ajout de matériel riche en nutriments pourrait aussi favoriser la croissance d'herbacées et de graminées au détriment des jeunes arbres. Dans une plantation juvénile de Douglas (*Pseudotsuga menziesii*), Cowley et al. (2005) ont attribué l'absence de différence en terme de croissance entre des parcelles amendées et les contrôles au prélèvement accru par la végétation de sous-bois au dépend des jeunes arbres — ces derniers n'ayant probablement pas un système racinaire suffisant pour accéder au nouvel apport de nutriments.

1.3 Objectifs de l'étude et hypothèses de travail

Les sols forestiers étudiés présentent des conditions sous-optimales à la croissance végétale suite à des perturbations anthropiques. La faible productivité du site d'étude découle essentiellement de la perte des horizons organiques de surface (couverture morte) et de l'appauvrissement en nutriments, ainsi que de l'affaiblissement de la structure dû au compactage par la machinerie lourde lors de la récolte forestière.

L'objectif général de cette étude est d'évaluer le potentiel des boues de fosse septique municipales pour la restauration de sols forestiers dégradés. Nous comparerons les effets de l'épandage des boues et de la couverture morte sur les processus physiques, chimiques et biologiques qui sous-tendent les fonctions écologiques des sols, de façon à déterminer si les boues peuvent remplacer la couverture morte d'origine.

Hypothèse 1 : L'épandage de boues et de couverture morte favorisera un enrichissement en C et en N dans les horizons minéraux, et par le fait même auront une incidence sur le cycle du C et du N dans les sols.

Hypothèse 2 : L'épandage augmentera la disponibilité des nutriments dans les horizons de surface du sol (0 - 10cm), dont l'azote, le phosphore, les cations basiques (calcium, magnésium, potassium). Les boues auront un effet plus marqué que la couverture morte.

Hypothèse 3 : Comparativement au témoin et à la couverture morte, les boues augmenteront la concentration et la disponibilité de certains métaux traces (cuivre, zinc, fer, aluminium, plomb) en surface du sol minéral (0 - 10cm).

Hypothèse 4 : Les boues et la couverture morte comportent une forte teneur en matière organique, ce qui contribuera à améliorer les propriétés physiques (masse volumique, porosité à l'air) des sols.

Hypothèse 5 : Les formes minérales de l'azote (NH_4^+ , NO_3^-) et du phosphore (PO_4^{3-}) sous les boues augmenteront la croissance de l'épinette blanche comparativement au témoin et à la couverture morte.

CHAPITRE II : RESTORING A DISTURBED CLAYEY FOREST SOIL: CAN
DEHYDRATED SEWAGE SLUDGE REPLACE THE NATIVE FOREST
FLOOR

Restoring a disturbed clayey forest soil: can dehydrated sewage sludge replace native forest floor

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2.1 Abstract

Sewage sludge contains organic matter and nutrients which could benefit forest soils that were adversely impacted by forest operations. We investigated the effects of dehydrated sewage sludge application on a compacted and severely impoverished forest clayey soil, and on white spruce (*Picea glauca* (Moench) Voss) seedling foliar nutrition and growth. Over a period of two years, the application of two types of dehydrated sludge (stored 1 and 5 years) was compared to the application of an original forest floor taken from the nearby forest stand and a control (no application). We characterized soil organic matter by determining total organic carbon (C) and nitrogen concentrations (N), C and N mineralization rates (net potential mineralization) and microbial biomass. Chemical structure was characterized using ^{13}C nuclear magnetic resonance (NMR) spectroscopy. Carbon and N mineralization rates and concentrations were also monitored in the mineral soil. Plant Root SimulatorTM probes were used to assess nutrient and trace metal supplies in the mineral soil. Soil bulk density and porosity were also assessed yearly. ^{13}C NMR spectroscopy revealed differences among amendments, with the mature sludge (stored 5 years) spectra displaying a higher proportion of aromatic compounds relative to the fresh sludge (stored 1 year), and the forest floor spectra a higher O-alkyl C and lower Alkyl C proportion relative to the sludges. Neither soil C concentrations and mineralization nor soil physical properties were significantly improved in the mineral soil, even at application rates exceeding 700 t ha^{-1} (dry mass). The sludges supplied more nitrate, phosphorous, calcium and sulphate and less ammonium and potassium to the mineral soil compared to the forest floor and control. High levels of nitrate indicate greater nitrification potential in sludges than in the forest floor. Increased nutrient availability under sludge and forest floor treatments also generally resulted in improved foliar nutrition and growth of white spruce seedlings. However, sludges supplied more copper, zinc and lead than the forest floor and control at most sampling periods. The results suggest that sludge

application could improve nutrition in disturbed soils, but there is a risk to surface and ground water quality if application rates exceed N and P requirements of the trees.

2.2 Résumé

Les boues de fosse septique constituent une source de matière organique et de nutriments qui pourrait servir à la restauration de sols dégradés suite à des opérations forestières. Nous avons évalué les effets de l'épandage en surface de boues de fosse septique déshydratées sur des sols forestiers argileux appauvris et compactés, et sur la nutrition foliaire et la croissance de semis d'épinette blanche (*Picea glauca* (Moench) Voss). Sur une période de deux ans, les effets de deux types de boues (entreposées 1 et 5 ans) ont été comparés à ceux de la couverture morte d'origine forestière prélevée à proximité et d'un témoin non amendé. Afin de caractériser les amendements, nous y avons déterminé les concentrations en carbone organique (C) et en azote total (N), les taux de minéralisation de C et de N (minéralisation potentielle nette) et la biomasse microbienne. La structure chimique des amendements a aussi été caractérisée par spectroscopie de résonance magnétique nucléaire (RMN) du ^{13}C . Des résines échangeuses d'ions (Plant Root SimulatorTM Probes) ont permis de suivre la biodisponibilité en nutriments et en métaux traces dans le sol minéral. La masse volumique et la porosité du sol y ont aussi été mesurées annuellement. Les spectres obtenus par RMN révèlent des différences dans la structure chimique des amendements; les boues matures ayant une plus importante proportion de composés aromatiques relativement aux boues fraîches, et la couverture morte une plus forte proportion de C alkyl et carbonyl ainsi qu'une plus faible proportion de O-alkyl par rapport aux boues. Pour la période couverte par l'étude, l'épandage de boues n'a pas eu d'effet ni sur les concentrations et la minéralisation du C, et ni sur les propriétés physiques du sol minéral, même avec une application excédant 700 t ha^{-1} (masse sèche). Les disponibilités en nitrate, phosphore, calcium et sulfate dans le sol minéral étaient plus élevées sous les boues, tandis que les apports en ammonium et en potassium étaient plus élevés sous la couverture morte et dans le témoin. Un flux important de nitrate sous les boues indique leur potentiel de nitrification plus important que celui de la couverture morte. L'augmentation de la disponibilité en

nutriments sous les boues et sous la couverture morte a d'ailleurs amélioré la nutrition foliaire et la croissance des semis suite aux amendements. Par contre, les apports en cuivre, en zinc et en plomb étaient plus élevés sous les boues durant la plupart des périodes d'échantillonnage. L'épandage de boues pourrait améliorer la nutrition sur des sols dégradés, mais il y a des risques de lessivage de nutriments et de métaux traces si les dosages dépassent les besoins des arbres.

2.3 Introduction

Soil application of sewage sludge is an economical alternative to inorganic fertilizers (Pritchard et al., 2010) and costly landfill disposal (Torri et al., 2014b). This practice is increasing worldwide (Giusti, 2009) as a result of more stringent laws for regulating water quality (Garcia-Delgado et al., 2007), and growing concerns about the environmental impacts of landfills, incineration and water disposal of these residual materials (Singh and Agrawal, 2008). However, application to soils is regulated in most countries based on the presence of contaminants such as trace metals and pathogens (Garcia-Delgado et al., 2007; Wang et al., 2008) and risks associated with N, P and trace metals leaching to surface and ground waters (Robinson and Polglase, 2005).

Degraded forest soils may benefit from the application of sewage sludges due to their high organic matter and nutrient contents (Larney and Angers, 2012). Following forest harvesting, site preparation is often performed to improve seedling microsite conditions. Site preparation can lead to removal of logging residues, forest floor and live vegetation (Fleming et al., 2006). In the boreal forest, soils typically have low N availability (Vitousek and Howarth, 1991) and the removal of the forest floor has long-lasting consequences on nutrient cycling and retention (Brais et al., 1995). Forest harvesting and site preparation can also lead to some damage of soil structure and to soil compaction, especially in heavy clay soils (Brais, 2001). In this context, sewage sludge application could replace surface organic matter and help re-establish soil properties and functions such as carbon levels, water and nutrient retention capacity, nutrient cycling, microbial diversity and ecosystem resilience (Bell, 2006).

The effect of sewage sludge application on soil properties depends on the chemical characteristics of the organic residue, namely C and N concentrations and pH (Hallett et al., 1999). The stabilizing process used prior to sludge application (e.g. composting, anaerobic or aerobic digestion) is very important in determining C and nutrient dynamics in soils as well as long-term effects of application (Mattana et al.,

2014). The response of the soil to sludge application also depends on its level of degradation (Larney and Angers, 2012; Page-Dumroese et al., 2006).

Despite large differences in sludge properties (Lashermes et al., 2009; Singh et al., 2011; Soriano-Disla et al., 2010; Torri et al., 2014a), several studies have reported short-term (2 to 3 years) improvements of soil nutrient availability in unproductive forest soils after sludge application (Bramryd, 2002; Cavaleri et al., 2004; Hallett et al., 1999; Varela et al., 2011). In both forest and agricultural soils, sewage sludges have been shown to increase soil organic carbon concentrations shortly after surface application (Soriano-Disla et al., 2010; Tarrasón et al., 2008; Virginia L. Jin, 2014) and incorporation into the soil surface using various site preparation techniques (Bolan et al., 2013; Tian et al., 2009). Increased soil organic matter has also been linked to a decrease in soil bulk density (García-Orenes et al., 2005; Neilsen et al., 2003; Tsadilas et al., 2005) increased aggregate stability (García-Orenes et al., 2005; Lindsay and Logan, 1998), and porosity (Aggelides and Londra, 2000; Pagliai et al., 1981). Carbon and N addition from sewage sludge may benefit soil microbial biomass (Kuznyakov et al., 2000; Singh and Agrawal, 2008), which, in turn, increases C and N mineralization rates (Gibbs et al., 2006; Kao et al., 2006; Khan and Scullion, 2002) and the release of other nutrients such as P, Ca and K (Borken et al., 2002). As a whole, sludge application improves tree nutrition and growth from increased soil organic C and nutrient availability (Gaulke et al., 2006; Holm and Heinsoo, 2013; Selivanovskaya and Latypova, 2006).

Changes in soil pH induced by sludges may also modify microbial biomass abundance (Aciego Pietri and Brookes, 2008) and composition (Bååth and Anderson, 2003; Rousk et al., 2009), and nutrient release dynamics (Kemmitt et al., 2006). However, the effect of sludge on soil pH may vary depending on initial soil pH and buffering capacity (Haynes et al., 2009). Studies have reported increased pH in acidic soils (McLaren et al., 2007; Morera et al., 2002; Zhang et al., 2006), and no effect (Morera et al., 2002) or decreased pH (Harrison et al., 1996; McLaren et al., 2004;

Richards et al., 2000) in alkaline soils following sludge application. A decrease in pH has been linked to soil solution acidification because nitrification and S oxidation by microbial biomass after sludge application generate H^+ ions (Qureshi et al., 2003).

At high sludge application rates, NO_3^- and PO_4^{3-} in excess of vegetation requirements can leach below the rooting zone (Samaras et al., 2008) and eventually reach surface waters, thus promoting eutrophication (Correll, 1998). Such problems are expected in regions receiving heavy rainfall where the nutrient runoff potential is high (Fredriksen et al., 1973; Grey and Henry, 2002). Increased trace metal concentrations in soil solutions and in drainage waters has been reported concurrently with soil acidification due to sludge mineralization of N and S oxidation (Egiarte et al., 2008; McLaren et al., 2004; Speir et al., 2003). However, metal sorption to soil particles and metal mobility/bioavailability (Antoniadis et al., 2007) depend on several factors including initial soil properties (e.g. pH, organic matter content, dissolved organic C, soil texture and cation exchange capacity) (Hooda and Alloway, 1994; Richards et al., 2001) and sludge application rates (Harrison et al., 2005). Surface accumulation of metals in the soil (i.e. 0 – 15 cm depth) has been observed (Yang et al., 2014), but other studies evaluating the long-term fate of trace metals after sludge application have shown that trace metals can be released into the environment over time due to organic matter decomposition and soil acidification (Antoniadis et al., 2007; Chang et al., 1997; McBride, 2003).

To the best of our knowledge, few studies to date have compared the effects of sewage sludge application to those of introducing natural forest floor to a disturbed soil. This comparison could be helpful to evaluate the potential of sewage sludge amendments to restore natural pre-disturbance soil properties and ecosystem functions. The objectives of this study were to assess and compare the effects of dehydrated sewage sludge application and of natural forest floor amendments on: (1) soil C and N dynamics, (2) soil nutrient availability, (3) soil physical properties, (4) bioavailability of trace metals, and (5) tree seedling foliar nutrition and growth.

2.4 Methods

2.4.1 Study area

The study site is located at the Lake Duparquet Research and Teaching Forest (LDRTF) about 45 km northwest of Rouyn-Noranda, Quebec (LDRTF: 48°47' to 48°44'N, 79°44' to 79°40'). The region is situated at the southern fringe of the boreal forest and belongs to the balsam fir (*Abies balsamea* [L.]), white birch (*Betula papyrifera* Marsh) and white spruce (*Picea glauca* (Moench) Voss) bioclimatic domain (MRNFQ, 2012). The climate is continental with a mean annual temperature of 0.5°C, and mean annual precipitation is 975.0 mm of which 30% falls as snow (Environnement Canada, 2010). Soils at the study site are Grey Luvisols (Soil Classification Working Group, 2002) which have developed on mesic to clayey glacio-lacustrine deposits left by the Barlow-Ojibway proglacial Lake (Veillette, 2000).

