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DIVERSITE DES CHAMPIGNONS SAPROXYLIQUES ET DECOMPOSITION DU BOIS MORT DANS DES PEUPLEMENTS NATURELS ET AMENAGES EN FORET BOREALE QUEBECOISE

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RESUME DE LA THESE

Au sein de l'écosystème forestier, le bois mort est considéré comme un attribut clé pour une cascade de processus écologiques qui affectent à la fois la diversité biologique et la productivité des sols forestiers. Les champignons saproxyliques sont les principaux responsables de la dégradation des débris ligneux. Cependant, la diversité moléculaire de la communauté dans son ensemble, ainsi que son rôle, n'ont été que peu étudiés. La plupart des études sur les communautés boréales de champignons saproxyliques proviennent des forêts fenno-scandinaves qui ont eu un long historique d'aménagement forestier intensif et une faible diversité d'essences forestières. A notre connaissance, peu d'études se sont intéressées à la diversité des champignons décomposeurs en fonction des caractéristiques du bois mort dans la forêt boréale d'Amérique du Nord. Ce projet visait donc à améliorer les connaissances sur les communautés de champignons associées à la dégradation du bois mort tout en évaluant l'effet des pratiques sylvicoles (dont celles issues de l'aménagement écosystémique) sur ces communautés fongiques.

Des billes et des chicots ont été échantillonnés dans différents peuplements que ce soit en conditions naturelles ou aménagées en utilisant une méthode moléculaire afin de détecter l'ADN fongique présent. La diversité moléculaire a été établie notamment selon un gradient de décomposition du bois, de perturbation et de récolte forestière par la technique de PCR-DGGE (denaturing gradient gel electrophoresis). L'activité métabolique des différentes communautés fongiques a également été étudiée pour les stades précoces de colonisation du bois grâce à des mesures de respiration. La décomposition du bois a été évaluée en s'intéressant à l'évolution des formes de carbone dans le bois par spectroscopie infrarouge.

Lors de l'analyse des communautés fongiques du bois mort au sol et en peuplement naturel, nous avons identifié 33 unités taxonomiques opérationnelles (UTOs) incluant deux espèces indicatrices. Les billes d'épinettes (*Picea* spp.) supportaient une plus grande diversité ainsi qu'un plus grand nombre d'UTOs. Nos résultats ont confirmé l'hypothèse que l'essence de la bille influence la richesse et la diversité fongique. La composition du peuplement, le volume et la composition chimique du bois mort structurent la composition des communautés fongiques saproxyliques. La présence de bois mort provenant d'une diversité d'essence forestière permettrait ainsi de maintenir une diversité dans les communautés de décomposeurs du bois. Plus précisément, le bois de sapin (*Abies balsamea*) et de tremble (*Populus tremuloides*) étaient rapidement colonisés par des communautés fongiques similaires, qui par la suite se différenciaient après une année de colonisation et ceci dans une grande gamme de peuplements allant des brûlis à des vieilles forêts naturelles. La perturbation dans ces peuplements affectait la composition des communautés de champignons mais seulement pour celles colonisant le sapin. Chaque UTO présentait des patrons de colonisation très variables. Durant les premiers mois de décomposition, le tremble se dégrade plus rapidement que le sapin. Bien que la respiration augmente avec le temps pour le sapin et le tremble, cette augmentation proviendrait de deux processus distincts: une augmentation de la compétition fongique chez le sapin et une augmentation de la décomposition chez le tremble. Dans les peuplements de tremble soumis à la récolte

forestière, les grosses billes bien décomposées avaient une diversité d'environ 10% supérieure comparativement aux billes de tremble moyennement décomposées (indépendamment de leur taille). L'effet du diamètre de la bille était dépendant du traitement sylvicole. Les plus grosses billes ont plus d'UTOs et une diversité plus grande mais seulement dans les brulis par rapport aux peuplements non coupés soulignant l'effet de bille refuge dans les traitements fortement aménagés. La relation négative entre la diversité des champignons sur les billes et les chicots et le volume de débris ligneux fins était reliée à l'intensité de la récolte forestière, ce volume augmentant avec l'intensité de récolte. Nos résultats soulignent l'effet des pratiques de récolte intenses qui diminuent la diversité fongique, la richesse spécifique et modifient la composition des communautés. Cependant, l'aménagement écosystémique par l'intermédiaire des coupes partielles pourrait contrebalancer ces effets en conservant un volume de bois mort plus important comparativement aux coupes totales.

Il ressort clairement de cette étude que le volume de bois mort occupe une grande importance au niveau des communautés fongiques saproxyliques mais moindrement sur les communautés pionnières, qui sont, quant à elles, très généralistes. Cette thèse apporte des connaissances nouvelles sur l'écologie du bois mort et des espèces fongiques saproxyliques associées. Les relations entre la diversité biologique (spécifique) des champignons et les processus de dégradation du bois ainsi qu'avec les perturbations anthropiques (type d'aménagement forestier) ont été éclaircies. Elle a permis aussi de mieux évaluer l'état des forêts quant à l'importance du bois mort pour la diversité fongique, entre autres, par le biais de nouvelles espèces indicatrices. Les recommandations qui découlent de notre étude faciliteront donc les objectifs de protection et de gestion durable des forêts canadiennes notamment quant à l'utilisation de l'aménagement écosystémique.

Mots-clés : champignons saproxyliques, diversité, décomposition, bois mort, perturbation, forêt boréale, lien diversité-fonction

INTRODUCTION GENERALE

Contexte de la thèse

La foresterie au Québec est un enjeu économique très important puisque cette région représente 2% des forêts mondiales (Coulombe, 2004) et qu'elle a apporté des retombées économiques de plus de 10 milliards de dollars en 2001. Elle fournit également des emplois directs à 1,5% de la population québécoise. Le Canada se classe aussi au deuxième rang mondial pour deux produits issus de la forêt, soit la pâte à papier et le bois d'œuvre. Cette activité commerciale est fortement liée aux marchés des États-Unis, puisque plus de 85% de ces exportations leur sont destinées. Cependant l'industrie forestière a longtemps pratiqué l'aménagement forestier de façon biaisée vers la récolte de matière ligneuse sans une vision écologique à plus long terme. C'est pourquoi le Conseil Canadien des Ministres des Forêts a énoncé six critères d'aménagement forestier durable qui ont été incorporés dans la loi québécoise sur les forêts modifiée en 2010. Le respect des critères d'aménagement forestier durable et des indicateurs correspondants (Kneeshaw *et al.*, 2000) pose des difficultés dans leur mise en œuvre par les exploitants forestiers.

L'aménagement forestier écosystémique (Bergeron & Harvey, 1997 ; Harvey *et al.*, 2002) peut apporter des éléments de solutions au niveau des stratégies d'aménagement et des pratiques sylvicoles à différentes échelles temporelles et spatiales. D'après Galindo-Leal et Bunnel (1995), l'aménagement écosystémique basé sur le fonctionnement des écosystèmes est durable, s'inspire des perturbations naturelles, permet la viabilité des espèces natives et s'applique à une échelle spatiale et temporelle large. Diverses études se sont penchées sur des pratiques sylvicoles basées sur la dynamique naturelle des forêts (Bergeron & Harvey, 1997). Les organismes et les écosystèmes sont adaptés à la gamme de perturbations qui affectent les systèmes naturels ; si les interventions humaines se rapprochent de la gamme d'intensité, d'étendue et de fréquence des perturbations naturelles, alors on minimise à moyen terme les impacts négatifs sur les organismes et les écosystèmes. A l'échelle du peuplement, les travaux sylvicoles réalisés visant à amener la succession des peuplements équiens vers une

structure plus complexe caractéristique des forêts naturelles plus anciennes favoriseraient aussi le maintien du fonctionnement de l'écosystème ainsi que la conservation de la biodiversité. Cette thèse s'inscrit dans cette optique en se focalisant sur la diversité et les fonctions écologiques des champignons décomposeurs du bois mort.