Prior to harvest, the stand at the study site was dominated by balsam fir, white birch and white spruce originating from a fire dating from 1760 (Dansereau and Bergeron, 1993). The forest floor was a 2 to 18 cm MOR humus type and contained up to 1400 kg ha⁻¹ of organic N (Brais et al., 2002). Intensive site preparation was undertaken following clear-cut harvesting in 2004 in order to establish a hybrid poplar plantation. First, winter windrowing (Brais et al., 2002) was used to pile surface organic matter such as stumps and branches. The cleared soil was then tilled and harrowed in preparation for planting. In the three years following plantation establishment, competing vegetation was removed mechanically by harrowing the soil surface between the trees. These operations have contributed to soil compaction and to a decrease in organic soil carbon content (Table 2.1), which, in turn, may have restricted root establishment and tree growth.

2.4.2 Experimental design

Septic tank sludge used in this study was obtained from the Abitibi-Ouest Regional County Municipality (AORCM, population = 21 200). Upon collection, the sludge is stored in dehydration basins ($45 \times 40 \times 3$ m) lined with wood chips until the basin is emptied (3 to 8 years). The sludge is then piled on the outer edges of the basins. We used a sludge that was extracted from a basin and piled for over five years (mature), and a sludge that was extracted one year before its application (fresh) (Table 2.2). The sludge was screened before application in order to remove coarse debris.

The sludge was applied on August 27th, 2013. The experimental design initially consisted of three replications of five treatments (2 types of sludge $\times$ 2 sludge thicknesses and control) applied to fifteen randomly distributed 3×15 m experimental plots (ExP; Fig. 2.1). The treatment loads were designed to reproduce a forest floor thickness similar to that of surrounding natural stands (i.e. 5 and 15 cm). However, the resulting treatment thicknesses were higher than the target thicknesses (i.e. 15 and 25 cm, see Table 2 for application rate equivalents and general characteristics). A sixth treatment consisting of a forest floor amendment in three additional and randomly distributed ExP of 3×3 m was applied in May 2014. The forest floor material was collected from a nearby (less than 500 m) natural stand similar to the one harvested in 2004 at the study site. Application rate equivalents and general characteristics of the forest floor are presented in Table 2.2.

In June of 2014, 241 white spruce seedlings were planted in all eighteen ExP and numbered. Fifteen seedlings were planted in two rows in each of the 3×15 m ExP where sludge was applied, and four seedlings were planted in each of the 3×3 m ExP where forest floor was added.

2.4.3 Field sampling

Sampling in 3×15 m ExP was done at 1 m from the plot end at intervals of 7 m, and at 0.75 m lengthwise from the plot end at intervals of 1 m in 3×3 m ExP, providing a

total of 3 sampling locations in each ExP (Fig. 2.1). Sampling described below applies to these three locations. Over the two-year study, sampling and/or measurements were conducted on five occasions: fall of 2013, spring of 2014, fall of 2014, spring of 2015 and fall of 2015. Sampling and measurements varied somewhat among occasions.

Organic amendments (sludge and forest floor) and mineral soil samples from each ExP were collected for respiration and potential N mineralization as well as for organic C and total N in the spring of 2014, fall of 2014 and spring of 2015. Organic amendment samples were collected for bulk composition analysis in the spring of 2014. Before sludge application in the fall of 2013, the top mineral soil (0 - 10 cm depth) was collected without disturbing its morphology using a double cylinder soil sampler (5 cm diameter, 100 cm³) to determine its porosity (Klute, 1986) and bulk density (Blake and Hart, 1986). Undisturbed soil samples were collected again in 2014 and 2015 after the organic amendments were carefully cleared over a small area.

In the fall of 2015, organic amendments were collected and pooled within each ExP for microbial biomass C and N.

In addition to soil sampling, ion-exchange resins [i.e. PRSTM probes (Western Ag Innovations, Saskatoon, SK, Canada)] were used to assess the soil solution ionic activity (NO₃⁻-N, NH₄⁺-N, H₂PO₄⁻-P, Ca²⁺, Mg²⁺, K⁺, Al³⁺, Fe³⁺, Mn²⁺, Cu²⁺, Zn²⁺, Pb²⁺, and Cd²⁺). The PRSTM probes allow for a dynamic measurement of ions flowing through the soil over time, and have been frequently used for forest ecology research (Bilodeau-Gauthier et al., 2013; Moukoui et al., 2012). In each ExP, 3 anion and 3 cation PRSTM probes were inserted vertically using a soil knife in the mineral soil surface (0 – 5 cm depth) directly under the amendments and at a 10 cm depth (Annexe A) after the amendment was carefully removed and placed back. Prior to sludge application in 2013, probes were inserted vertically within the top mineral soil (0 – 5 cm depth) only. The probes were left in the soil for a period of four weeks each

time in the fall of 2013, spring of 2014, fall of 2014 and spring of 2015. Upon collection, excess soil was removed from the probes with a knife. Cation and anion probes from each ExP were placed in a single bag. Probes were brought back to the laboratory where they were immediately cleaned with deionized water and placed in clean bags in a cooler and sent to Western Ag laboratories for analysis.

White spruce seedling height (cm) and diameter at the collar (mm) were measured at the time of planting in June of 2014. Height and diameter were measured once more for all seedlings and needles were collected and pooled within each ExP for foliar nutrients in October of 2014 and August of 2015.

2.4.4 Laboratory analysis

Upon collection, organic amendments and mineral soil samples were placed in a refrigerator (4°C) pending analyses. With the exception of microbial C and N which were done within a month after collection (described later), analyses of fresh samples were done within 7 days of collection. For each fresh sample, a subsample was weighted and oven-dried (at 65°C for amendments, and 105°C for mineral soil samples) for 48 hours to calculate moisture level and sample dry weight (g) using the following equations:

$$\text{Moisture level (\%)} = \left(\frac{\text{Sample wet weight (g)} - \text{Sample dry weight (g)}}{\text{Sample dry weight (g)}} \right) \times 100$$

$$\text{Sample dry weight (g)} = \text{sample weight (g)} - (\text{sample weight (g)} \times \text{moisture level})$$

2.4.4.1 Bulk chemical composition

Subsamples of organic amendments and mineral soil samples were air-dried, sieved at 2 mm and finely ground for total N and organic C analysis (Bremney, 1996). Carbon and N concentrations were then determined on these samples by combustion and infrared detection using a EA1108 CHNS-O Analyzer (Thermo Fisons, MA, USA).

Bulk chemical composition (P, K, Ca, Mg, Al, Mn, Cu, Zn, Cd, Pb, As) of organic amendments was determined by X-ray fluorescence spectrometry using a S8 Tiger WD XRF (Bruker, Billerica, MA, USA) equipped with a high-intensity X-ray tube operating at 4 kW. The fused beads were prepared from a 1:10 soil:lithium tetra(meta)borate mixture which was heated for 18 min at 1000°C.

2.4.4.2 NMR shift regions

For each amendment type (i.e. fresh sludge, mature sludge and forest floor), one air-dried and sieved (2 mm) sample was randomly selected from samples collected in the spring of 2015. The dry solid samples were ground and packed into Bruker 4 mm thin-walled rotors. Solid-state ^{13}C cross-polarization with magic angle spinning (CP-MAS) NMR spectra were acquired using a Bruker Avance 300 spectrometer ($B_0 = 7.05\text{ T}$, $\nu_L(^{13}\text{C}) = 75.5\text{ MHz}$). Spectra were acquired using the following parameters: ^1H 90° pulse length of 4 μs , a cross-polarization contact time of 1 ms, acquisition time of 25.6 ms, ^{13}C transmitter offset of 150 ppm, and a sweep width of 40 kHz. The samples were spun at 10 kHz. A recycle delay of 5 s was used between scans, and approximately 7800, 22600 and 24600 scans were collected for the fresh sludge, forest floor and mature sludge, respectively. A line-broadening factor of 100 Hz was applied to the three spectra. ^{13}C NMR spectra were referenced to tetramethylsilane (TMS) at 0 ppm using adamantane as an external reference (38.56 ppm). Bruker's TopSpin™ package for NMR data analysis was used to estimate the integrated areas for seven chemical shift regions (Table 2.3) according to Nelson and Baldock (2005).

2.4.4.3 Microbial respiration

Respiration of organic amendments and mineral soils was measured by the soda lime method (Keith and Wong, 2006). Fresh samples (100 – 200 g of organic amendment and 300 – 400 g of mineral soil) were incubated for 24 hours at a constant temperature (18.5°C) in sealed polyethylene containers (14 cm in height and 9 cm in diameter) with dried and weighted soda lime granules placed in open-topped glass jars.

Subsequently, soda lime granules were oven-dried at 105°C for 14 hours and weighted again. To account for CO₂ absorbed by the soda lime granules due to leakage from polyethylene containers during the incubation, blank measurements were made in 9 empty sealed polyethylene containers in which soda lime was placed. Mineralized carbon (CO₂ (g)) over a 24 h period was estimated using the following equations:

$$\begin{aligned} \text{Soil CO}_2 \text{ efflux (gC m}^2 \text{ day}^{-1}) &= \\ &\left(\frac{(\text{soda lime weight gain (g)} - \text{mean blank weight gain (g)}) \times 1.69}{\text{chamber area (m}^2\text{)}} \right) \times \left(\frac{24 \text{ (h)}}{\text{time of exposure}} \right) \\ &\times (12/44) \\ \text{Mineralized C (mg CO}_2 \text{ gC}^{-1} \text{ day}^{-1}) &= \frac{\text{Soil CO}_2 \text{ efflux (gC m}^{-2} \text{ day}^{-1})}{\text{Soil dry weight (g)}} \end{aligned}$$

2.4.4.4 Nitrogen mineralization potential

Nitrogen mineralization potential was measured on organic amendments and mineral soil samples according to Curtin and Campbell (2008). Fresh samples (5 g) were incubated under anaerobic conditions at 40°C for 7 days in 50 ml deionized water. Samples were then extracted with 50 ml 2 M KCl. Initial NH₄⁺ concentrations (i.e. prior to incubation) were determined on equivalent subsamples (10 – 15 g) extracted with 100 ml 1 M KCl. Extracts were analyzed colorimetry for NH₄⁺ using a QuickChem 8500 series 2 FIA System (Lachat Instruments, Loveland, CO, USA). The potential net N mineralization rate is calculated as the difference between final and initial subsample concentrations over the total N level.

2.4.4.5 Microbial biomass

Microbial biomass C and N (MBC and MBN) were determined on fresh samples collected in the fall of 2015 using the chloroform fumigation-extraction method (Jenkinson, 1982). Each sample (5 – 10 g of forest floor and 10 – 15 g of sludge) was weighed in triplicates. Non-fumigated samples were extracted with 0.5 mol L⁻¹ K₂SO₄. Remaining samples were placed in the dark in a vacuum-sealed desiccator

with 50 mL chloroform for 24 h, and then extracted with a 0.5 mol L⁻¹ K₂SO₄ solution. Total organic C (TOC) measured as non-purgeable organic C was determined by acidifying and then sparging the samples to strip off purgeable organic and inorganic C. Samples were placed in a combustion tube with a catalyst material in order to detect the CO₂ that had evolved from the redox reaction using a non-dispersive infrared detector for C. Total N (N_t) was measured by combustion of NO and reaction with O₃ to form NO₂, and measurement of the resultant photon emission by a chemiluminescence detector. Total organic C and N_t were determined on these samples with a Shimadzu TOC-V CHS/CSN Model Total Organic Carbon Analyzer (Shimadzu Corporation, Kyoto, Japan). Both MBC and MBN were estimated using the following equations (Jenkinson, 1982) without using a correction factor (k_{EC} or k_{EN}) (McMillan et al., 2007):

$$MBC = DOC_{fumigated} - DOC_{unfumigated}$$

$$MBN = N_{t-fumigated} - N_{t-unfumigated}$$

2.4.4.6 Soil nutrient and trace-metal supplies

PRSTM probes were eluted using a 0.5N HCl solution. Ammonium (NH₄) and NO₃ concentrations were determined colorimetrically with an automated flow injection analysis system, whereas all other ions were analyzed using inductively coupled plasma spectrometry (Western Ag Innovations inc., 2010).

2.4.4.7 Mineral soil physical properties

To assess soil macroporosity, undisturbed mineral soil samples collected with the double cylinder soil sampler were placed in water in a vacuum-sealed desiccator for 48 h to reach saturation and then weighted (W_1) (Klute, 1986). Soil samples were then placed in a sandbox apparatus for 48 h (Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands) to reach equilibrium at a tension of -10 kPa (field capacity) and weighted again (W_2). Samples were then oven dried (105°C, 48 h) and

weighted (W_3) a third time for bulk density measurements. Bulk density, macroporosity and total porosity were estimated with the following equations:

$$\text{Bulk density (g/cm}^3\text{)} = \frac{W_3 \text{ (g)}}{100 \text{ cm}^3}$$

$$\text{Macroporosity (\%)} = \frac{(W_2 - W_1)}{100 \text{ cm}^3} \times 100$$

$$\text{Total porosity (\%)} = W_2 - W_3$$

2.4.4.8 Seedling foliar nutrients and growth

White spruce needles were oven dried at 40°C to a constant weight. For each sample, two hundred needles were weighted before they were finely ground. A mean mass per needle was calculated for each sample. Total N and organic C concentrations were determined with the EA1108 CHNS-O Analyzer. Ground samples were also digested with concentrated HNO₃ to determine Ca, K and Mg concentrations using atomic absorption spectroscopy (model AA-1475, Varian, Palo Alta, CA, USA), whereas P was analyzed colorimetrically (molybdate based method) using the QuickChem 8500 series 2 FIA System.

2.4.5 Statistical analysis

Data were analyzed according to a completely random experimental design. Linear mixed models with random effects were used to account for subsampling at the Exp scale (random effect). Variance estimates were based on the maximum likelihood and significance of treatment effects on the Type 1 test of hypothesis. Individual analyses were conducted for each sampling period. Mean comparisons using *a priori* contrasts were conducted among treatments (control, fresh sludge, mature sludge and forest floor) and amendment thickness (15 cm versus 25 cm) in sludge-amended plots. Contrasts associated with thickness were eventually removed from the analyses because it had no significant effect (i.e. $p > 0.05$) on soil properties.

A priori contrasts (df = 3) used to test for significantly different properties between organic amendments were: (1) forest floor versus sludge amendments, and (2) fresh

sludge versus mature sludge. *A priori* contrasts ($df = 4$) were also used to assess whether there was a significant difference in properties between mineral soils under: (1) control versus sludge amendments, (2) forest floor versus sludge amendments, and (3) fresh sludge versus mature sludge. Since the forest floor plots were established in the spring of 2014 ($df = 3$), contrasts pertaining to fall of 2013 were: (1) control versus sludge amendments, and (2) fresh sludge versus mature sludge.

Normality and equal variance were assessed for each variable using the Anderson-Darling test for normality ($p < 0.05$) and graphical tools (QQ plot, distribution of residuals). Data were log-transformed when required. Data were analysed with the R freeware (R Core Team, 2015) using the *nlme* (Linear and nonlinear mixed-effects models) and the *gmodels* (Various R programming tools for model fitting) packages.

2.5 Results

2.5.1 Organic amendments

2.5.1.1 Bulk chemical composition

Organic C concentrations (OC) ranged from 397 to 415 mg g⁻¹ in the forest floor material and from 68 to 113 mg g⁻¹ in the sludge, whereas total N (N_t) concentrations varied from 15.6 to 18.6 mg g⁻¹ and from 4.4. to 7.5 mg g⁻¹, respectively in the forest floor and the sludge (Table 2.4). For the duration of the experiment, OC and N_t concentrations as well as C/N ratios remained significantly higher in the forest floor than in the sludges. With the exception of OC at the first sampling date, mature sludge had significantly lower OC and N_t concentrations than fresh sludge at all sampling dates, with OC concentrations being 27% and 34 % higher in the fresh sludge than in the mature sludge in the fall of 2014 and in the spring of 2015, respectively. Fresh and mature sludges had similar C/N ratios from fall of 2014 and onward.

Phosphorous, Mg, Al and Cu concentrations were significantly higher in the sludges than in the forest floor, while K, Ca, Mn, Cd and Pb concentrations were significantly higher in the forest floor than in the sludges (Table 2.5). Mean P concentrations in the sludges ranged from 1743 to 2116 mg kg⁻¹; K concentrations from 447 to 574 mg kg⁻¹, Ca from 6295 to 6504 mg kg⁻¹ and Mg from 2395 to 2700 mg kg⁻¹. No significant difference were found in Zn and As concentrations among all organic amendments and in the concentration of all elements measured between fresh and mature sludges. Trace metal concentrations remained below maximum limit values according to legislation (Table 2.5, MDDEP, 2012)

2.5.1.2 NMR spectroscopy

The O-alkyl C region of the NMR spectra was the dominant region for all organic amendments, but the relative abundance of O-alkyl C differed among treatments with the forest floor being the lowest and the fresh sludge being the highest (Fig. 2.2, Table 2.6). The sharpest peak for all three amendment types was located at 73 ppm, which is indicative of polysaccharides making up cellulose and hemicelluloses (Quideau et al., 2001).

The forest floor was richer in alkyl C than both sludges (Table 2.6). The peak at 30 ppm observed for the forest floor that corresponds to the methylenic C in long chain aliphatic compounds (Bartoszek et al., 2008; Kögel-Knabner, 1997) found in waxes and cutins, polyesters of roots and bark, condensed tannins and sidechains of proteins (Preston, 1996). The small peaks in the 20 – 30 ppm region for the sludges are associated to C-CH₃ moieties (Keeler and Maciel, 2000), and the sharper peak at 22 ppm likely corresponds to terminal methyl groups of alkyl chains and to acetyl methyl groups in hemicelluloses (Keeler and Maciel, 2000).

The mature sludge had the highest abundance of aromatics and a major peak at 135 ppm corresponds to C-substituted aromatic carbons (Quideau et al., 2001). Relative

abundance in phenolic C was also highest in the mature sludge. Peaks at 148 ppm for all three amendment types correspond to C₃ in guaiacyl units of condensed and hydrolysable tannins and lignins (Preston, 1996). All spectra have a peak at 56 ppm in the N-alkyl/methoxy C region which is indicative of O-CH₃ or methoxyl in lignin. N-alkyl C was more abundant in the sludges than in the forest floor. Aromaticity was higher in the mature sludge than in the fresh sludge.

2.5.1.3 Carbon and nitrogen mineralization

Over the study period, C mineralization rates in organic amendments ranged from 2.89 to 9.15 mg CO₂ g C⁻¹ day⁻¹. Few significant differences in mineralization rates were observed among amendments. The fresh sludge had a higher mineralization rate than the mature sludge in the spring of 2014, while the sludges had a significantly higher C mineralization rate than the forest floor in the fall of 2014 (Table 2.7). Net N mineralization potential in organic amendments ranged from 3.10 to 7.17 mg N g⁻¹ (Fig. 2.3a, Table 2.8). Again, few differences were found among amendments during the study period. However, in spring of 2014, the net N mineralization potential was 1.6 times higher in the fresh sludge than the mature sludge.

2.5.1.4 Microbial biomass

In the fall of 2015, MBC and MBN were significantly higher in the forest floor than in the sludges; MBC values were 4 and 5.8 times higher and MBN values were 4.5 and 6 times higher in the forest floor than in the fresh sludge and the mature sludge, respectively (Table 2.7). However, MBC:OC ratios derived from values reported in Tables 2.4 and 2.7 were quite similar between organic amendment types (0.33 – 0.37). The MBC:MBN ratios did not differ statistically among amendment types despite that the ratios were generally higher in the fresh sludge (Table 2.7).

2.5.2 Soils

2.5.2.1 Organic carbon and total nitrogen

In the mineral soil, OC, N_t concentrations and C/N ratios were not significantly different below all three amendment types at all measurement periods (Table 2.8). The values obtained for OC and N_t ranged from 25.1 to 47.7 mg g⁻¹ and from 1.93 to 4.17 mg g⁻¹, respectively, while the mineral soil C/N ratios ranged from 10.5 to 13.4.

2.5.2.2 Microbial respiration and potential nitrogen mineralization

Mineral soil respiration rates per C unit were not significantly different between organic amendments. Rates ranged from 2.8 to 10.6 mg CO₂ g C⁻¹ day⁻¹ over the study period (results not shown). Net N mineralization potential of mineral soils under the control, sludges and forest floor did not differ between the fall of 2013 and fall of 2014 (Fig.2.3b, Table 2.9). It ranged from 2.04 to 4.42 mg NH₄-N g N⁻¹. However, mineralization potential of NH₄-N was greater for soils receiving sludges compared to control soils in the spring of 2015 by a factor of 1.8 (Fig. 2.3b).

2.5.2.3 Nutrient supply rates immediately after organic amendments

Immediately following sludge application (i.e. fall of 2013), significant increases in nutrient supply rates below sludges were observed for NO₃⁻, PO₄³⁻, Ca²⁺ and SO₄²⁻ (Fig. 2.4, Table 2.10), while K⁺ supply was higher in the control than under the sludges. No difference in supply rates was observed between the control and the sludges for NH₄⁺, Mg²⁺ and Mn²⁺.

Comparing both sludges, higher supplies of NO₃⁻, NH₄⁺, PO₄³⁻ and Mg²⁺ were observed below the mature sludge than the fresh sludge. Hence, soils receiving the mature sludge supplied 14 times more NO₃⁻, 1.4 times more NH₄⁺, 3.8 times more PO₄³⁻ and 1.4 times more Mg²⁺ than the soils receiving the fresh sludge. Conversely, soil SO₄²⁻ and Ca²⁺ supply rates were significantly higher under the fresh sludge than under the mature sludge, while no significant difference in supply rates was observed between the two sludges for K⁺ and Mn²⁺. The soil SO₄²⁻ supply rate was 1.8 times higher under the fresh sludge than under the mature sludge.

2.5.2.4 Nutrient supply rates 1 to 2 years after organic amendments

In the spring following amendments application, soils under the sludges supplied significantly more, NO_3^- , PO_4^{3-} , SO_4^{2-} and Ca^{2+} than the soils under the forest floor and in the control. However, by the fall of 2014, supply rates of NO_3^- and PO_4^{3-} in control soils and of soils under sludges were no longer significantly different (Fig. 2.4, Table 2.10). Comparisons between the sludges and the forest floor led to similar results. However, soil NH_4^+ supply rate was higher under the forest floor than under the sludges in the fall of 2014 and higher in the control than under the sludges in the spring of 2015.

Soil Ca^{2+} supply rates remained significantly higher under the sludges than in the control until the fall of 2014, while no significant difference in Ca^{2+} supply rate was observed between the sludges and the forest floor after the spring of 2014. The supply rate of K^+ remained higher for the whole duration of the experiment in the control soils and in soils under the forest floor than in soils receiving the sludges. Soil Mg^{2+} supply rate was 1.24 times higher under the mature sludges than under the fresh sludge in the spring of 2014.

Soils receiving the sludges supplied more SO_4^{2-} than control soils for all of the study period and more than soils under the forest floor in the spring of 2014 and 2015. However, differences among treatments decreased with time and were largely due to the high supply rate observed under the fresh sludge rather than under the mature sludge. In the fall of 2014, soil SO_4^{2-} supply rate was 2.3 times higher under the fresh sludge than under the mature sludge, while no difference between sludges was observed in the fall of 2015.

Besides differences in soil SO_4^{2-} supply rates, few significant differences were observed between the sludges after the fall of 2013. Soil Mn^{2+} supply rate was significantly higher under the forest floor than under the sludges in the spring of 2015.

2.5.2.5 Trace metal supply rates

Immediately following organic amendment application (i.e. fall of 2013), soil supply rates of Cu^{2+} , Zn^{2+} and Pb^{2+} were significantly higher under the sludges than in the control soils (Table 2.11, Fig. 2.5). Initially, soil Cu^{2+} , Zn^{2+} and Pb^{2+} supply rates were respectively 4.1, 20 and 6.5 times higher under the sludges than in the control soils. These differences remained significant until the end of the experiment in the spring of 2015. Comparison between the sludges and the forest floor led to similar results until the spring of 2015, whereas soil Zn^{2+} and Cu^{2+} supply rates under the sludges were similar to those under the forest floor. With the exception of higher soil Pb^{2+} supply rate in the fall of 2013 under the mature sludge compared to the fresh sludge, no significant difference in trace metal supply was observed between the sludges. No difference was found among treatments in the soil supply rate of Al^{3+} , whereas soil Cd^{2+} supply rate was negligible under all treatments (data not shown).

2.5.2.6 Soil physical properties

No significant difference among treatments was observed for bulk density, macroporosity and total porosity of the top mineral soil (0 – 5 cm) (Table 2.12). Soil bulk density, macroporosity and total porosity ranged from 1.08 to 1.28 g cm^{-3} , 5.82 to 9.90 % and 49.0 to 55.2 %, respectively, over the study period.

2.5.3 Foliar nutrition and seedling growth

At the end of the first growing season, foliar N, P, K and Ca concentrations of white spruce seedlings were higher in sludge amended plots than in the control plots by factors of 0.87, 2.1, 3.4 and 1.6, respectively (Table 2.13). Significantly higher foliar N concentrations were observed in the forest floor amended plots than in the sludge amended plots, while no significant difference was found for P, K Ca and Mg. No difference in foliar concentrations was observed between seedlings in the sludge amended plots, except for K which was higher under the fresh sludge than under the

mature sludge. Finally, treatments had no significant effect on foliar Mg concentrations.

At the end of the second growing season, foliar Ca and Mg concentrations were significantly higher in the sludge than in the control plots, but did not differ significantly from the forest floor plots. Foliar Mg concentrations were higher in the mature than fresh plots in 2015.

At the end of the first growing season (i.e. 2014), no difference in seedling total height, yearly height growth and diameter was observed among treatments. However, seedling annual height growth and relative height growth measured at the end of the second growing season (i.e. 2015) were higher in the sludge amended plots than in the control plots. No difference in growth was observed between the sludge and forest floor amended plots or between the fresh sludge and mature sludge.

2.6 Discussion

2.6.1 Chemical characteristics and microbial activity of organic amendments

The dehydrated sewage sludges used in this study have low OC and N_t concentration in comparison to other municipal biosolids. As a whole, municipal sewage sludges contain between 200 and 500 mg g⁻¹ of OC and between 20 to 50 mg g⁻¹ of N_t (Table 2.4; Haynes et al., 2009; Torri et al., 2014b; Weetman et al., 1993). The dominance of O-alkyl groups in sewage sludge amendments indicates a high carbohydrate content, which is in agreement with the findings of several authors for sewage sludges (e.g. Rowell et al., 2001; Smith et al., 2008; Zbytniewski et al., 2002). Carbohydrates from fibers are the main component of primary sewage sludge (Jimenez et al., 2013), and include sugars and organic acids that are readily available for uptake by microorganisms (Lessa et al., 1996).