Le bois mort

Au sein de l'écosystème forestier, le bois mort est considéré comme un facteur clé dans le maintien de la biodiversité. De nombreux organismes comme les insectes (Similä, Kouki & Martikainen, 2003), les oiseaux et les petits mammifères (Bowman *et al.*, 2000 ; Drapeau *et al.*, 2009), ainsi que les champignons et les bactéries (Heilmann-Clausen & Christensen, 2004 ; Sippola & Renvall, 1999) utilisent ce compartiment que ce soit comme source d'alimentation ou comme habitat (Harmon *et al.*, 1986). Il se compose de matériel ligneux que l'on retrouve sur le sol (chablis, branches), dans le sol (racines mortes, bois enterré, débris fins) et sur pied (chicots, souches). Ces débris sont créés par les perturbations telles que le feu, les bourrasques de vents, les attaques d'insectes et de maladies mais aussi de la sécheresse, de la compétition ou tout simplement de la sénescence des individus. Le volume de bois mort dans un peuplement dépend donc des apports par mortalité des arbres et des pertes dues à la respiration et aux exportations. Généralement, après une perturbation sévère, la quantité de débris ligneux suit un modèle en forme de « U » (Brais *et al.*, 2005 ; Harmon *et al.*, 1986 ; Ranius *et al.*, 2003) car au début on retrouve un haut niveau de débris dû à la perturbation puis comme les seuls apports immédiats proviennent de la végétation qui est restée debout, il y a une chute dans l'approvisionnement de matériel et enfin le taux d'apport de bois mort remonte avec le vieillissement des peuplements. Dans un peuplement forestier, on observe un gradient de décomposition constitué par l'ensemble du bois qui se retrouve à des états de détérioration plus ou moins avancés. Ainsi on retrouve des chicots quasiment intacts jusqu'aux débris dans un état très avancé de désagrégation et intégrés à la matière organique du sol.

Rôles écologiques du bois mort

Tout d'abord le bois mort est une ressource importante en ce qui concerne la biodiversité que ce soit comme habitat, substrat ou source d'énergie et de nutriments. Par exemple, dans les forêts finlandaises, il existe entre 4000 et 5000 espèces dites saproxyliques, c'est-à-dire les espèces qui sont dépendantes durant une partie de leur cycle de vie du bois mort (debout ou couché) ou qui sont dépendantes d'autres espèces saproxyliques (Davies *et al.*, 2008 ; Persiani *et al.*, 2010 ; Speight, 1989). Les champignons saproxyliques représentent un groupe très diversifié comptant plus de 1 500 espèces (Siitonen, 2001). Dans de nombreux cas, les espèces dépendantes du bois mort ont besoin d'une certaine quantité de bois mort (Lonsdale, Pautasso & Holdenrieder, 2008). Cependant le rôle écologique des débris ligneux grossiers (DLG), dépend de plusieurs facteurs dont leur état de décomposition (Harmon & Sexton, 1996). Les débris ligneux interviennent également au niveau de la biodiversité végétale en servant de substrat pour la régénération de certaines essences forestières (Lonsdale, Pautasso & Holdenrieder, 2008). Le bois mort joue également un rôle essentiel en ce qui concerne les champignons mycorhyziens (Buée *et al.*, 2007 ; Paul, Chapman & Chanway, 2006 ; Tedersoo *et al.*, 2008), la fixation d'azote (Crawford, Li & Floyd, 1997 ; Feller, 2003 ; Li, Crawford & Chang, 1997 ; Spano *et al.*, 1982), mais aussi le cycle des nutriments et la séquestration du carbone (Ganjegunte *et al.*, 2004 ; Harmon *et al.*, 1986).

Durant l'altération du bois, plus la dégradation avance et plus la concentration en azote et en phosphore augmente car la teneur en carbone diminue à cause de la dégradation des sucres, de l'amidon et des autres nutriments directement utilisables (Boddy & Watkinson, 1995). Par ce truchement, les débris ligneux influencent la teneur en azote du sol sous-jacent. En effet, Hafner et Groffman (2005) ont trouvé que les taux d'azote étaient moins importants dans le sol sous les débris ligneux que dans le sol sous la litière, ce qui pourrait participer à l'établissement et au maintien d'une certaine hétérogénéité des conditions environnementales et donc d'une diversité spécifique des forêts. Frey, Six et Elliott (2003) ont également montré que, par les champignons décomposeurs, des transferts de carbone et d'azote avaient lieu entre le sol et la litière. Les filaments mycéliens peuvent ainsi transporter des nutriments entre ces différents compartiments (Boddy & Watkinson, 1995 ; Lindahl, Finlay & Olsson, 2001).

Les organismes décomposeurs du bois mort : les champignons saproxyliques

Les communautés de champignons saproxyliques sont les principaux décomposeurs du bois mort en forêt boréale (Moore *et al.*, 2004) et représentent un groupe très diversifié. En effet, ces microorganismes sont capables de dégrader les composants récalcitrants du bois tels que la lignine, la cellulose et les hémicelluloses (Martínez *et al.*, 2005). Selon des critères macroscopiques et leur capacité à dégrader la lignine, la cellulose et les autres polysaccharides du bois, on distingue quatre catégories de champignons : les moisissures de surface et les colorations de l'aubier, les champignons de caries brunes, les champignons de carie molle et enfin les champignons de carie blanche (Boulet, 2003 ; Martínez *et al.*, 2005). Ces derniers sont les plus fréquents (90% des espèces fongiques liées au bois mort) et sont capables de dégrader la lignine, la cellulose et les hémicelluloses. Les moisissures brunes et blanches sont causées par les *Basidiomycota* qui sont considérés comme les principaux agents décomposeurs. Les groupes de plantes vasculaires (Angiospermes et Gymnospermes ligneux) sont attaqués tous les deux par les champignons, mais la carie brune se développe presque exclusivement sur les bois tendres (Gymnospermes) (Green III & Highley, 1997 ; Martínez *et al.*, 2005). Ce type de dégradation n'attaque pas la lignine, seulement la cellulose et les hémicelluloses. Le bois se fractionne en petits blocs d'où le nom de « carie brune cubique ». La coloration brune provient de la lignine, composante foncée du bois, qui a résisté aux champignons. La biodégradation fongique des composés lignocellulosiques a été revue par Martinez *et al.* (2005). La pourriture blanche est la plus destructrice car elle s'attaque en même temps à la lignine, à la cellulose et aux hémicelluloses. Elle touche surtout les feuillus. Par ce processus de décomposition, les champignons décomposeurs du bois créent différentes niches écologiques et fournissent les ressources et un habitat pour de nombreux autres organismes. Lonsdale, Pautasso et Holdenrieder (2008) ont recensé les études soulignant l'importance du bois mort décomposé par les champignons pour de nombreux organismes (Tableau A).

Tableau A Exemples d'études indiquant le bois mort comme étant la principale ressource pour les organismes vivants mentionnés. Tiré de Lonsdale, Pautasso et Holdenrieder (2008).

Principal organisme	Région	Références
Gorilla beringei	Ouganda	Rothman *et al.* (2006)
Petits mammifères	Canada	Bowman *et al.*(2000)
Martes americana	Canada	Porter, St. Clair et De Vries (2005)
Petite musaraigne (*Cryptotis parva*)	Etats-Unis	McCay et Komoroski (2004)
Campagnol à dos roux de Gapper (*Clethrionomys gapperi*)	Etats-Unis	Ucitel, Christian et Graham (2003)
Souris sylvestre (*Peromyscus maniculatus*)	Etats-Unis	Lee (2004)
Marsupiaux arboricoles	Australie	Wormington *et al.*(2003)
Chauves-souris	Canada	Parsons, Lewis et Psyllakis (2003)
Sittelle à poitrine rousse (*Sitta canadensis*)	Canada	Steeger et Hitchcock (1998)
Grand pic (*Dryocopus pileatus*)	Etats-Unis	McClelland et McClelland (1999)
Pic tridactyle (*Picoides tridactylus*)	Suisse	Bütler *et al.*(2004)
Pic à face blanche (*Picoides borealis*)	Amérique du Nord	Jackson et Jackson (2004)
Vertébrés du sol forestier	Etats-Unis	Butts et McComb (2000)
Salamandre	Etats-Unis	Alkaslassy (2005)
Macro-arthropodes du sol	Allemagne	Jabin *et al.* (2004)
Diptères	Norvège	Økland (1996)
Insectes en forêt boréale	Suède	Ehnström (2001)
Insectes dans les polypores	Suède	Jonsell et Nordlander (2002)
Insectes dans *Fomitopsis pinicola*	Finlande	Komonen (2003)

La décomposition du bois est un processus essentiel pour le cycle des nutriments, la formation du sol, la régénération de la forêt et le bilan en carbone des écosystèmes forestiers. En effet, les champignons décomposeurs modulent la disponibilité des ressources pour plusieurs groupes fonctionnels (Moore *et al.*, 2004). L'importance écologique de la décomposition du bois mort par les champignons a été soulignée dans de nombreuses études et revues de littérature (Tableau B).