Despite having similar C/N ratios to those of the fresh sludge on most sampling periods, the mature sludge contained less OC and N_t , and a higher proportion of OC

was composed of aromatic and phenolic C (Tables 2.4 and 2.6). Greater proportions of aromatic and phenolic C structural groups, as well as higher aromaticity, are indicative of more recalcitrant C such as in lignin (Preston 1996). They also imply a greater degree of maturity associated with humification, which results from the decomposition of more labile aliphatic compounds during storage (Bartoszek et al., 2008). Conversely, a higher proportion of aliphatic compounds in the fresh sludge and its lower aromaticity indicate the greater abundance of labile C.

Microbial biomass has been shown to increase with increasing nutrient availability (Joergensen et al., 1995). Accordingly, the lower microbial biomass in the mature sludge in comparison to fresh sludge was consistent with its lower OC concentrations and higher aromaticity (Flavel and Murphy, 2006). The higher C mineralization rate and N mineralization potential of fresh sludge observed early in the experiment was likely caused by its higher labile C availability and higher MBC, whereas lower C availability in the mature sludge likely limited organic matter mineralization (Lessa et al., 1996). However, differences in C and N mineralization rates between sludges were of short duration and disappeared in the fall of 2015.

Relative to the sewage sludge, the forest floor material had a high OC concentration and C/N ratio. Similarly to our findings, (Zbytniewski and Buszewski, 2005) found the alkyl C, O-alkyl C and carbonyls C regions of the NMR spectra to be dominant in forest floor material. It is probable that the higher alkyl C content of the forest floor compared with sludges (Table 2.6) resulted from the decomposition of leaf constituents such as lipids and cutins (Kögel-Knabner, 2002) and amino-acids derived from microbes (Miltner et al., 2009). These compounds are generally resistant to decomposition. Carbonyls are functional groups of humic acids and their abundance has been reported to increase with increasing organic matter humification and decomposition (Bartoszek et al., 2008). In contrast, O-alkyl C components typically decrease as a result of decomposition and were found in somewhat higher proportions in the sludges (Baldock et al., 1997; Kögel-Knabner, 1997). However,

higher N-alkyl content of the sludges compared to that of the forest floor could be caused by their high protein content (Jimenez et al., 2013). These findings indicate that the forest floor amendment may be more stable, as a result of organic matter humification (Caricasole et al., 2011), than the sludges. Higher stability may be beneficial for soil restoration because nutrient mineralization and subsequent release occurs more slowly, which could translate into more long-lasting effects (Larney and Angers, 2012).

Given the differences in the origin of amendments, large differences in MBC and MBN and microbial activity were expected (Dilly, 2004). However, the higher microbial biomass in the forest floor only reflected its greater C and alkyl C concentrations (Miltner et al., 2009), and the proportion of MBC to OC was similar between amendments. The MBC:MBN ratios did not differ among organic amendments and values ranging from 8 to 9.21 indicated the dominance of fungi over bacteria in all three amendment types (Paul and Clark, 1996). Moreover, few differences in C mineralization rates and no difference in net N mineralization potential were observed among organic amendments. Low C mineralization rate and N mineralization potential were expected in the forest floor due to its higher proportion of recalcitrant organic matter (Lessa et al., 1996) compared to the more bioavailable organic matter in sewage sludge. It is possible that anaerobic incubation took place within sludge piles before they were sieved and applied onto the soil surface, thus leading to a decrease in labile organic matter (Barrena et al., 2011) and mineralization of C and N (Wang et al., 2003). Additionally, at high metal concentrations, especially Cu and Zn, microbial inhibition may occur in sewage sludge due to the destructive effects of these metals on enzymes (Kao et al., 2006; Khan and Scullion, 2002). This can lead to decreased MBC and MBN and microbial activity. Complexation of metals with sewage sludge organic matter may also result in lower C mineralization rates in amended soils (Kao et al., 2006).

2.6.2 Soil organic carbon and total nitrogen

Sludge application rates were very high and amounted to net C additions that were 7 to 14 times higher than with the forest floor treatment (see Table 2.2). It also resulted in net N additions that were 13 to 25 times higher than with the forest floor treatment despite that the forest floor had the highest N_t concentrations (Table 2.4). Yet, few differences in mineral soil C and N mineralization and concentrations were observed among treatments.

Although OC and N_t concentrations varied in the mineral soil throughout the experiment, all treatments had returned to their initial concentration by the second year of the experiment. Egiarte et al. (2005) and Martínez et al. (2003) also found soil OC and N_t to decrease or to remain unchanged one to three years after sludge application. This response was explained by an increase in the decomposition rate of soil organic matter within the mineral soil due to the N added from the sludge. The incorporation of organic matter to soils may induce the rapid mineralization of native soil C and N (Woods et al., 1987), also known as the priming effect (Dalenberg and Jager, 1989; Fontaine et al., 2003), thus inducing soil C and N losses and preventing sequestration (Gibbs et al., 2006). As a result, the effect of sludge on C accumulation in amended soils may be delayed by three to four years after application (White et al., 1997; Martínez et al., 2003).

However, neither C mineralization rates nor OC concentrations in the mineral soil were affected by sludge and forest floor amendments. In clayey soils with small pore size ($<75\mu\text{m}$), organic substrates may be protected from degradation by soil organisms (Mtambanengwe et al., 2004). As indicated in the previous section, complexation of metals with organic matter in sewage sludge may prevent C mineralization by microorganism in amended soils (Kao et al., 2006). Additionally, root establishment into the mineral soil of the vegetation present may have been reduced due to the high sludge thickness ($>15\text{cm}$) created in our experiment. Roots

are an important source of soil organic matter and CO₂ via respiration (Singh et al., 2009).

2.6.3 Soil nutrient supplies

As expected, sludge application increased the supply of some essential nutrients, (i.e. N, P, S and Ca) at the mineral soil surface (0 – 5 cm), although this effect had diminished by the second year of the experiment. The nutrient pulse following sludge application is associated with rapid mineralization and transformation of the organic nutrient fraction in the sludge (Epstein, 2002d). High NO₃⁻, PO₄³⁻ and SO₄²⁻ supplies observed shortly after sludge application (fall of 2013) also reflected high sludge application rates. In our study, sludge application rates exceeded 700 t ha⁻¹, while the highest sewage sludge field application rates to forest soils in other studies ranged from 0.28 to 500 t ha⁻¹ (Bramryd, 2002; Cavaleri et al., 2004; Dumbrell and McGrath, 2002; Harrison et al., 2005; Rowell, 1996; Varela et al., 2011). However, the two sewage sludge application rates (i.e. 15 cm and 25 cm thicknesses) did not differ in regard to nutrient supply rates. In spite of our efforts to apply the sewage sludge uniformly over each plot, the thicknesses varied within plots (as reflected by the wide range of minimum and maximum thicknesses, Table 2.2). The occasional overlap in thicknesses between sludge treatments may explain why we were unable to detect differences in nutrient supplies.

2.6.3.1 Nitrogen

The NO₃⁻ supply rate of the top mineral soil (0 – 5 cm) was higher in the sludge amended plots immediately after application than in the control plots, possibly due to more favorable conditions for nitrification, namely the higher pH of the sludge (Sahrawat, 2008; Schmidt, 1982). However, most of the difference was caused by the high rates observed under the mature sludge. The higher soil NO₃⁻ supply rates below the mature sludge when compared to the fresh sludge may be the result of oxygenation of a material that stored with poor aeration for five years. Manipulations

(screening, transport and spreading) of the material may have induced rapid organic N mineralization due to increased O_2 availability for microbes (Sahrawat, 2008). However, higher soil NO_3^- supply rates under sludges were of short duration. Our results are similar to other studies that have reported initial soil NO_3^- pulses following sludge application that diminished within one to two years (Grey and Henry, 2002; Hallett et al., 1999; Martínez et al., 2003; Robinson and Polglase, 2005).

In contrast to the sludges, the forest floor and control plots supplied more soil NH_4^+ than NO_3^- . The supply rate of NH_4^+ was also higher in the control plots and under the forest floor than under sludges for a longer period. Nitrification in soils is controlled by NH_4^+ availability and occurs when soil NH_4^+ concentrations exceed plant and microbial uptake (Robertson and Groffman, 2015). However, in boreal forest soils, nitrification may be low even at high NH_4^+ availability due to acidic conditions limiting the presence of nitrifiers and hindering nitrification (Ste-Marie and Paré, 1999). The relatively higher soil NO_3^- supply rates compared to that of NH_4^+ in the sludge amended plots suggests rapid use of NH_4^+ by nitrifying bacteria, thus resulting in lower soil NH_4^+ supplies.

2.6.3.2 Phosphate

The mature sludge released high levels of PO_4^{3-} within the top mineral soil in the month following application. McLaren et al. (2007) and Wang et al. (2004) both reported an initial increase in soil P availability following the application of high loads of digested sludges in *Pinus radiata* plantations. However, both studies applied sludges to sandy soils, which generally have low sorption capacity (Haynes et al., 2009). Phosphorus leaching is generally limited in acidic ($pH < 5.5$) and fine-textured forest soils due to retention of PO_4^{3-} by adsorption/precipitation onto/with amorphous oxides of iron and aluminum (Johnson et al., 1986), and soil colloids (Haynes et al., 2009). High soil PO_4^{3-} in the sludge amended plots may therefore be due to saturation from the high application rates resulting in increased potential for P release (McDowell et al., 2001), presumably from acidification of the soil solution induced

by nitrification, and a high soil P to Al ratio exceeding critical values (Pellerin et al., 2006). Similarly, Islas-Espinoza et al. (2013) found P leaching to increase with application rates in an acidic sandy clay loam soil. In their study, the largest P pulse was observed 15 days after sludge application.

2.6.3.3 Sulphate

Soil SO_4^{2-} supply rates were higher in the sludge amended plots than the forest floor amended and control plots. This difference was mostly due to the very high supply rates under the fresh sludge. A large proportion of S in sewage sludge can be mineralized readily (Tabatabai and Chae, 1991). Once applied to the soil surface, microbial activity may induce sludge acidification through the oxidation of S by S-oxidizing bacteria, thus leading to S mobilization (Qureshi et al., 2003). In a laboratory sewage sludge column leaching study, Qureshi et al. (2004) associated the greatest SO_4^{2-} release to an increase in acidity induced by microbial oxidation and respiration rates. Heavy leaching of SO_4^{2-} from sewage sludge has also been reported in loamy and sandy soils, and linked to water flowing through macropores (McLaren et al., 2003). On the other hand, H_2S may be released and lost to the atmosphere during storage and dewatering of the sludge (Dewil et al., 2009). Sludge storage time may thus explain the lower soil SO_4^{2-} supply rates in the mature sludge amended plots compared to the fresh sludge amended plots.

Zhang et al (2006) also reported increased SO_4^{2-} leaching shortly after the application of high loads of sewage sludge and progressive lowering of SO_4^{2-} activity in the soil solution over the following two years. They attributed the reductions in SO_4^{2-} activity to precipitation with Ca^{2+} as SO_4^{2-} moved down the soil profile. Since the high soil SO_4^{2-} supply rates in the fresh sludge amended plots were recorded only at a shallow depth (i.e. 5 - 10 cm depth), it is possible that SO_4^{2-} eventually precipitated with Ca^{2+} as it continued to leach deeper down the soil profile.

2.6.3.4 Calcium, potassium and magnesium

Sewage sludge generally contains small amounts of Mg and K (Epstein, 2002d), which may explain the low soil supply rates of these nutrients in the sludge amended plots. Potassium is highly soluble in water and consequently, a large amount may be lost to the sewage effluents during dewatering (Haynes et al., 2009). The remaining K^+ should be readily available for plant uptake. In contrast, the sewage sludge amendments increased soil Ca^{2+} supply rates in comparison to the control soils during the first year following application. This was observed despite the high exchangeable Ca status of the clayey soil at the study site (Brais et al., 1995).

2.6.4 Soil trace metal supplies

Sewage sludge application increased trace metal bioavailability in the mineral soil at a 0 – 5 cm depth as shown by consistently high supply rates in comparison to the control soil. Other studies have found increased metal concentrations at the soil surface (Harrison et al., 2005; McLaren et al., 2007; Yang et al., 2014; Zhang et al., 2006) and in soil leachates (McLaren et al., 2004) as a result of sewage sludge application, especially at high application rates (Brenton et al., 2007; Zhang et al., 2006).

The solubility of most trace metals including Zn, Cu and Pb increases at low pH (McBride et al., 2004; Sauvé et al., 1997; Torri and Corrêa, 2012). However, trace metal mobility and availability can decrease with the formation of Al, Fe and Mn hydrous oxides, chelates and phosphates that bound trace metals as well as in soils with high organic matter concentrations due to sorption of metals onto organic particles (Chuan et al., 1996; Epstein, 2002e).

Until the spring of 2015, soil trace metal supply was higher in the sludge amended plots compared to the forest floor amendments plots. Low organic matter concentrations in the sludge amended plots and very high application rates relative to the forest floor may have resulted in the addition of trace metals in amounts that

exceeded the adsorption capacity of the mineral soil despite its high clay content (Alloway, 1995; Gascó et al., 2007). Moreover, trace metal solubilization and release into the soil solution may have been accelerated in the sludge amended plots in the year following application because of increased nitrification, S oxidation, and rapid decomposition of readily available organic matter can all contribute to sludge and soil acidification (Speir et al., 2003; Stacey et al., 2001). Besides lower application rates, metal binding onto exchange sites that arise from the dissociation of carboxylic and phenolic functional groups present at the surface of humic and fulvic acids and complexation with organic matter likely account for the low trace metal concentrations in the soil solution in the forest floor amended plots (Hooda, 2010).

By the end of the experiment, however, soil Cu and Zn supply rates were no longer significantly different in the plots receiving sludges compared to soils in the forest floor amended and control plots. A large amount of the mobilized metals in the sludges had already been released and leached to lower soil depths early in the experiment, thus contributing to lower trace metal supplies later in the experiment.

2.6.5 Soil physical properties

Soil physical properties were not improved by sludge application. Aggelides and Londra (2000) found bulk density to decrease and porosity to increase proportionally with application rates in a soil amended with a mixture of sewage sludge and urban waste. However, the effects were not as large in the clayey soil compared to the loamy soil. Changes induced by sewage sludge application may take place more slowly in fine-textured than coarse-textured soils (Khaleel et al., 1981). For instance, previous studies have found no effect of sludge on top soil bulk density up to 5 years after a one-time surface application to a silt loam soil (Wallace et al., 2009) and up to 8 years after annual applications to silt loam and silty clay loam soils (Jin et al., 2015).

Bioturbation by earthworms and other larger fauna has been shown to contribute to soil structure and macroporosity improvement in compacted soils (Lal, 1991, 1988; Lal and Kimble, 2000). Formation of stable soil aggregates by soil fauna also enhances soil C sequestration (Jiménez and Lal, 2006; Lavelle et al., 2006) and is thus key to restoring soil structure. However, boreal soils with their mor humus form are devoid of macrofauna (Ponge et al 2002), which results in very little vertical mixing (Lindahl et al., 2007; Trumbore and Harden, 1997). The absence of mixing could very well explain the lack of changes in soil structure following organic amendments.

White spruce root systems are thought to be restricted to the upper 15 cm of soil (Safford and Bell, 1972) and tend to develop laterally (Strong and Roi, 1983). In our experiment, white spruce seedlings most likely did not reach the mineral soil in sludge amended plots due to the sludge layer exceeding a thickness of 15 cm. As a result, bioturbation by roots and C additions from root exudates (Jiménez and Lal, 2006) were likely limited. Moreover, in compacted soils, soil bulk density above 1.2-1.4 mg m⁻³ may limit root growth (Gebauer and Martinkova, 2005; Rosolem et al., 2002). Since root activity contributes to improving soil physical properties (Reisinger et al., 1988), the fact that the growth of roots was already hindered in the control probably did not help in regard to exhibiting the benefits of sludge application on mineral soil bulk density and porosity.

2.6.6 White spruce seedling foliar nutrition and growth

Sludge application improved white spruce seedling growth in the second growing season (2015) in comparison to the control. Increased growth could have resulted from higher soil nutrient availability and improved foliar nutrition observed the previous year (2014). Similarly, Bramryd (2002) reported increased foliar N and P concentrations in Scots pine needles as well as improved growth in the year following the application of 20 tons ha⁻¹ of sewage sludge. Weetman et al. (1993) also found a

positive correlation between sludge application rates and both soil and pine needle nutrient concentrations.

Moreover, as a result of the thick layer of sludge in the experimental plots, seedlings were rooted mainly in the sludge material and their roots barely reached the mineral soil. As such, the improved growth in white spruce seedlings could be linked to a greater rooting ability in a softer substrate (i.e. sewage sludge), while the highly compacted clay soil likely hindered root development in the control (DesRochers and Tremblay, 2009; Larcheveque et al., 2011). Greater moisture retention in both the sludge and forest floor plots could also have enhanced growth conditions.

In spite of overall lower supply of essential nutrients in soils receiving forest floor, white spruce seedlings had a yearly growth and foliar nutrient concentrations comparable to that of sludge amended plots. Improved growth in forest floor amended soils may be attributed in part to higher NH_4^+ supplies in soils receiving forest floor than sewage sludge and in turn, to NH_4^+ preferential assimilation (Kronzucker, 1997) by white spruce as observed by the higher foliar N concentration in 2014. Moreover, the forest floor treatment may have enhanced soil nutrient bioavailability to white spruce seedlings, notably N and P, by allowing for mycorrhizae associations to develop more readily (Smith and Read, 2010).

2.7 Conclusions

Sewage sludge enhanced nutrient supplies and white spruce growth in a degraded forest soil two years after its application, but had limited effects on mineral soil properties. While rapid nutrient mineralization and release takes place shortly after sludge application, organic matter incorporation and improvement of soil physical conditions may take longer in these fine-textured boreal forest soils. Consequently, long-term effects of sewage sludge application need to be monitored to assess changes in soil C and N dynamics and physical properties.

Carefully selecting sludge application rates prior to their application to a forest soil may contribute to reducing the risks of a flush of nutrients such as N and P and trace metals upon application. For instance, taking into account site P requirements in planning sludge application rates (Lu et al., 2012) could contribute to limiting risks associated with P leaching and percolation into groundwater in acidic forest soils. Using stabilized and matured sewage sludge could reduce the risks of NO_3^- and PO_4^{3-} leaching and of trace metal contamination in the short-term, while ensuring long-term nutrient supplies.

Despite differences in their chemical structure and nutrient and trace metal supplies, sewage sludge and forest floor amendments had similar effects on foliar nutrition and tree growth. However, the lower nutrient supply under the forest floor suggests a more steady and gradual release due to its more recalcitrant organic matter. With application rates much lower than that of the sewage sludge, the forest floor provided nitrogen under a form that is more adapted to white spruce nutrition. Nonetheless, sewage sludge could replace the natural forest floor by providing essential nutrients and promoting seedling growth, although the risks associated with trace metal contamination should be closely monitored. Our findings also suggest that sewage sludge somewhat altered N dynamics in the mineral soil by favouring nitrification. However, we did not assess soil microbial community composition. Doing so might have improved our understanding of the effects of sewage sludge on soil biogeochemical processes.

2.9 References

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Table 2.1 Average physical and chemical properties of the mineral topsoil (0-10 cm) sampled at the study site and at an undisturbed location from a nearby natural stand.

	Bulk density (10 cm)	Total N (mg/g)	Organic C (mg/g)	C/N	pH*
Study site	1.28 (0.03)	2.00 (0.02)	23.0 (0.4)	11.2 (0.7)	5.89
Undisturbed soil	0.84*	6.80 (0.20)	118.0 (4.8)	15.4 (0.9)	5.09

Mean (SE). $N = 38$, Bulk density: $N = 135$. *Brais, unpublished data. Bulk density: $N = 45$, pH: $N = 15$.

Table 2.2 Initial sludge and forest floor characteristics and application rate.

	Fresh sludge (FS)		Mature sludge (MS)		Forest floor (FF)
<i>Parameter</i>	FS@15 cm	FS@25 cm	MS@15 cm	MS@25 cm	
C/N ^A	12.4 (0.3)		14.0 (0.3)		22.6 (0.9)
pH ^B (H ₂ O)	7.17 (0.05)		6.74 (0.05)		5.31 (0.06)
Thickness ^C (cm)					
mean	16.0 (5.0)	24.0 (1.6)	14.0 (0.4)	22.0 (1.1)	8.20 (0.8)
min	7.00	19.0	11.0	13.5	3.00
max	27.0	30.0	15.0	26.0	12.0
Avg. application rate ^C					
(<i>tm ha⁻¹</i>)	702 (123)	1043 (68)	818 (26)	1333 (67)	10.4 (0.9)
N dose ^C (<i>tm ha⁻¹</i>)	2.60 (0.36)	3.86 (0.36)	3.19 (0.38)	5.20 (0.38)	0.19 (0.02)
C content ^C (<i>tm ha⁻¹</i>)	33.6 (5.9)	49.9 (3.3)	42.2 (1.3)	68.8 (3.4)	4.55 (0.38)
SiO ₂ (<i>tm ha⁻¹</i>)	443 (15)		450 (15)		

Mean (SE). ^AN = 54; ^BN = 45; ^CN = 15.

Table 2.3 Chemical shift regions (ppm) of the CP-MAS ^{13}C spectra of organic amendments and their main structural group assignments (source: Nelson and Badock (2005)).

Spectra region	Carbon structural groups	Peaks (ppm)	Structural group assignments
7	Alkyl	0 – 42	Lipids, waxes
6	<i>N</i> -alkyl/methoxy	42 – 60	
5	O-alkyl	60 – 95	Carbohydrates
4	Di-alkyl	95 – 110	
3	Aromatic	110 - 140	Lignins, polyphenols
2	Phenolic	140 – 165	
1	Carbonyl	165 – 210	

Table 2.4 Forest floor and municipal sewage sludge properties measured at different periods during the experiment. Contrasts between forest floor (FF) and sludges (S) and between fresh sludge (FS) and mature sludge (MS) were assessed by means of mixed linear model based on a Type 1 test of hypothesis.

<i>Parameter</i>	Treatment means			Contrasts	
	Forest floor	Fresh sludge	Mature sludge	FF vs. S	FS vs. MS
Organic C (mg g ⁻¹)					
Spring 2014	415 (9)	98.0 (6.6)	86.0 (6.6)	***	NS
Fall 2014	438 (5)	93.2 (5.7)	68.3 (5.7)	***	**
Spring 2015	397 (8)	113 (5)	74.1 (5.3)	***	***
Total N (mg g ⁻¹)					
Spring 2014	18.6 (0.1)	7.50 (0.41)	5.78 (0.41)	***	***
Fall 2014	18.3 (0.4)	7.45 (0.30)	5.03 (0.30)	***	***
Spring 2015	15.6 (0.3)	6.61 (0.24)	4.36 (0.24)	***	***
C/N					
Spring 2014	22.6 (0.9)	12.9 (0.3)	14.7 (0.4)	***	*
Fall 2014	24.1 (0.5)	12.3 (0.4)	13.1 (0.5)	***	NS
Spring 2015	25.6 (0.6)	17.0 (0.4)	16.8 (0.5)	***	NS

Mean (SE), $N = 45$. Significant differences are designated as followed: (*) $p < 0.05$; (**) $p < 0.01$; and (***) $p < 0.001$, and NS (not significant).

Table 2.5 Forest floor and municipal sewage sludge bulk chemical composition (P, K, Ca, Mg, Al, Mn, Cu, Zn, Cd, Pb, As) measured in the spring of 2014. Contrasts between forest floor (FF) and sludges (S), and between fresh sludge (FS) and mature sludge (MS) were assessed by means of a linear model based on a Type 1 test of hypothesis.

<i>Parameter</i>	Treatment means (mg kg ⁻¹)			Contrasts		Max. value ^A (mg kg ⁻¹)
	Forest floor	Fresh sludge	Mature sludge	FF vs. S	FS vs. MS	
Al	2152 (766)	7112 (442)	6015 (442)	***	NS	NA
As	3.78 (1.77)	3.91 (1.02)	4.22 (1.02)	NS	NS	150
Cd	3.42 (0.47)	1.63 (0.27)	1.32 (0.27)	**	NS	10.0
Ca	17684 (2312)	6295 (1335)	6504 (1335)	***	NS	NA
Cu	50.3 (39)	165 (23)	135 (23)	*	NS	1000
Fe	2577 (851)	8715 (491)	7617 (491)	***	NS	NA
K	973 (121)	574 (70)	447 (70)	**	NS	NA
Mg	1580 (360)	2700 (208)	2395 (208)	*	NS	NA
Mn	614 (64)	170 (37)	147 (37)	***	NS	NA
P	674 (527)	2116 (304)	1743 (304)	*	NS	NA
Pb	64.8 (6.4)	48.1 (3.7)	41.4 (3.7)	*	NS	300
Zn	316 (95)	402 (55)	311 (55)	NS	NS	1850

Mean (SE). $N = 14$. Significant differences are designated as followed: (*) $p < 0.05$; (**) $p < 0.01$; and (***) $p < 0.001$, and NS (not significant). NA is not applicable. ^AMaximum trace metal limit values allowed in municipal sewage sludge according to legislation of the province of Quebec (MDDEP, 2012).

Table 2.6 Relative abundance (%) of C structural groups, alkyl:O-alkyl C ratio and aromaticity (%) of forest floor and sludges (%) measured by solid-state CP-MAS ^{13}C in the spring of 2015.

	Forest floor	Fresh sludge	Mature sludge
Alkyl	21.5	15.4	13.1
N-alkyl	10.2	14.1	13.6
O-alkyl	32.6	37.2	35.4
Di-O-alkyl	7.23	7.34	7.78
Aromatic	12.9	13.6	17.8
Phenolic	7.57	7.68	9.77
Carbonyl	7.94	4.69	2.69
Aromaticity ^A	22.3	22.3	28.3

$N = 3$. ^A [Aromatic C (110-165ppm) / (Aromatic C (110-165ppm) + Aliphatic C (0-110ppm))] $\times$ 100 (source: (Bartoszek et al., 2008)).

Table 2.7 Forest floor and municipal sewage sludge C and N microbial biomass measured in the fall of 2015, and respiration measured at different periods during the experiment. Contrasts between forest floor (FF) and sludges (S) and between fresh sludge (FS) and mature sludge (MS) were assessed by means of mixed linear model based on a Type 1 test of hypothesis.

Parameter	Sampling	Treatment means			Contrasts	
		Forest floor	Fresh sludge	Mature sludge	FF vs. S	FS vs. MS
MBC (mg C kg soil ⁻¹) ^A	Fall 2015	146 (5)	36.3 (4.6)	25.1 (4.6)	***	*
MBN (mg N kg soil ⁻¹) ^A	Fall 2015	18.0 (0.6)	4.00 (0.55)	3.19 (0.55)	***	NS
MBC:MBN	Fall 2015	8.22 (0.52)	9.21 (0.52)	8.00 (0.52)	NS	NS
Respiration	Spring 2014	3.57 (0.47)	5.45 (0.35)	2.89 (0.33)	NS	***
(mg CO ₂ g C ⁻¹ day ⁻¹)	Fall 2014	4.66 (1.35)	9.15 (0.95)	8.83 (0.95)	**	NS
	Spring 2015	5.45 (0.60)	5.19 (0.43)	4.82 (0.43)	NS	NS

MBC is microbial biomass carbon; MBN is microbial biomass nitrogen. Mean (SE), $N = 45$. Significant differences are designated as followed: (*) $p < 0.05$; (**) $p < 0.01$; and (***) $p < 0.001$, and NS (not significant).^ALog transformed. $N = 27$.