Tableau B Exemple d'articles de synthèse soulignant le rôle écologique fondamental du bois mort en décomposition dans les forêts. Tiré de Lonsdale, Pautasso et Holdenrieder (2008).

Objectif principal	Région	Références
Ecosystèmes tempérés	Amérique du Nord	Harmon *et al.* (1986)
Directives pour la sylviculture	Allemagne	Ammer (1991)
Canopées	Amérique du Nord	Parks et Shaw (1996)
Directives pour la sylviculture	Grande-Bretagne	Hoddge et Peterken (1998)
Maintien de la biodiversité	Amérique du Nord	McComb et Lindenmayer (1999)
Réserves forestières	Allemagne	Meyer (1999)
Forêts aménagées	Suède	Fridman et Walheim (2000)
Fonctions des DLG	Chine	Hou et Pan (2001)
Statut et écologie du bois en décomposition	Royaume-Uni	Butler (2002)
Insectes saproxyliques	Australie	Grove (2002)
Dynamique de la décomposition	Scandinavie	Kruys, Jonsson et Ståhl (2002)
Gestion des habitats	Grande-Bretagne	Bratton (2003)
Vieilles forêts	Canada	Feller (2003)
Billes d'hêtre en décomposition	Danemark	Heilmann-Clausen et Christensen (2003)
Insectes forestiers	Europe	Bouget et Duelli (2004)
Coupes de rétention	Finlande	Hautala et al (2004)
Plantations de conifères	Grande-Bretagne	Humphrey (2005)
Gestion du bois mort	Scandinavie	Jonsson, Kruys et Ranius (2005)

Takahashi et Kaagya (2005) ont montré que les patrons de diversité des communautés fongiques se développant sur les DLG se modifient selon le stade de dégradation du substrat. D'autres études se sont intéressées à la diversité des champignons en relation avec le bois mort. Par exemple, la diversité fongique a été examinée selon le type d'habitat (Sippola, Mönkkönen & Renvall, 2005), l'âge du peuplement (Nordén & Paltto, 2001), l'essence forestière impliquée (Heilmann-Clausen, Aude & Christensen, 2005 ; Yamashita, Hattori & Abe, 2010), la taille des débris ligneux (Heilmann-Clausen & Christensen, 2004 ; Nordén *et al.*, 2004) et selon l'aménagement forestier (Küffer & Senn-Irlet, 2005 ; Lindhe, Åsenblad & Toresson, 2004 ; Sippola & Renvall, 1999 ; Sippola *et al.*, 2004 ; Vasiliauskas *et al.*, 2004).

Cependant la plupart des études sur les communautés boréales de champignons saproxyliques proviennent des forêts fenno-scandinaves ayant un long historique d'aménagement forestier intensif et une faible diversité d'essences forestières. A notre connaissance, peu d'études ont été conduites concernant la diversité des champignons décomposeurs en fonction des caractéristiques du bois mort dans la forêt boréale d'Amérique du Nord (Lumley, Gignac & Currah, 2001) ou alors concernant les champignons mycorhiziens (Kernaghan, Sigler & Khasa, 2003 ; Kernaghan *et al.*, 2003). Dans notre étude, les peuplements sont naturels et nos expériences sont les seules interventions anthropiques. Nos résultats reflètent donc ce qu'il se passe en forêt boréale naturelle ou suite à des interventions sylvicoles directement dans ces peuplements. Nos résultats ne sont donc pas influencés par un long historique d'aménagement tel qu'effectué dans les forêts scandinaves.

Interactions fongiques

Les interactions entre les champignons colonisant les débris ligneux dans les écosystèmes forestiers ont lieu lors de la phase de croissance mycélienne (Rayner & Boddy, 1988). La compétition fongique s'observe pour la capture d'une ressource non utilisée en y accédant le plus rapidement possible ou alors pour la défense (attaque) d'un territoire vis-à-vis d'un autre individu. Les mécanismes responsables de ces relations interspécifiques sont divers : antagonisme à distance par composés volatiles, interférence des hyphes, parasitisme et croissance mycélienne par contact (Boddy, 2000). Les différentes espèces fongiques présentent des capacités compétitrices variables (Woods, Woodward & Redfern, 2005). Ces relations sont importantes dans la mesure où elles conduisent à une succession des espèces lors de la dégradation du bois mort (Boulet, 2003). Les colonisateurs primaires puis les colonisateurs secondaires plus compétiteurs remplacent les champignons de première ligne. Au fur et à mesure de la dégradation, les propriétés du bois changent et des polypores plus compétitifs colonisent le substrat. Avec la prolifération des microorganismes, la disponibilité des ressources énergétiques diminue et les espèces luttent contre leurs concurrents. Ensuite viennent les champignons de dernière ligne qui interviennent pour attaquer les composés carbonés récalcitrants dans les états de décomposition du bois très avancés (matériel enfoui et désagrégé) (Boulet, 2003).

Rôle des décomposeurs pionniers

Parmi les communautés de champignons décomposeurs, les espèces pionnières jouent un rôle important. En effet, Heilmann-Clausen et Christensen (2003) ont observé que l'importance du type de DLG pour la richesse des espèces fongiques menacées était reliée à la présence de certains décomposeurs pionniers. Ces auteurs ont suggéré qu'une grande diversité de décomposeurs primaires pourrait être un élément clé dans la conservation des champignons décomposeurs du bois mort. Chez les angiospermes, les décomposeurs primaires auraient une influence sur l'établissement des champignons arrivant à un stade subséquent de la succession (Heilmann-Clausen & Boddy, 2005). Fukami *et al.* (2010) ont trouvé de grandes différences dans le fonctionnement de l'écosystème (taux de décomposition) causés par de petites différences dans la composition de la communauté de champignons pionniers. De plus les facteurs influençant la diversité fongique dans le bois en décomposition varient selon le stade successionel des champignons impliqués (colonisateurs primaires vs. secondaires) (Jönsson, Edman & Jonsson, 2008). On voit donc le rôle non négligeable de ces décomposeurs primaires à la fois dans le processus de décomposition du bois mais aussi dans la structure des communautés saproxyliques.

Lien entre diversité et fonction

Le lien entre la diversité biologique et les fonctions associées à cette dernière ont reçu un intérêt grandissant ces dernières années (Coleman & Whitman, 2005 ; Fitter *et al.*, 2005 ; Gray *et al.*, 2003 ; Hooper, 2002 ; Naeem, 2002 ; O'Connor & Crowe, 2005). Plus précisément, il s'agit d'évaluer la perte de biodiversité sur le fonctionnement d'un écosystème (O'Connor & Crowe, 2005). Cependant peu d'études ont examinées les relations entre la diversité microbienne et le processus de décomposition (Hättenschwiler, Tiunov & Scheu, 2005). Une corrélation positive entre la diversité microbienne et la décomposition a été démontrée pour la cellulose (Wohl, Arora & Gladstone, 2004) et dans les écosystèmes du sol (Bonkowski & Roy, 2005). Cependant il n'existe pas de consensus permettant de décrire comment la perte de diversité va affecter la décomposition dans la mesure où il existe des résultats contradictoires dans la littérature (Mikola & Setälä, 1998 ; Müller *et al.*, 2002 ; Setäla & McLean, 2004 ; Wardle *et al.*, 1999). De plus, la plupart de ces études concerne les

microorganismes du sol et les processus impliquant la diversité des champignons saproxyliques et les processus de décomposition sont rares.

La diversité microbienne pourrait augmenter le processus de décomposition dans la mesure où certaines réactions biochimiques nécessitent des conditions particulières, et donc, ne peuvent être assurées par un seul organisme (Van Der Heijden, Bardgett & Van Straalen, 2008). C'est le cas de la dégradation de la lignine (qui est présente en grande quantité dans le bois). En effet, pour décomposer ce composant, il est nécessaire d'avoir recours à plusieurs réactions chimiques qui ne peuvent pas être réalisées par un seul organisme (De Boer *et al.*, 2005).