Table 2.8 Organic carbon and total nitrogen levels of the top mineral soil (0-10 cm) measured at different periods following organic amendments. Contrasts between control (C) and sludges (S), between forest floor (FF) and sludges (S), and between fresh sludge (FS) and mature sludge (MS) were assessed by means of mixed linear model based on a Type 1 test of hypothesis.

<i>Parameter</i>	Treatment means				Contrasts		
	Control	Fresh sludge	Mature sludge	Forest floor ^A	C vs. S	FF vs. S	FS vs. MS
Organic C ^B (mg g ⁻¹)							
Spring 2014	43.5 (7.3)	43.9 (4.5)	47.7 (5.8)	43.9 (7.5)	NS	NS	NS
Fall 2014 ^B	42.5 (8.8)	39.6 (5.5)	43.5 (6.0)	46.9 (7.5)	NS	NS	NS
Spring 2015	25.1 (7.0)	32.1 (5.0)	29.6 (4.6)	33.5 (6.5)	NS	NS	NS
Total N ^B (mg g ⁻¹)							
Spring 2014	3.92 (0.43)	4.10 (0.28)	4.12 (0.31)	4.17 (0.43)	NS	NS	NS
Fall 2014	3.13 (0.48)	3.07 (0.31)	3.14 (0.33)	3.42 (0.42)	NS	NS	NS
Spring 2015 ^B	1.93 (0.36)	2.66 (0.30)	2.34 (0.25)	2.69 (0.34)	NS	NS	NS
C/N							
Spring 2014	10.9 (0.7)	10.5 (0.5)	11.1 (0.6)	12.2 (0.9)	NS	NS	NS
Fall 2014	12.9 (0.8)	12.5 (0.6)	13.4 (0.5)	13.3 (0.8)	NS	NS	NS
Spring 2015	12.0 (1.1)	11.8 (0.6)	12.0 (0.7)	11.9 (0.9)	NS	NS	NS

Mean (SE), $N = 45$. No significant difference ($p < 0.05$) (NS) was found using a mixed model with random effects.

^A The forest floor plots were sampled in 2014 and 2015. ^B Log transformed.

Table 2.9 Nitrogen (NH_4^+) mineralization potential of the forest floor and municipal sewage sludge as well as of the top mineral soil (0-10 cm) measured at different periods following organic amendments. Contrasts between control (C) and sludges (S), between forest floor (FF) and sludges (S), and between fresh sludge (FS) and mature sludge (MS) were assessed by means of mixed linear model based on a Type 1 test of hypothesis.

	Sampling	C vs. S	FF vs. S	FS vs. MS
Organic amendments	Spring 2014	NA	NS	***
	Fall 2014	NA	NS	NS
	Spring 2015	NA	NS	NS
Mineral soil	Spring 2014	NS	NS	NS
	Fall 2014	NS	NS	NS
	Spring 2015	*	NS	NS

Mean (SE), $N = 53$. NA is not applicable as there is no organic amendment in the control. Significant differences are designated as followed: (*) $p < 0.05$; (**) $p < 0.01$; and (***) $p < 0.001$, and NS (not significant). NA (not available): no organic amendments in the control.

Table 2.10 Nutrient supply rates ($\mu\text{g } 10\text{cm}^2 \text{ 4weeks}^{-1}$) of the top mineral soil (0 - 10cm) measured with PRSTM probes at different periods following organic amendments. Contrasts between control (C) and sludges (S), between forest floor (FF) and sludges (S), and between fresh sludge (FS) and mature sludge (MS) were assessed by means of mixed linear model and are based on a Type 1 test of hypothesis.

	Sampling	C vs. S	FF vs. S	FS vs. MS
Nitrate	Fall 2013 ^A	***	NA	***
	Spring 2014 ^A	**	**	NS
	Fall 2014	NS	NS	NS
	Spring 2015 ^A	NS	NS	NS
Ammonium	Fall 2013	NS	NA	**
	Spring 2014	**	*	NS
	Fall 2014	NS	*	NS
	Spring 2015	*	NS	NS
Phosphate	Fall 2013 ^A	**	NA	*
	Spring 2014	**	*	NS
	Fall 2014 ^A	NS	NS	NS
	Spring 2015 ^A	NS	NS	NS
Potassium	Fall 2013	**	NA	NS
	Spring 2014	*	*	NS
	Fall 2014	***	*	*
	Spring 2015	**	*	NS
Calcium	Fall 2013	***	NA	*
	Spring 2014	***	**	NS
	Fall 2014 ^A	**	NS	NS
	Spring 2015	NS	NS	NS
Magnesium	Fall 2013	NS	NA	*
	Spring 2014	NS	NS	*
	Fall 2014	NS	NS	NS

	Sampling	C vs. S	FF vs. S	FS vs. MS
	Spring 2015	NS	NS	NS
Sulphate	Fall 2013	***	NA	***
	Spring 2014	***	**	***
	Fall 2014	**	NS	**
	Spring 2015 ^A	***	**	NS
Manganese	Fall 2013 ^A	NS	NA	NS
	Spring 2014	NS	NS	NS
	Fall 2014 ^A	NS	NS	NS
	Spring 2015	NS	*	NS

$N = 18$. Significant differences are designated as followed: (*) $p < 0.05$; (**) $p < 0.01$; and (***) $p < 0.001$, and NS (not significant). NA is not applicable because the forest floor material was added in the spring of 2014. ^ALog transformed.

Table 2.11 Trace metal supply rates ($\mu\text{g } 10\text{cm}^2 \text{ 4weeks}^{-1}$) of the top mineral soil (0 - 10cm) measured with PRSTM probes at different periods following organic amendments. Contrasts between control (C) and sludges (S), between forest floor (FF) and sludges (S), and between fresh sludge (FS) and mature sludge (MS) were assessed by means of mixed linear model and are based on a Type 1 test of hypothesis.

	Sampling	C vs. S	FF vs. S	FS vs. MS
Copper	Fall 2013	*	NA	NS
	Spring 2014 ^A	***	***	NS
	Fall 2014 ^A	***	***	NS
	Spring 2015 ^A	**	NS	NS
Lead	Fall 2013	*	NA	*
	Spring 2014	***	***	NS
	Fall 2014 ^A	***	***	NS
	Spring 2015	**	**	NS
Zinc	Fall 2013	**	NA	NS
	Spring 2014	***	***	NS
	Fall 2014	**	**	NS
	Spring 2015 ^A	**	NS	NS

Mean (SE). $N = 18$. Significant differences are designated as followed: (*) $p < 0.05$; (**) $p < 0.01$; and (***) $p < 0.001$, and NS (not significant). NA is not applicable because the forest floor was added in the spring of 2014.

^ALog transformed.

Table 2.12 Physical properties of the top mineral soil (0 - 10cm) one and two years following organic amendments (spring sampling). Contrasts between control (C) and sludges (S), between forest floor (FF) and sludges (S), and between fresh sludge (FS) and mature sludge (MS) were assessed by means of mixed linear model and are based on a Type 1 test of hypothesis.

<i>Parameter</i>	Treatment means				Contrasts		
	Control	Fresh sludge	Mature sludge	Forest floor	C vs. S	FF vs. S	FS vs. MS
Bulk density (g cm ⁻³)							
2014	1.23 (0.08)	1.26 (0.05)	1.28 (0.05)	1.21 (0.06)	NS	NS	NS
2015	1.08 (0.07)	1.15 (0.04)	1.16 (0.04)	1.08 (0.07)	NS	NS	NS
Macroporosity ^A (%)							
2014	9.90 (2.03)	9.42 (1.22)	5.82 (0.74)	7.97 (1.68)	NS	NS	NS
2015	6.54 (1.63)	8.00 (0.97)	7.07 (0.86)	8.54 (1.12)	NS	NS	NS
Total porosity (%)							
2014	52.4 (2.4)	53.0 (1.6)	54.0 (3.4)	49.0 (5.6)	NS	NS	NS
2015	54.8 (2.0)	54.9 (0.9)	54.4 (1.3)	55.2 (2.0)	NS	NS	NS

Mean (SE). *N* = 54. No significant difference ($p < 0.05$) (NS) found using a mixed model with random effects.

^AMacroporosity and total porosity were not measured in the fall of 2013.

Table 2.13 White spruce foliar nutrients and growth response one and two years following organic amendments and seedling establishment. Contrasts between control (C) and sludges (S), between forest floor (FF) and sludges (S), and between fresh sludge (FS) and mature sludge (MS) were assessed by means of mixed linear model and are based on a Type 1 test of hypothesis.

<i>Parameter</i>	Treatment means				Contrasts		
	Control	Fresh sludge	Mature sludge	Forest floor	C vs. S	FF vs. S	FS vs. MS
Nitrogen (mg g ⁻¹)							
2014	7.97 (0.91)	11.78 (0.64)	11.00 (0.64)	15.13 (0.91)	**	**	NS
2015							
Phosphorous (mg g ⁻¹)							
2014	0.68 (0.21)	1.68 (0.15)	1.30 (0.15)	1.41 (0.21)	**	NS	NS
2015							
Potassium (mg g ⁻¹)							
2014	1.14 (0.43)	4.48 (0.30)	2.89 (0.30)	3.29 (0.43)	***	NS	**
2015	0.24 (0.02)	0.19 (0.01)	0.26 (0.01)	0.17 (0.02)	NS	*	**
Calcium (mg g ⁻¹)							
2014	3.97 (0.61)	6.77 (0.43)	6.00 (0.43)	6.10 (0.61)	**	NS	NS
2015	0.12 (0.03)	0.17 (0.02)	0.23 (0.02)	0.13 (0.03)	*	NS	NS
Magnesium (mg g ⁻¹)							
2014	0.77 (0.16)	0.66 (0.11)	0.82 (0.11)	0.94 (0.16)	NS	NS	NS
2015	0.03 (0.003)	0.02 (0.002)	0.03 (0.002)	0.02 (0.003)	*	NS	*
Total height (cm)							
2014	30.0 (2.1)	27.0 (1.5)	27.5 (1.5)	28.6 (2.4)	NS	NS	NS
2015	31.6 (1.6)	35.4 (1.1)	33.8 (1.1)	38.3 (2.1)	NS	NS	NS

<i>Parameter</i>	Treatment means				Contrasts		
	Control	Fresh sludge	Mature sludge	Forest floor	C vs. S	FF vs. S	FS vs. MS
Diameter (mm)							
2014	4.62 (0.28)	4.04 (0.20)	4.40 (0.20)	4.31 (0.34)	NS	NS	NS
2015	5.16 (0.28)	5.28 (0.20)	5.19 (0.20)	5.78 (0.44)	NS	NS	NS
Yearly height growth (cm)							
2014	9.24 (1.67)	9.73 (1.18)	9.34 (1.18)	9.05 (1.89)	NS	NS	NS
2015	1.57 (1.29)	8.41 (0.91)	6.27 (0.91)	9.75 (1.49)	**	NS	NS
Relative growth ^{A,B}							
2015	0.05 (0.06)	0.34 (0.04)	0.25 (0.04)	0.35 (0.07)	**	NS	NS

Mean (SE), $N = 18$. Significant difference are designated as followed: (*) $p < 0.05$; (**) $p < 0.01$; and (***) $p < 0.001$, and NS (not significant). ^A Log transformed. ^B Relative growth = [Height growth (cm) in 2015 / total height (cm) in 2014].

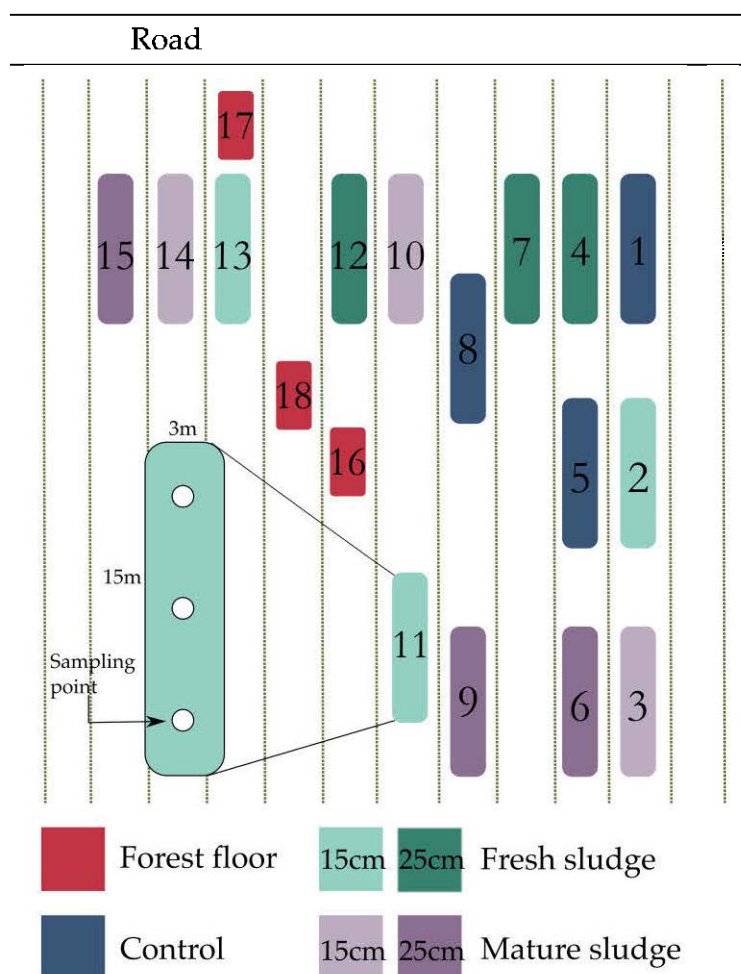


Figure 2.1 Experimental design consisting of 18 randomly distributed experimental plots corresponding to forest floor, control, fresh sludge (15 and 25 cm) and mature sludge (15 and 25 cm). Distance between sampling points and plot edge is 1.5 m.

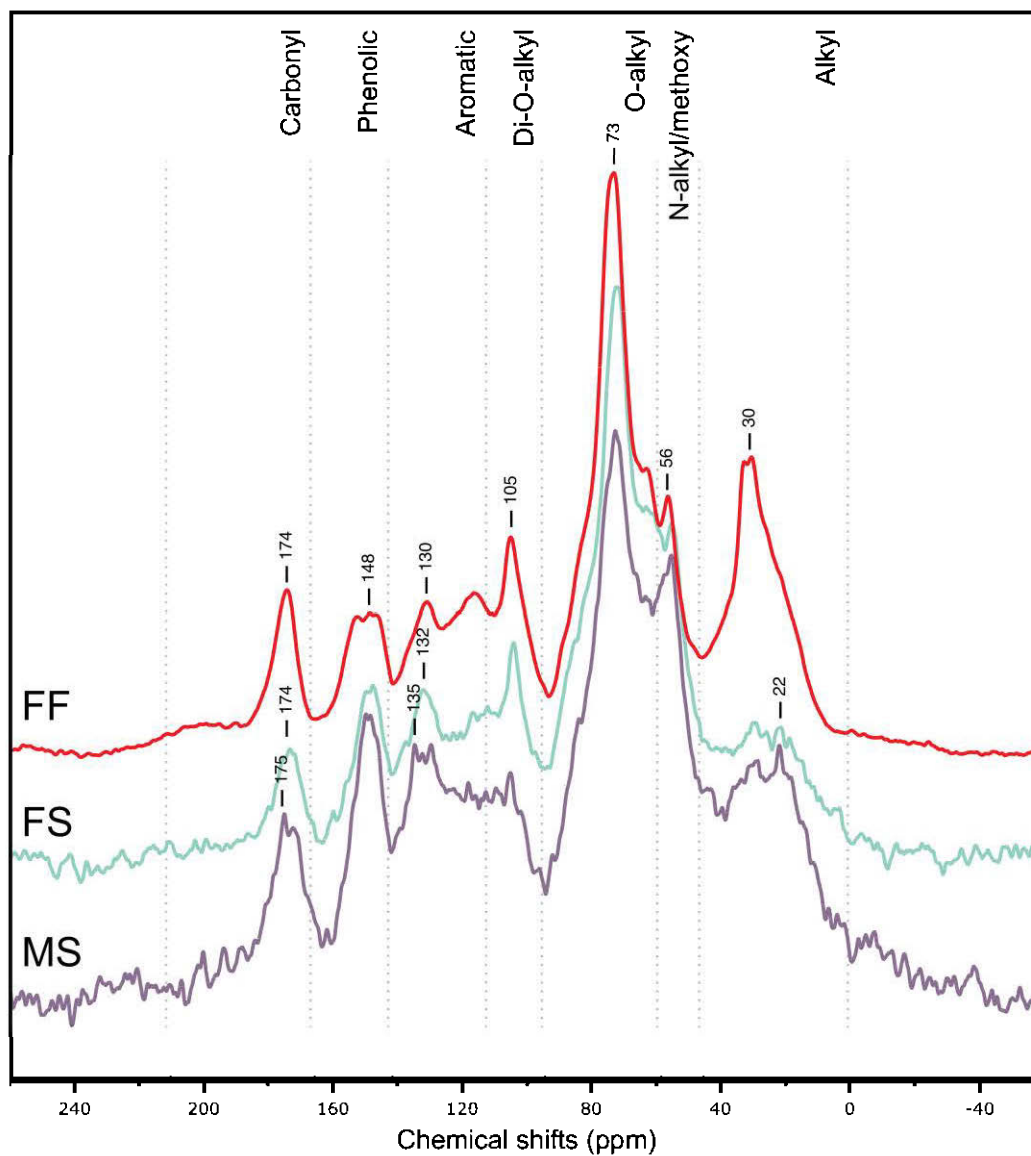


Figure 2.2 Solid-state CP-MAS ^{13}C spectra acquired for the organic amendments: FF (forest floor); FS (fresh sludge); MS (mature sludge) with seven chemical shifts (ppm) region assigned to the main structural groups.

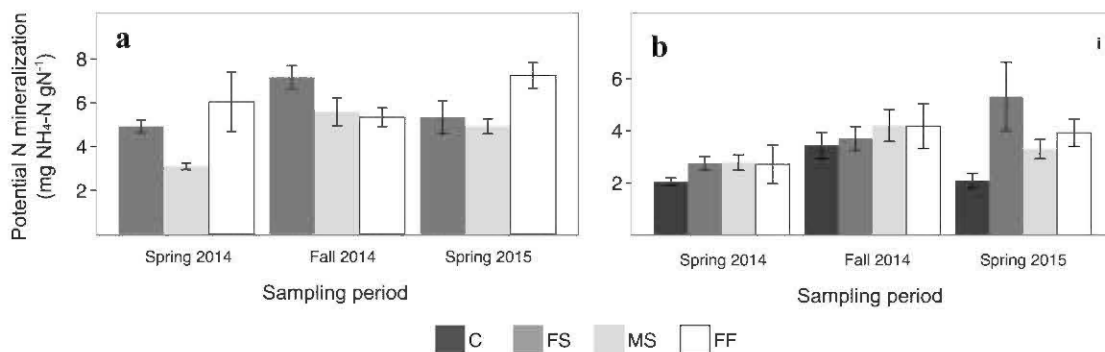


Figure 2.3 Nitrogen (ammonium) mineralization potential (mgNH₄-N gN⁻¹) in organic amendments (a) and effect of organic amendments on nitrogen (ammonium) mineralization potential (mgNH₄-N gN⁻¹) in the mineral soil (b) for the control (C), fresh sludge (FS), mature sludge (MS) and forest floor (FF) treatments. Data were analyzed by means of a linear mixed model with random effects (plot level) based on Type 1 hypothesis. $N = 53$.

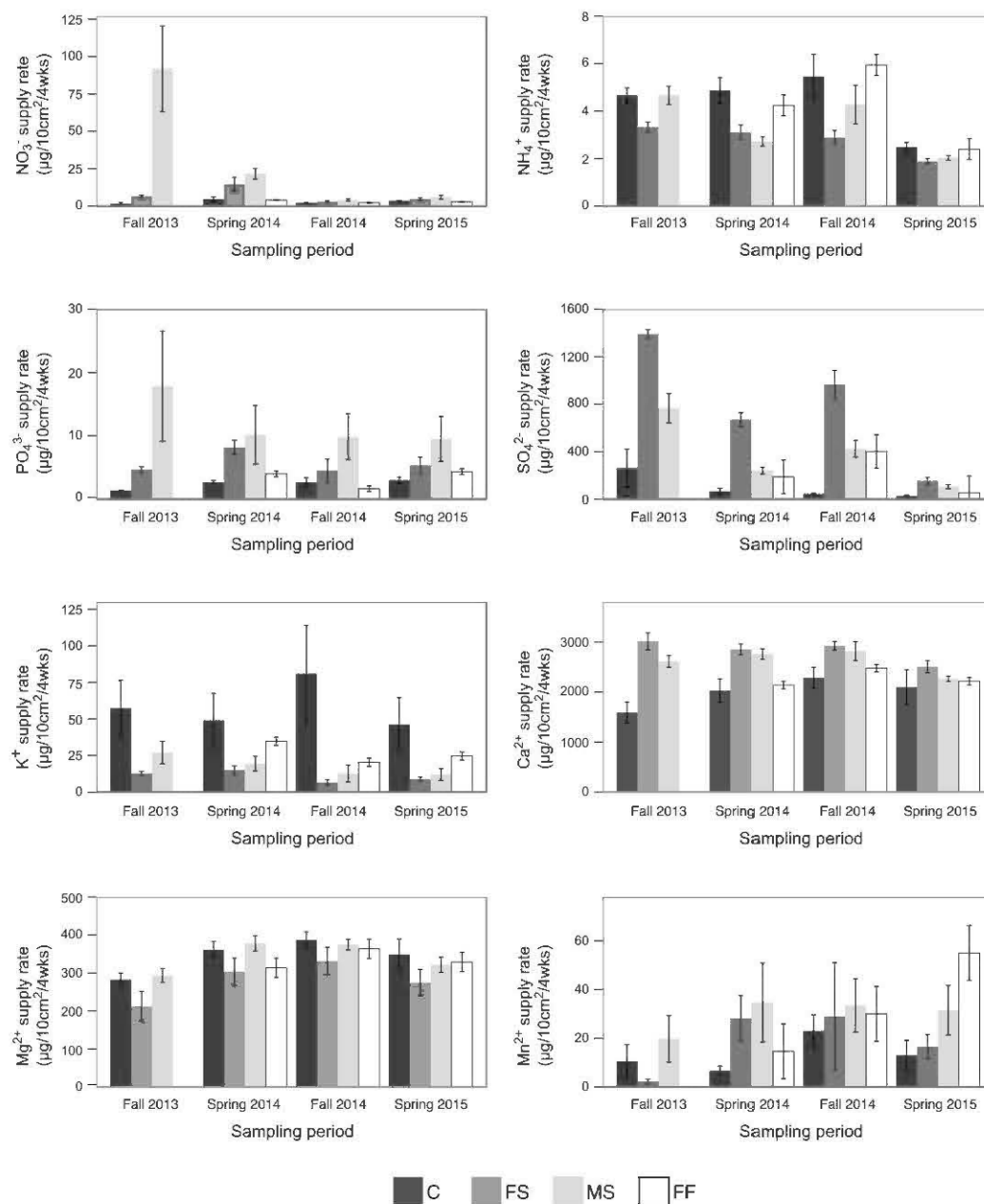


Figure 2.4 Effects of organic amendments on nutrient supply rate (µg/10cm²/4weeks) measured with PRSTM probes at the mineral soil surface (0 - 10cm) for the treatments: control (C), fresh sludge (FS), mature sludge (MS) and forest floor (FF). The forest floor (FF) plots were sampled in 2014 and 2015. Data were analyzed by means of a mixed linear model with random effects (plot level) and based on Type 1 hypothesis ($N = 18$).

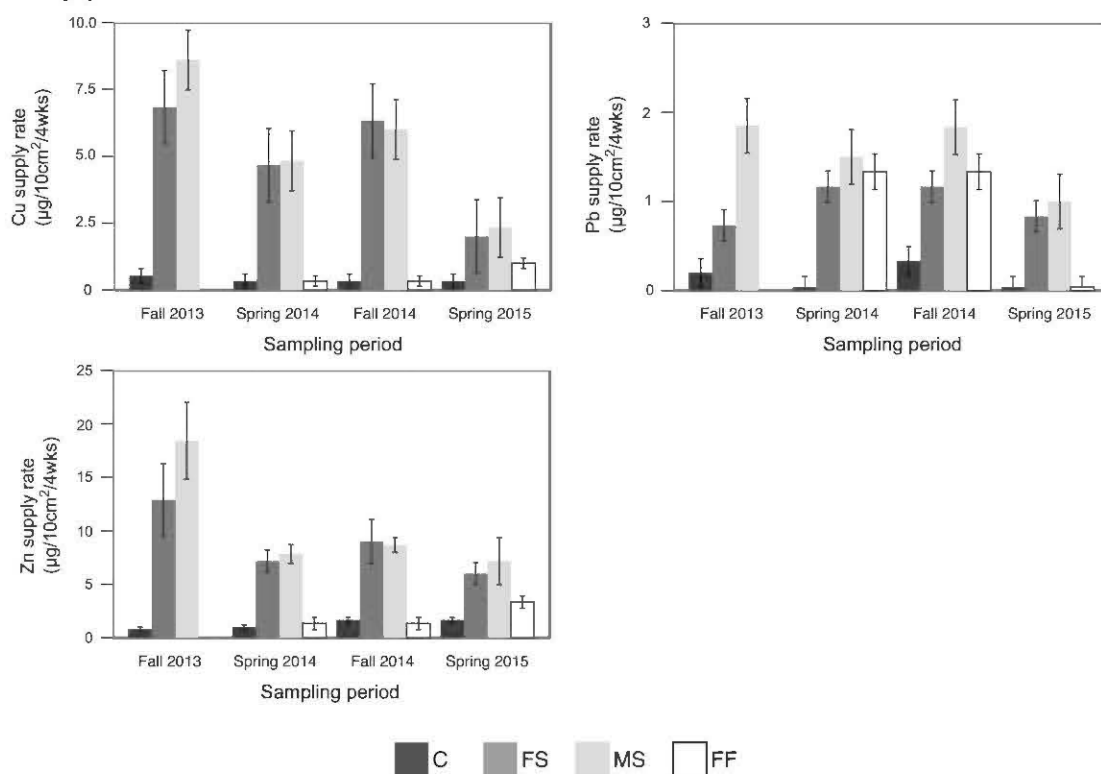


Figure 2.5 Effects of organic amendments on trace metal supply rate ($\mu\text{g}/10\text{cm}^2/4\text{weeks}$) measured with PRSTM probes at the mineral soil surface (0 - 10cm) for the treatments: control (C), fresh sludge (FS), mature sludge (MS) and forest floor (FF). The forest floor (FF) plots were sampled in 2014 and 2015. Data were analyzed by means of a mixed linear model with random effects (plot level) and based on Type 1 hypothesis ($N = 18$).