Influence de l'aménagement forestier

Les coupes forestières intensives réalisées à l'échelle du paysage entrainent une augmentation de la proportion des jeunes forêts par rapport aux vieilles forêts et une réduction importante de la densité des gros arbres vivants (Drapeau *et al.*, 2009). En effet, en changeant la distribution des classes d'âge des peuplements, la proportion de vieilles forêts diminue à l'échelle du paysage. En Fenno-Scandinavie, le long historique d'aménagement forestier intensif (depuis le XIXème siècle, utilisation d'herbicides pendant 30 ans, plantations, fertilisation en azote) a fait drastiquement chuté la proportion de ces vieilles forêts et changé la composition forestière (Ostlund, Zackrisson & Axelsson, 1997). En forêt boréale canadienne, la surreprésentation des coupes totales et des peuplements équiens en résultant est un des principaux enjeux de l'aménagement forestier (Gauthier *et al.*, 2008). Pour répondre à cela, la diversification des méthodes de récolte forestière, incluant l'utilisation des coupes partielles, a été proposée comme une approche d'aménagement des écosystèmes forestier boréaux (Bergeron *et al.*, 2002 ; Franklin, 1997 ; Harvey *et al.*, 2002). Les objectifs de la coupe partielle visent notamment l'augmentation de la diversité des essences forestières et des classes de taille ainsi que l'établissement et la croissance des espèces tolérantes à l'ombre tout en maintenant le fonctionnement et la biodiversité des écosystèmes. Les coupes partielles pourraient mener les peuplements vers des structures de cohortes plus vieilles ou maintenir la cohorte au même stade. Dans les forêts boréales, une grande quantité de bois mort est caractéristique des vieux peuplements (Linder, Elfving &

Zackrisson, 1997). Cependant l'aménagement forestier entraine une diminution de la quantité de ces débris ligneux et en particulier concernant la qualité et la quantité des billes en décomposition (Brais *et al.*, 2004 ; Brassard & Chen, 2008 ; Siitonen *et al.*, 2000). La sylviculture entraîne en général une augmentation de l'apport de certains types de DLG après l'éclaircie. Certaines structures clés comme les chicots ou les grands chablis sont remplacées par les souches (Montes & Cañellas, 2006) et le bois frais de petite dimension. Les coupes partielles permettent de conserver certaines structures et de maintenir un recrutement minimum de bois mort plus proche des forêts non aménagées comparativement aux pratiques sylvicoles plus intensives (Harvey & Brais, 2007).

L'aménagement forestier par l'intermédiaire des coupes a des effets sur la diversité biologique et en particulier sur la diversité des communautés de champignons saproxyliques dans les peuplements aménagés (Bader, Jansson & Jonsson, 1995 ; Penttilä, Siitonen & Kuusinen, 2004). À l'échelle mondiale, la diminution de la disponibilité en matière ligneuse morte dans les forêts aménagées est considérée comme l'une des principales causes de perte de diversité biologique (Drapeau *et al.*, 2009). En Finlande, 20 à 25% de toutes les espèces vivantes en forêt sont associées au bois mort (Siitonen, 2001). De plus, en Scandinavie, le champignon menacé *Phellinus nigrolimitatus*, est très fortement dépendant des débris ligneux grossiers bien décomposés, qui sont des caractéristiques rencontrées dans les forêts non aménagées (Stokland & Kauserud, 2004). En comparant les forêts aménagées et non aménagées, les auteurs ont observé une diminution de 82% de la fréquence de cette espèce de champignon sur les sites soumis aux coupes forestières. De même, en Finlande, Sippola *et al.* (2004) ont noté une diminution de l'occurrence des champignons polypores, dans les sites aménagés. Lorsque l'intensité d'exploitation dépassait un certain seuil de tiges coupées, plus aucun individu des espèces menacées n'était observé. Bien que de nombreuses études se sont penchées sur l'effet de l'aménagement forestier sur la diversité des champignons décomposeurs comparativement à des forêts non-aménagées, l'effet des pratiques sylvicoles alternatives et notamment les coupes partielles sur les communautés fongiques a reçu que très peu d'intérêt (Lõhmus, 2011 ; Nordén *et al.*, 2008), tout particulièrement en Amérique du Nord.

D'autre part, les communautés microbiennes sont caractérisées par une grande redondance fonctionnelle (décomposition, respiration, nitrification) (Degens, 1998 ; Griffiths *et al.*, 2000 ; Griffiths *et al.*, 2001 ; Müller *et al.*, 2002) et on ne connait pas dans quelle mesure une réduction de la diversité spécifique causée par l'aménagement forestier aura des conséquences sur le fonctionnement de l'écosystème (Baptist *et al.*, 2008 ; Bonkowski & Roy, 2005 ; Setäla & McLean, 2004 ; Wertz *et al.*, 2006). Ceci montre à quel point une meilleure compréhension des processus associés aux communautés saproxyliques est nécessaire dans l'élaboration des stratégies d'aménagement forestier fondées sur la conservation des fonctions de l'écosystème forestier.

Objectifs de la thèse

L'objectif général de cette thèse est d'améliorer les connaissances sur l'écologie du bois mort et des champignons décomposeurs qui lui sont associés en évaluant les facteurs qui structurent les communautés fongiques dans les peuplements naturels et sous aménagement. Il s'agit également de vérifier les relations entre la diversité fongique et l'activité de décomposition; et enfin de documenter les effets des coupes partielles sur les communautés de champignons saproxyliques. Dans la plupart des études précédentes, la diversité et la structure des communautés fongiques se développant sur les DLG ont surtout été étudiées à partir de l'observation de sporocarpes (fructifications) et ont souvent été basées sur une seule essence de bois mort. Cependant, les sporocarpes ne révèlent pas la richesse entière présente mais seulement celle des espèces qui ont fructifié au moment de l'échantillonnage et il est reconnu que les études basées sur l'observation des sporocarpes ont des limitations certaines (Boddy, 2001). Pour pallier ce problème, d'autres travaux ont été réalisés grâce à des méthodes d'isolement et de culture du mycélium provenant du bois (Hood *et al.*, 2004). Cependant certains champignons ne peuvent pas être cultivés sur milieux synthétiques (Rayner & Boddy, 1988). C'est pourquoi des méthodes de biologie moléculaire ont été développées pour identifier la présence fongique directement dans le bois mort (Johannesson & Stenlid, 1999 ; Råberg *et al.*, 2005). Une technique en particulier a été utilisée pour comparer les fragments d'ADN selon leur séquence : le gel d'électrophorèse en gradient dénaturant (Muyzer & Smalla, 1998). Cette technique offre la possibilité d'utiliser l'ADN pour réaliser du séquençage afin d'identifier les organismes. Cette technique a été utilisée

avec succès pour évaluer les changements dans la composition des communautés microbiennes du sol (Anderson, Campbell & Prosser, 2003) et du bois (Kulhánkova *et al.*, 2006). Cependant peu d'études ont appliqué ces techniques moléculaires sur les successions de champignons saproxyliques (Kubartová *et al.*, 2007 ; Kubartová *et al.*, 2009). A la différence des inventaires des fructifications, cette méthode permet de détecter des espèces fongiques importantes mais discrètes, qui restent sous forme de mycélium et ne fructifient pas (Rajala *et al.*, 2010 ; Stenlid, Penttilä & Dahlberg, 2008). Les inventaires des fructifications fongiques sont considérés comme « la partie visible de l'iceberg » dans la mesure où la distribution et l'abondance des sporocarpes ne reflètent pas forcement la distribution et l'abondance du mycélium. Ainsi, l'absence de sporocarpes dans l'échantillon ne signifie pas que le champignon est absent (Lindner *et al.*, 2011). Dans cette thèse, nous avons utilisé la méthode du gel d'électrophorèse en gradient dénaturant afin de caractériser la composition des communautés fongiques. Les mesures de diversité sur les communautés ont été mises en relation avec les caractéristiques chimiques du bois (mesurée par spectroscopie infrarouge) et avec des variables propres aux peuplements comme le stade successionel ou le volume de bois mort par exemple.

Toute notre étude a été conduite dans le projet SAFE (Sylviculture et Aménagement Forestier Ecoystémique) (Brais *et al.*, 2004 ; Haeussler *et al.*, 2007 ; Harvey & Brais, 2007), qui consiste en une série d'expériences menées à la Forêt d'Enseignement et de Recherche du Lac Duparquet (FERLD) dans la partie sud-est de la forêt boréale canadienne. Le projet SAFE teste un modèle d'aménagement écosystémique basé sur la dynamique naturelle des écosystèmes (Bergeron & Harvey, 1997 ; Harvey *et al.*, 2002).

Dans le premier chapitre « Species composition of saproxylic fungal communities on decaying logs in the boreal forest », publié en 2011 dans Microbiol Ecology (Kebli *et al.*, 2011), nous avons analysé la structure et la composition des communautés fongiques dans des peuplements naturels et leurs relations avec les facteurs environnementaux responsables des changements de ces communautés le long d'un gradient de décomposition du bois mort au sol. Il s'agissait d'évaluer l'état des communautés saproxyliques en s'affranchissant des problèmes méthodologiques liés à l'identification (tous les champignons de fructifient pas) ou à la culture des champignons (certains ne sont pas cultivables). D'après les études

précédentes, on s'attendait à ce que la diversité des champignons saproxyliques sur les billes soit maximale aux stades de dégradations intermédiaires, et que les plus grosses billes supportent une plus grande diversité. On a également posé l'hypothèse que la composition de la communauté serait influencée par l'âge du peuplement et le volume de bois mort. Pour répondre à ces suppositions, nous avons donc choisi une chronoséquence de bois mort au sol (102 billes) pour cinq essences forestières différentes identifiées par microscopie à l'aide de lames minces (deux feuillus et trois conifères) et dans trois types de peuplements : des vieux peuplements issus d'un feu de 1760 et des peuplements plus jeunes (des peuplements mixtes de 1910 et des tremblaies issus d'un feu de 1923). Nous avons également relié les paramètres de diversité aux conditions chimiques du bois en plus des variables explicatives à l'échelle du peuplement et de la bille.

Dans le deuxième chapitre « Patterns of saproxylic fungal colonization during wood decomposition along a gradient of forest disturbance », on s'est intéressé aux effets des perturbations du peuplement sur la diversité et l'activité des communautés fongiques lors des premières étapes de colonisation dans des peuplements de composition différente en utilisant une approche moléculaire. Dans ce chapitre nous nous sommes également intéressés à la relation entre la diversité des champignons saproxyliques et la fonction de décomposition. Nous avions émis l'hypothèse qu'il existait une corrélation positive entre la diversité et l'activité des champignons due au rôle complémentaire des différentes espèces fongiques. On s'attendait également à trouver des patrons de décomposition différents selon les trois intensités de perturbation (traitements sylvicoles) et aussi entre les deux essences de bois hôte (feuillu et conifère). Enfin, on s'attendait à ce que la diversité et l'activité des champignons saproxyliques suivent les changements de structure du peuplement (surface terrière résiduelle et volume de bois mort au sol) causé par le stade successionel du peuplement et par la perturbation. On a donc choisi les peuplements selon un gradient de perturbation dans une approche plus expérimentale dans la mesure où l'on a déposé 480 blocs de bois frais (non plus des billes), c'est-à-dire issus d'arbres non décomposés, dans les différents traitements (témoins, partiellement coupés, brûlis) pendant 2 ans et demi.

Le troisième chapitre s'intitule « Impact of harvesting intensity on wood-inhabiting fungi in aspen boreal forests of Eastern Canada » (soumission prévue à Microbial Ecology en

2011). Comme la gestion du bois mort en forêt aménagée est perçue comme un enjeu majeur de l'aménagement forestier durable, il s'agissait d'évaluer plus précisément la réponse des communautés fongiques 10 ans après la réalisation de traitements sylvicoles. Nous avons donc choisi une essence (le peuplier faux-tremble) et un peuplement homogène (même stade successionel) dans lequel diverses pratiques sylvicoles ont été effectuées (coupes partielles, totales et brûlis). Pour ce chapitre nous avons également analysé les communautés fongiques du bois mort sur pied, à savoir les chicots. Nous nous sommes aussi penchés sur les capacités de dispersion des champignons en mesurant la distance géographique entre les échantillons pour savoir si les communautés plus proches géographiquement étaient plus semblables. On s'attendait à ce qu'une plus grande connectivité de bois mort dans le peuplement conduise à une plus grande similarité de la composition des communautés fongiques. Nous avons aussi émis l'hypothèse que la richesse spécifique et la diversité des champignons saproxyliques était corrélée négativement avec l'intensité des pratiques sylvicoles. On s'attendait aussi à ce que le volume de bois mort résiduel minimise l'impact de la récolte sur les communautés fongiques. Finalement on s'attendait que les billes les plus grosses et les plus décomposées supporteraient une plus grande diversité.

Les trois chapitres s'articulent donc selon un lien logique qui va de l'évaluation des communautés fongiques en conditions naturelles suivi par l'influence des perturbations sur la diversité, la composition, la colonisation et l'activité des champignons pionniers et pour se terminer par l'effet des pratiques sylvicoles sur les communautés fongiques. Toutes les analyses ont été effectuées en utilisant des méthodes de biologie moléculaire très peu utilisées dans ce domaine. Le premier chapitre apporte donc des connaissances fondamentales concernant l'écologie du bois mort et des champignons décomposeurs en peuplements naturels; le second des informations sur une thématique qui reçoit un intérêt important et grandissant dans la littérature sur les liens entre diversité et fonction et sur le processus de colonisation du bois en lien avec la perturbation du peuplement; enfin le troisième chapitre permet d'évaluer l'effet des pratiques sylvicoles sur les champignons saproxyliques et donc de guider les exploitants forestiers et d'établir des recommandations de conservation de la diversité fongique dans le cadre de l'aménagement écosystémique.

CHAPITRE I

SPECIES COMPOSITION OF SAPROXYLIC FUNGAL COMMUNITIES ON DECAYING LOGS IN THE BOREAL FOREST

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1.1 Abstract

Coarse woody debris support large numbers saproxylic fungal species. However most of the current knowledge comes from Scandinavia and studies relating the effect of stand or log characteristics on the diversity and composition of decomposer fungi have not been conducted in Northeastern Canada. Logs from five tree species were sampled along a decomposition gradient in nine stands representing three successional stages of the boreal mixed forest of Northwestern Quebec, Canada. Using a molecular fingerprinting technique, we assessed fungal community Shannon diversity index, richness and composition. We used linear mixed models and multivariate analyses to link changes in fungal communities to log and stand characteristics. We found a total of 33 operational taxonomic units (OTU) including an indicator species for balsam fir (similar to *Athelia* sp.) and one found only in aspen stands (similar to *Calocera cornea*). Spruce logs supported the highest fungal Shannon diversity index and OTU number. Our results support the hypothesis that log species influences fungal richness and diversity. However log decay class does not. Stand composition, volume of coarse woody debris and log chemical composition were all involved in structuring fungal communities. Maintaining the diversity of wood decomposing communities in boreal mixedwood forests should then require the presence of dead wood from diverse log host species.

1.2 Introduction

Saproxylic (Speight, 1989) fungi are the main agents of wood decomposition and an essential component of forest ecosystem food webs (Moore *et al.*, 2004), influencing nutrient cycling and carbon sequestration (Harmon *et al.*, 1986). They represent a highly diverse group, for example over 1500 species were identified from Finnish forests alone (Siitonen, 2001). Forest harvesting decreases the amount of deadwood and species diversity of saproxylic fungi in managed fennoscandian forests (Penttilä, Siitonen & Kuusinen, 2004 ; Sippola & Renvall, 1999) and wood-decaying fungi are considered good indicators of effective conservation (Lonsdale, Pautasso & Holdenrieder, 2008), as they are sensitive to changes in forest structure.