CHAPITRE III: CONCLUSION GÉNÉRALE

Le recyclage des matières résiduelles représente un enjeu environnemental de premier plan. En 2012, 34% des boues de fosse septique municipales produites au Québec ont été recyclées par épandage en agriculture, en sylviculture ou sur des sites dégradés, ce qui représente un écart important par rapport à l'objectif de 60% fixé par la politique du gouvernement provincial sur les matières résiduelles fertilisantes (MDDEP, 2014). Pourtant, certaines initiatives mises en place par le gouvernement du Québec ou par certaines municipalités témoignent d'une volonté d'augmenter le recyclage des matières résiduelles fertilisantes, dont les boues de fosse septique municipales. Par exemple, le Fonds vert du gouvernement du Québec offre maintenant un appui financier aux municipalités ou entreprises privées désirant installer des infrastructures de recyclage des matières résiduelles fertilisantes. Par ailleurs, le recyclage en milieu forestier demeure marginal, bien qu'il s'agisse d'un potentiel d'épandage sur d'importantes superficies. En plus de la réduction d'importantes quantités de boues enfouies ou incinérées, l'épandage de boues dans les sols forestiers pourrait constituer un apport nutritif non négligeable pour la croissance végétale.

Cette étude visait à évaluer le potentiel d'épandage de boues de fosse septique déshydratées pour restaurer des sols argileux dégradés par des opérations forestières. Notre objectif était de rétablir les processus pédologiques essentiels à la séquestration de carbone et à la croissance végétale, dont le cycle des nutriments et les propriétés physiques des sols. Les sols amendés de boues ont été comparés à un témoin, ainsi qu'aux sols ayant reçu des amendements de couverture morte, afin de comparer l'effet des boues à celui de matière organique issues d'un écosystème naturel. En raison de la teneur élevée des boues en matière organique et nutriments facilement assimilables, nous avons émis cinq hypothèses. Comparativement aux sols dépourvus de matière organique en surface, les boues (1) rétabliraient les dynamiques du carbone et de l'azote, (2) augmenteraient la disponibilité des nutriments, (3) augmenteraient la disponibilité des métaux traces en surface, (4) amélioreraient les propriétés physiques des sols, (5) augmenteraient la nutrition foliaire et favoriseraient la croissance de semis.

Contrairement à nos attentes, les boues de fosse septique ont eu peu d'effet sur les propriétés du sol minéral durant les deux années suivant l'épandage. L'épandage de boues n'a augmenté ni l'accumulation et ni la minéralisation du carbone et d'azote de façon significative en surface du sol minéral. De plus, deux ans après l'épandage, la masse volumique et la porosité

du sol demeuraient inchangées. Cependant, l'incorporation de la matière organique est lente dans les sols fins en forêt boréale et par conséquent ces propriétés devraient être suivies à plus long terme afin de détecter les changements induits par l'épandage des boues.

Par ailleurs, l'épandage a augmenté l'apport en nutriments à la surface du sol et a eu un effet positif sur la croissance et la nutrition foliaire des épinettes blanches. Cet apport en nutriments facilement assimilables pourrait donc permettre d'améliorer la productivité de sites forestiers dégradés et par le fait même de favoriser la restauration du sol. Toutefois, le flux de nitrate et de phosphate sous les boues était très élevé bien que de courte durée, ce qui indique un risque potentiel de contamination qui pourrait être associé à l'application d'une quantité très élevée de boues. La forte proportion de carbone labile dans les deux types de boues aurait pu faciliter la décomposition rapide de la matière organique suivant l'épandage, contribuant ainsi à générer ce flux important de nutriments. Inversement, la plus importante proportion en carbone récalcitrant dans la couverture morte a engendré un apport en nutriment plus lent, mais favorisant la croissance végétale. Des taux d'application de boues plus faibles pourraient contribuer à limiter les risques de contamination. Cependant, l'épandage fréquent de quantités moindres de boues n'est pas toujours envisageable en milieu forestier dû à l'accessibilité restreinte et aux superficies importantes. Par conséquent, il serait souhaitable d'optimiser à la fois la quantité de boues appliquées et le flux de nutriments à court et à long terme. L'utilisation de boues stabilisées (c.-à-d. compostées ou méthanisées) pourrait réduire les risques de lessivage, tout en assurant un apport en nutriment à long terme.

Les objectifs de l'épandage – fertilisation vs restauration – doivent être examinés. D'autres études seraient nécessaires entre autres pour évaluer l'impact de la structure chimique des boues sur l'apport en nutriment et sur les processus biogéochimiques des sols amendés. De plus, les taux d'application devraient refléter les conditions naturelles d'un site donné. Ainsi, d'autres études pourraient évaluer les effets de différents taux d'application de boues reflétant mieux le volume et l'épaisseur de matière organique (couverture morte) naturellement présente en forêt boréale. Dans les peuplements voisins de notre site d'étude, l'épaisseur moyenne de matière organique varie typiquement entre 2 et 18 cm alors que celle des boues épandues au site d'étude était plutôt de 7 à 30 cm. Elle ne reflétait donc pas les conditions originales du site. Les taux d'application de boues étaient aussi plusieurs fois supérieurs à

ceux de la matière organique épandue dans les placettes de couverture morte. Dans une optique de restauration écologique, il serait donc intéressant de comparer des quantités équivalentes de boues et de matière organique naturelle, de façon à rétablir une couverture organique similaire. L'épandage de boues pourrait aussi être intéressant pour rétablir le couvert végétal de milieux perturbés tels les sites miniers. Par exemple en Alberta, les compagnies d'exploitation de sables bitumineux sont contraintes de rétablir les écosystèmes forestiers et habitats en place avant l'exploitation (Government of Alberta, 1999). L'utilisation des boues directement sur les résidus miniers constituerait une alternative moins coûteuse au prélèvement et au transport de la couche superficielle du sol afin de remplacer un sol très perturbé et possiblement contaminé (Brown et al., 2003).

L'épandage des matières résiduelles fertilisantes en milieu forestier ou minier constitue une solution encourageante pour éliminer l'enfouissement et l'incinération de boues de fosse septique, ainsi que pour réduire les émissions de gaz à effet de serre associées aux matières résiduelles (Brown et al., 2010; SYLVIS, 2009). Cependant, des avenues permettant de limiter les flux initiaux en nutriments et contaminants doivent être développées au préalable.

ANNEXE A: RÉSULTATS ADDITIONNELS

Table A.1 Effects of soil amendments on nutrient and trace metal supply rates ($\mu\text{g } 10\text{cm}^2 \text{ 4weeks}^{-1}$) of the top mineral soil (0-10 cm) measured with PRSTM probes at different periods during the experiment. Contrasts between control (C) and sludges (S), between forest floor (FF) and sludges (S), and between fresh sludge (FS) and mature sludge (MS) were assessed by means of mixed linear model and are based on a Type 1 test of hypothesis.

Sampling	Treatment means				Contrasts		
	Control	Fresh sludge	Mature sludge	Forest floor	C vs. S	FF vs. S	FS vs. MS
Nitrate							
Spring 2014	4.38 (3.07)	11.4 (2.2)	23.1 (2.2)	4.26 (3.07)	*	*	*
Fall 2014 ^A	1.96 (1.24)	3.41 (0.88)	4.32 (0.88)	3.03 (1.24)	NS	NS	NS
Spring 2015	3.96 (1.12)	5.75 (0.79)	4.62 (0.79)	3.70 (1.12)	NS	NS	NS
Ammonium							
Spring 2014	3.44 (0.29)	2.91 (0.21)	2.80 (0.21)	3.97 (0.29)	NS	**	NS
Fall 2014 ^A	4.39 (1.00)	4.36 (0.70)	4.04 (0.70)	5.87 (1.00)	NS	NS	NS
Spring 2015	2.73 (0.36)	2.25 (0.26)	2.20 (0.26)	2.50 (0.36)	NS	NS	NS
Phosphate							
Spring 2014 ^A	4.67 (1.57)	6.33 (1.11)	7.83 (1.11)	3.00 (1.57)	NS	*	NS
Fall 2014	1.67 (1.83)	4.83 (1.29)	4.17 (1.29)	2.00 (1.83)	NS	NS	NS
Spring 2015	1.00 (0.58)	2.67 (0.41)	2.83 (0.41)	2.00 (0.58)	*	NS	NS
Potassium							
Spring 2014 ^A	18.2 (5.6)	3.60 (4.00)	3.54 (4.00)	9.93 (5.61)	NS	NS	NS
Fall 2014	10.2 (2.0)	3.22 (1.38)	5.13 (1.38)	7.73 (2.00)	*	NS	NS
Spring 2015	11.9 (2.67)	5.71 (1.89)	6.19 (1.89)	11.2 (2.7)	NS	NS	NS

Sampling	Treatment means				Contrasts		
	Control	Fresh sludge	Mature sludge	Forest floor	C vs. S	FF vs. S	FS vs. MS
Calcium							
Spring 2014	2728 (204)	3030 (144)	2862 (144)	2747 (204)	NS	NS	NS
Fall 2014	2655 (156)	2660 (110)	2767 (110)	2609 (156)	NS	NS	NS
Spring 2015	2150 (154)	2420 (109)	2401 (109)	2141 (154)	NS	NS	NS
Sulfate ^A							
Spring 2014	46.4 (10.8)	766 (104)	223 (11)	192 (65)	***	**	***
Fall 2014	29.7 (47.2)	1071 (208)	218 (33)	374 (265)	***	NS	***
Spring 2015	25.7 (2.1)	176 (37)	64.4 (10.2)	25.7 (2.1)	***	***	***
Magnesium							
Spring 2014	386 (40)	394 (40)	377 (29)	392 (29)	NS	NS	NS
Fall 2014	389 (48)	335 (34)	390 (34)	367 (48)	NS	NS	NS
Spring 2015	312 (41)	310 (29)	358 (29)	300 (41)	NS	NS	NS
Boron							
Spring 2014	2.33 (0.66)	2.5 (0.47)	2.5 (0.47)	3.00 (0.66)	NS	NS	NS
Fall 2014	1.67 (0.43)	1.17 (0.31)	2.00 (0.31)	1.33 (0.43)	NS	NS	NS
Spring 2015	0.67 (0.47)	0.50 (0.34)	1.33 (0.34)	0.67 (0.47)	NS	NS	NS
Manganese							
Spring 2014	6.67 (1.78)	31.8 (7.92)	22.5 (7.2)	22.5 (7.2)	**	NS	NS
Fall 2014	17.0 (3.3)	22.7 (10.1)	16.0 (7.4)	32.0 (11.4)	NS	NS	NS
Spring 2015	17.3 (6.2)	36.2 (13.3)	29.0 (7.4)	38.7 (5.2)	NS	NS	NS

Sampling	Treatment means				Contrasts		
	Control	Fresh sludge	Mature sludge	Forest floor	C vs. S	FF vs. S	FS vs. MS
Zinc							
Spring 2014	1.00 (1.33)	5.17 (0.94)	5.33 (0.94)	1.00 (1.33)	*	*	NS
Fall 2014 ^A	0.67 (1.38)	3.83 (0.98)	4.50 (0.98)	1.00 (1.38)	**	**	NS
Spring 2015	1.33 (0.67)	2.83 (0.47)	3.00 (0.47)	1.33 (0.67)	NS	NS	NS
Copper							
Spring 2014	1.67 (0.95)	4.67 (0.67)	6.00 (0.67)	1.33 (0.95)	**	**	NS
Fall 2014	2.00 (1.24)	3.83 (0.88)	5.00 (0.88)	0.33 (1.24)	NS	*	NS
Spring 2015	1.33 (0.50)	2.17 (0.36)	2.50 (0.36)	0.67 (0.50)	NS	*	NS
Lead							
Spring 2014	0.33 (0.51)	1.50 (0.36)	2.33 (0.36)	0.33 (0.51)	*	*	NS
Fall 2014	0.67 (0.48)	1.00 (0.34)	2.33 (0.34)	0.33 (0.48)	NS	*	*
Spring 2015 ^A	0.33 (0.30)	1.17 (0.22)	1.17 (0.22)	0.33 (0.30)	*	*	NS
Aluminum							
Spring 2014	58.3 (11.5)	55.5 (8.14)	32.5 (8.14)	74.7 (11.5)	NS	*	NS
Fall 2014	41.0 (8.8)	41.7 (6.2)	26.5 (6.2)	44.7 (8.8)	NS	NS	NS
Spring 2015	27.7 (6.6)	33.3 (4.6)	26.2 (4.6)	35.0 (6.6)	NS	NS	NS

Mean (SE). $N=18$.

Significant difference are designated as followed: (*) $p<0.05$; (**) $p<0.01$; and (***) $p<0.001$, and NS (not significant).

^A Log transformed

Table A.2 Effects of soil amendments on vegetation cover and on litter decomposition at different periods during the experiment. Contrasts between control (C) and sludges (S), between forest floor (FF) and sludges (S), and between fresh sludge (FS) and mature sludge (MS) were assessed by means of mixed linear model and are based on a Type 1 test of hypothesis.

Sampling	Treatment means			Contrasts			
	Control	Fresh Sludge	Mature Sludge	Forest floor	C vs. S	FF vs. S	FS vs. MS
Vegetation cover ^A (%)							
Aug.14	22.3 (5.7)	13.3 (4.0)	35.4 (4.0)	10.3 (5.7)	NS	NS	**
Aug.15	34.6 (7.9)	25.1 (5.6)	42.3 (5.6)	18.5 (7.9)	NS	*	*
Litterbag mass loss (g)							
Oct.14	0.14 (1.04) ^B	1.54 (0.71)	2.08 (0.70) ^B	2.69 (1.04)	NS	NS	NS
May.15	1.47 (1.11)	2.16 (0.77)	0.42 (0.79)	3.48 (1.11)	NS	NS	NS
Sep.15	1.06 (1.17)	1.49 (0.78)	1.38 (0.80) ^B	4.09 (1.11)	NS	NS	NS

Mean (SE). $N = 18$.

Significant difference are designated as followed: (*) $p < 0.05$; (**) $p < 0.01$; and (***) $p < 0.001$, and NS (not significant).

^A Log transformed. ^B Mass gain.

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