Wood structure and transformations in chemical composition over the course of decomposition can induce changes in fungal species composition, as some species utilize specific substrates for a limited time according to their biochemical requirements (Boddy, 1992). Succession of saproxylic fungal communities during decomposition has been described in spruce logs in natural boreal old-growth forests of Sweden (Berglund, Edman & Ericson, 2005) and in spruce and trembling aspen logs in the boreal mixedwood forest of Alberta (Canada) (Lumley, Gignac & Currah, 2001). Log host species (Lumley, Gignac & Currah, 2001) and stand characteristics, such as age (Nordén & Paltto, 2001) and abundance of deadwood (Junninen *et al.*, 2006), can also influence fungal composition and diversity.

Factors influencing fungal diversity in decomposing logs vary according to fungal species (Hottola, Ovaskainen & Hanski, 2009), fungal successional status (early vs. secondary) (Jönsson, Edman & Jonsson, 2008) and the scale of the study (Berglund, Edman & Ericson, 2005). Also, some species, especially heart-rot agents, prefer larger logs (Nordén *et al.*, 2004) and species diversity has been shown to increase with log size (Edman, Kruys & Jonsson, 2004). Large logs may have a buffering effect, which contributes to the stability of microclimatic conditions. Conversely, the interiors of small logs are subject to greater variation in sun exposure, temperature, and precipitation, especially in the most open stands (Bader, Jansson & Jonsson, 1995 ; Sippola & Renvall, 1999). On the other hand, logs at intermediate decay stages have also been found to harbour more species (Heilmann-Clausen

& Christensen, 2003) due to the lack of energy resources when the cellulose is depleted (Stenlid, Penttilä & Dahlberg, 2008) and to the availability of multiple niches caused by heterogeneous decomposition (Pyle & Brown, 1999).

However, much of the information available on boreal saproxylic fungal communities comes from Fennoscandian forests with a long history of forest management and poor tree diversity. To our knowledge, few studies on saproxylic fungal diversity in relation to deadwood characteristics have been conducted in the boreal forests of North America (Lumley, Gignac & Currah, 2001). Moreover, fungal diversity and community structure of coarse woody debris (CWD) have, so far, mainly been assessed from observations of sporocarps and based on single log species. For example, investigations of fungal communities in relation to habitat type (Sippola *et al.*, 2004), stand age (Nordén & Paltto, 2001), successional status and total volume of dead wood (Junninen *et al.*, 2006), and host tree species (Yamashita, Hattori & Abe, 2010) were all based on fungal fructifications. Community fingerprinting techniques have recently been applied to studies of soil fungi and a few studies have used molecular techniques to study saproxylic fungal diversity on dead wood (Kubartová *et al.*, 2007 ; Kubartová *et al.*, 2009). Denaturing/Temperature Gradient Gel Electrophoresis (DGGE/TGGE) has been used successfully for evaluating changes in community composition in soils (Anderson, Campbell & Prosser, 2003) and in wood (Kulhánkova *et al.*, 2006). Moreover, this technique allows DNA to be recovered and sequenced for fungal identification.

We used DGGE to separate fungal Internal Transcribed Spacer (ITS) regions amplified from total CWD DNA to evaluate saproxylic fungal diversity of five species of decomposing logs from natural boreal stands of different successional status in relation to chemical and physical characteristics of CWD. Our objectives were (i) to characterize how diversity and species composition vary along a decomposition gradient for different wood species in natural stands, (ii) to link fungal communities to morphological and chemical features of CWD and other stand features, and (iii) to identify indicator species of log species, stand features (successional stage) or environmental conditions (decay stage). We hypothesized that the saproxylic fungal diversity of individual logs would be maximal at intermediate decay stages, and that higher diversity would occur in larger logs. We also

expected fungal composition to be influenced by log species and stand conditions such as age and CWD volume.

1.3 Materials and methods

1.3.1 Field sites description

The study area was located within the Lake Duparquet Research and Teaching Forest (Harvey, 1999) in the Abitibi region of northwestern Québec, 45 km northwest of Rouyn-Noranda, Québec (48°86′N–48°32′N, 79°19′W–79°30′W). The climate is moist continental with a mean annual temperature of 0.8 °C and annual precipitation is 890 mm (Environment Canada; Canadian climatic normals 1971-2000). The region was situated in the mixedwood zone of the boreal shield. Forest succession on rich mesic sites generally begins with the establishment of pure or mixed stands of paper birch (*Betula papyrifera* Marsh.), trembling aspen (*Populus tremuloides* Michx.) and jack pine (*Pinus banksiana* Lamb.) that can maintain dominance for a period of over 100 years. In the absence of a major disturbance, these species are gradually replaced by a mixture of shade tolerant species such as white spruce (*Picea glauca* (Moench) Voss), black spruce (*Picea mariana*, (Mill) B.S.P.), and balsam fir (*Abies balsamea* (L.) Mill.) (Bergeron & Dubuc, 1989).

1.3.2 Field methods

Sampling took place in the control stands of the SAFE experiment in early summer (Brais *et al.*, 2004). The SAFE study is set in natural stands of fire origin representing a gradient of composition typical of the natural succession on rich mesic clayey sites. The oldest stand types (mixed post budworm outbreak stands) dates from a fire in 1760 and was later effected by the 1970-1987 spruce budworm outbreak (*Choristoneura fumiferana*) (Morin, Laprise & Bergeron, 1993) resulting in a mixture of white birch, white spruce, and balsam fir (Table 1.1). The experimental design of the SAFE study includes three, 1-2 ha, unharvested plots in each stand type, for a total of nine experimental units. In all experimental units, five permanent circular sampling plots (radius = 11.28 meters) were established and all tree stems greater than 5.0 cm in diameter at breast height (dbh) were identified and measured (dbh) for basal area estimation.

Table 1.1 Mean volume of CWD by decay class and tree species basal area in sampled stands of different successional status

	Post spruce budworm outbreak stand (1760)	Mixed trembling aspen stand (1910)	Trembling aspen stand (1923)
	Stand CWD volume (m^3 ha^{-1})		
Decay class			
Fresh	8.8 (n=6)	10.3 (n=9)	7.3 (n=10)
Medium	55.4 (n=8)	15.4 (n=6)	48.9 (n=22)
Advanced	25.5 (n=20)	85.1 (n=10)	71.7 (n=11)
All categories	89.7	110.8	127.9
	Basal area (m^2 ha^{-1})		
Tree species			
Trembling aspen	0.7	30.2	37.3
Paper birch	9.6	0.2	0.7
Spruce	6.1	4.0	1.4
Balsam fir	2.6	2.6	0.6

Number of logs sampled are given in parenthesis.
Fresh wood: decomposition classes I and II.
Medium decayed wood: decomposition class III.
Advanced decay stage: decomposition classes IV and V.

The volume of downed wood was estimated using triangular-transects. One triangle (30 m side, (Van Wagner, 1982)) was sampled in each experimental plot. Along each transect line, the frequency of downed wood was recorded by species, diameter class (5-cm: 2.5-7.6 cm, 10-cm: 7.6-12.5 cm, 15-cm: 12.6-17.5 cm, and greater than 17.5 cm) and five decomposition classes (Daniels *et al.*, 1997).

Logs of spruce, paper birch, jack pine, balsam fir and trembling aspen with diameter over 10 cm and from five decay classes were located and identified. Log length and diameter (both ends) were measured. Material for DNA extraction was collected by drilling one hole in each log in the selected decomposition class with a flat drill bit (12.7 mm). The bark and the uppermost layer of wood were first removed and precautions taken to prevent cross-contamination of samples; drill bits were cleaned, rinsed with sterile water, soaked in 95% ethanol and flame sterilised between samples. Additional samples from each log were taken for physical and chemical laboratory analyses. For well-decomposed logs, where visual species identification was impossible, an additional wood sample was taken from a less

decomposed part of the log for laboratory identification. All samples were kept frozen at −20°C until analyzed.

Of the 102 sampled logs, 43 were sampled in the youngest stands types (1923 fire), 34 in the oldest stands (1760 fire) and 25 in the second youngest stands (1910 fire). Not all combinations of species and decomposition classes could be found in all stand types. For example, no trembling aspen logs were found in the 1760 stand or jack pine in the 1910 stand.

1.3.3 Log species identification

Woody species were identified from structural and anatomical features from sub-samples cut using a microtome (Hoadley, 1990). However, this technique did not allow us to distinguish between white and black spruce.

1.3.4 Wood physical and chemical characteristics

Log samples were cut into 5 x 5 cm pieces. Wood density was estimated from volume, determined by water displacement after immersing samples in hot paraffin, and weight corrected for moisture content estimated from a second sample. The latter sample was then air-dried and ground with a cutting mill (Retsch, SM2000) for chemical analyses.

Total N and C were measured by dry combustion using a LECO CNS 2000 analyzer (LECO Corporation, St. Joseph, MI). Lignin and cellulose were determined by near infrared spectroscopy (Foley *et al.*, 1998) using a FOSS NIRSystems (model 6500). A calibration equation was first developed following wet chemical measurements from 100 samples to predict the chemical composition of every sample (Appendix A). The wet chemical analyses used to calibrate the multivariate spectroscopic method were based on the Acid Detergent Fiber/Neutral Detergent Fiber method (Goering & Van Soest, 1970). Lignin determination was based on the method of Brinkmann (2002).

1.3.5 DNA extraction from wood

Wood samples were lyophilized for 48 hours before disruption in a Qiagen TissueLyser (QIAGEN, Mississauga, Ontario, Canada), then run for 2 min at 26 Hz, or until the wood was reduce to a fine powder. DNA was extracted with the DNeasy Plant Mini Kit (QIAGEN) according to the manufacturer's instructions. DNA was eluted in 100 µL of elution buffer and stored at -20°C.

1.3.6 PCR amplification of fungal-specific genes

The Internal Transcribed Spacer (ITS) region of the fungal rDNA was polymerase chain reaction-amplified using the fungal specific primers ITS1-F (Gardes & Bruns, 1993 ; Jasalavich, Ostrofsky & Jellison, 2000) and ITS2 (White *et al.*, 1990) to obtain a sequence of 280-bp-length. A GC clamp (CGC CCG CCG CGC CCC GCG CCC GGC CCG CCG CCC CCG CCC CC) was added to the 5' end of the ITS1-F primer to avoid complete separation of DNA strands during the subsequent denaturing electrophoresis. Polymerase chain reactions (50-µL) contained 2 µL of template, 5 µL of PCR reaction buffer (ThermoPol, New England Biolabs), 1 µl (10 mM) of each dNTP, 1 µL of each primer (50 µM), 0.2 µL of Taq polymerase (5 $U.\mu L^{-1}$, New England Biolabs). Cycling parameters were an initial denaturation cycle of 3 min at 95 °C followed by 35 cycles of denaturation at 94 °C for 45s, annealing at 55 °C for 45 s, and extension at 72 °C for 1 min 15 s, ending by a final elongation at 72 °C for 8 min (Kubartová *et al.*, 2007). Negative (no DNA) and positive controls (fungal DNA from pure culture) were included in each set of reactions. All amplification products were analysed by electrophoresis with 1% (w/v) agarose gels in TAE (40 mM Tris-acetate, 1 mM EDTA), stained with Gelgreen (Biotium) and visualized under UV light.

1.3.7 Separation of fungal ITS amplicons by DGGE

Electrophoresis was performed according to a slight modification from the protocol of Julien *et al.* (2008). We used a DCode universal mutation detection system (Bio-Rad Laboratories, Hercules, CA) and an acrylamide gel (8% [wt/vol] acrylamide–bis-acrylamide, 37.5:1) with a linear vertical gradient of 20–55% denaturing agents (100% denaturant

corresponding to 7 M urea and 40% [v/v] deionized formamide) and increasing in the direction of the electrophoretic run with a stacking gel (4% [w/v] acrylamide–bis-acrylamide, 37.5:1) on top. Approximately 400 ng of each PCR product was loaded and electrophoresis was performed in tris–acetate–ethylenediaminetetraacetic acid (EDTA) (TAE) buffer (40 mM Tris-acetate, 1 mM EDTA) at 75 V and 60 °C for 16h. Gels were stained for 15 min with SYBR gold (Invitrogen, Carlsbad, CA), visualized under UV illumination, and digitized using a ChemiDoc XRS System molecular imager (Bio-Rad Laboratories, Hercules, CA).

1.3.8 Gel analysis

The software package GelCompar II (version 5.0, Applied Maths, Belgium) was used to analyse ITS DGGE banding patterns. In order to minimize migration differences and to normalize for distortions between gels, we aligned the gels using an external reference pattern comprised of mixed ITS amplicons from five different fungi. A band-matching process was used to obtain a presence-absence matrix for statistical analyses. A 5% band intensity threshold was set for the band selection process. Individual bands were grouped into classes based on melting behaviour (positions in the gels). Each band class was then considered to be an operational taxonomic unit (OTU), allowing calculation of their frequency among log samples. We also calculated the relative intensity of each band, applying a value between 0 and 1 by dividing the intensity of the band by the sum of the intensity of all the bands within the lane, thus eliminating the variation in band intensity due to difference in amplification and amount of DNA loaded on the DGGE gel.

1.3.9 Cloning and sequencing of excised DGGE bands

Amplicons which generated prominent DGGE bands were selected for cloning and sequencing. Bands were excised from DGGE gels and the DNA eluted in 20 µL of sterile deionized water and subsequently used as a template for PCR using the primers ITS1-F and ITS2 as described above. Amplicons (280 bp) obtained with the fungal specific primers were purified on agarose gels using the Geneclean Turbo kit as recommended by the manufacturer (Q-bio gene, USA). The purified DNA was cloned by ligation to the pGEM-T Easy vector system (Promega, Madison, WI) and transformed into competent *Escherichia coli* cells (JM109) according to the manufacturer's instructions. Positive clones were selected on

appropriate LB agar plates and their plasmids isolated with the Wizard Plus SVMinipreps system (Promega). Sequencing reactions were performed with a BigDye Terminator cycle sequencing kit 3.1 with a genetic analyzer (3130XL; Applied Biosystems, Foster City, CA) by the Plate-forme d'Analyses Biomoléculaires (Université Laval, Québec, Canada). All clones were sequenced in both directions using Sp6 and T7 universal primers. Sequences were aligned using Bioedit software (Hall, 1999) and vector plasmid related sequence were removed. Sequence identity searches were performed using BLASTn against the GenBank database of the National Center for Biotechnology Information (NCBI).

1.3.10 Statistical analysis

A matrix of relative abundance (and presence/absence) was obtained for fungal species. From this, the number of OTUs (i.e. DGGE band classes per sample) and the Shannon diversity index (H′) were calculated.

Shannon indices were calculated according to Eichner *et al.* (1999). In order to link OTU number and H′ with explanatory variables, data were analyzed with a linear mixed-effects model using the "nlme" package (R package version 3.1-90; nlme: Linear and Nonlinear Mixed Effects Models) from R software (R Development Core Team, 2010). Five models were tested based upon our hypothesis (Table 1.2).

The global statistical model (model 5) included all possible explanatory factors: wood density, log volume, species (paper birch, trembling aspen, balsam fir, spruce, jack pine), decay stage (fresh deadwood (class I and II); medium decay (class III) and well decomposed wood (class IV and V)), stand deadwood volume, deciduous and coniferous basal area, lignocellulose index (LCI = lignin/(lignin + cellulose)) (Preston & Trofymow, 2000) and the carbon:nitrogen ratio. All other models are subsets of the global model each corresponding to different hypothesis. All explanatory variables were entered as fixed factors whereas stand, replicates and experimental units were considered random factors - each one nested in the former. Models were compared on the basis of Akaike's information criteria (AIC) (Burnham & Anderson, 2004). The ''best'' model, is the model with the lowest AIC and the highest Akaike weight. Akaike weights (w_i) indicate the level of support in favor of any given model being the most parsimonious and most probable among candidate models

(Mazerolle, 2006). For model selection, we used "AICcmodavg" R package (R package version 1.01; AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c), http://CRAN.R-project.org/package=AICcmodavg).

Table 1.2 Linear mixed models relating species richness (OTU number) and Shannon diversity index to stand and log characteristics.

Model	Variables included in each model	Corresponding hypothesis
1	Log species	Effect of log species
2	Decomposition class + log volume + wood log density	Effect of logs physical variables
3	LCI + C:N ratio	Effect of log chemical variables
4	CWD Volume + deciduous basal area + coniferous basal area	Effect of stand features
5	Model 1+ model 2 + model 3 + model 4	Global model (all variables)

Multivariate analyses of fungal community composition were computed using the "vegan" package (R package version 1.15-2; vegan: Community Ecology Package, http://CRAN.R-project.org/package=vegan). We used CCA (canonical correspondence analysis) (Fromin *et al.*, 2002 ; Ramette, 2007) because it positions DGGE profiles (OTU scores and logs/site scores) in relation to environmental factors and ordinates fungal communities in such a way that the relationship between samples, species and environmental variables on the ordination diagram can be assessed from angles between vectors (quantitative environmental variables) or distances between points (samples and species) and vectors (Salles, Van Veen & Van Elsas, 2004). The analysis shows the way in which changes in fungal community composition are associated with changes in environmental variables. The method assumes a unimodal distribution of species. Quantitative environmental variables were standardized before performing the analysis by subtracting the mean and dividing by the standard deviation. CCA was done on the presence-absence matrix of fungal DGGE bands because it gave the best separation between different samples (Dilly *et al.*, 2004).

Finally, indicator species analysis was carried out with the duleg function of the "labdsv" package (R package version 1.3-1; labdsv: Ordination and Multivariate Analysis for Ecology, http://ecology.msu.montana.edu/labdsv/R). We also applied a Holm correction on these probabilities. All statistical analyses were called significant when $P < 0.05$.

1.3.11 Nucleotide sequence accession numbers

The sequences generated during this study have been deposited in GenBank under accession numbers HM195119 to HM195131.

1.4 Results

1.4.1 Stand characteristics

Stand types were characterized by different amounts of deadwood (Table 1.1), as well as tree species composition. The 1923 fire trembling aspen stands had the largest amount of total deadwood while the largest volume of well decomposed wood was found in the mixed trembling aspen stands (1910 fire stands). The oldest stand types (mixed post budworm outbreak stands from the 1760 fire) had the lowest volume of CWD but the largest volume in the medium decay stage (Table 1.1).

1.4.2 Dead wood properties

Log species differed in their physical and chemical features (Table 1.3). Higher C:N ratios were found among spruce and jack pine logs, while the lignocellulose index (LCI) was higher in jack pine. The largest logs were spruce, jack pine and trembling aspen in decreasing order. C:N ratio and wood density were higher in fresh wood and lower in well-decomposed wood. Inversely, LCI index and water content was higher for well-decomposed wood (data not shown).

Table 1.3 Physical and chemical characteristics (mean and sd values) of decomposing logs by decay stage found in natural stands.

Wood characteristics	Decay stage	Spruce	Balsam fir	Paper birch	Trembling aspen	Jack pine
Log volume (m^3)	Fresh	0.27 ± 0.25	0.16 ± 0.14	0.19 ± 0.26	0.51 ± 0.80	0.39 ± 0.26
	Medium	0.36 ± 0.19	0.25 ± 0.19	0.11 ± 0.22	0.16 ± 0.14	0.14 ± 0.09
	Advance	0.32 ± 0.31	0.14 ± 0.08	0.16 ± 0.16	0.12 ± 0.07	0.67 ± 0.58
Density ($g\ cm^{-3}$)	Fresh	0.32 ± 0.10	0.31 ± 0.05	0.31 ± 0.15	0.37 ± 0.05	0.32 ± 0.06
	Medium	0.30 ± 0.10	0.25 ± 0.04	0.32 ± 0.11	0.20 ± 0.09	0.28 ± 0.10
	Advance	0.20 ± 0.09	0.20 ± 0.06	0.19 ± 0.10	0.19 ± 0.07	0.29 ± 0.09
LCI [1]	Fresh	0.39 ± 0.03	0.39 ± 0.08	0.23 ± 0.07	0.18 ± 0.03	0.44 ± 0.03
	Medium	0.38 ± 0.02	0.43 ± 0.07	0.27 ± 0.11	0.27 ± 0.09	0.58 ± 0.16
	Advance	0.53 ± 0.11	0.46 ± 0.10	0.44 ± 0.15	0.34 ± 0.10	0.54 ± 0.07
C:N ratio	Fresh	1586 ± 709	754 ± 662	615 ± 355	908 ± 799	1805 ± 862
	Medium	1812 ± 787	660 ± 260	570 ± 238	597 ± 376	945 ± 230
	Advance	273 ± 74	592 ± 283	188 ± 140	321 ± 306	876 ± 772

[1]Ligno-cellulose index : LCI=lignin/(lignin+cellulose)
Fresh correspond to the decay class 1 and 2, medium to the decay class 3 and advance to the decay class 4 and 5.

1.4.3 Fungal richness and diversity

We found a total of 33 different DGGE bands (OTUs) in the 102 logs sampled. The mean number of OTUs per log was 6.3, with a minimum of one and a maximum of 16 (Fig. 1.1). The maximum number of OTUs in the same log (16 OTUs), was from a non-decomposed (fresh wood) spruce log in the oldest stand (1760 fire) and the second highest (15 OTUs) was from a trembling aspen log of the medium decay class in the mixed aspen stands (1910 fire). Seven logs had only one OTU: two trembling aspen, one jack pine and four balsam fir logs (Fig. 1.1). The mean number of OTUs per decomposition class was 6.6 for fresh wood, 7.0 for medium decayed logs and 6.4 for advanced decay stage. With respect to log species, the mean OTU number was 9.0 for spruce, 6.7 for trembling aspen, 6.0 for paper birch, 5.8 for jack pine and 5.5 for balsam fir (Fig. 1.2).

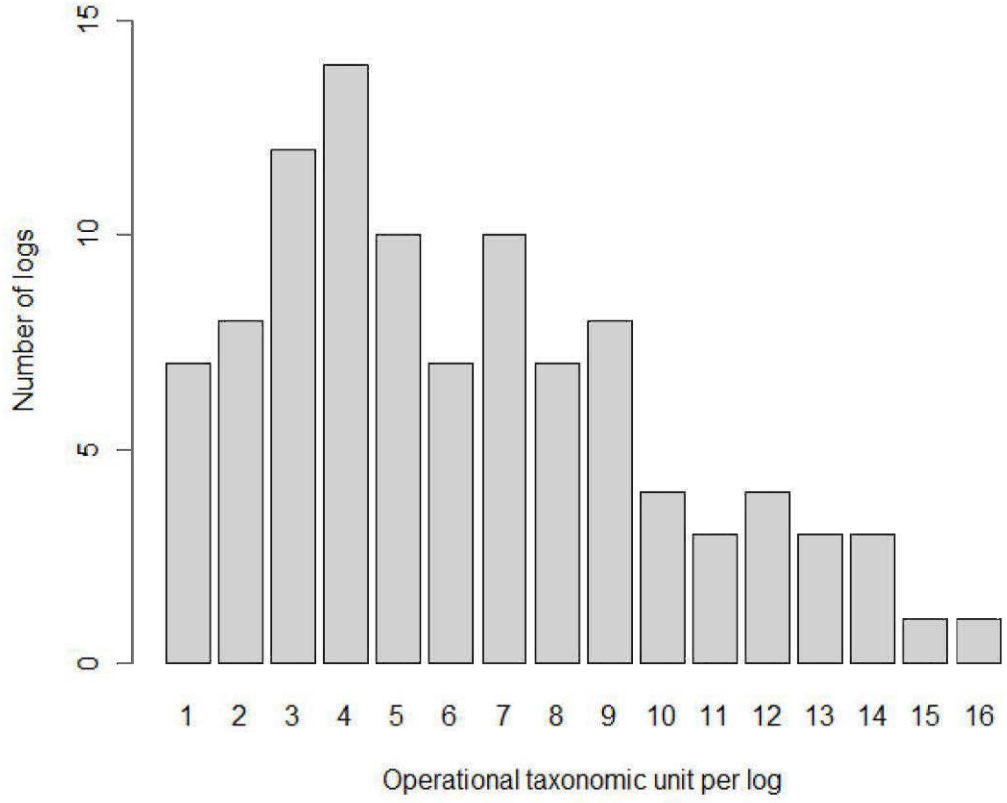

Figure 1.1 Histogram of the number of OTUs per log for the five log species obtained from PCR-DGGE profiles. A total of 102 logs have been sampled in all natural stands (three successional stages) and decomposition classes.

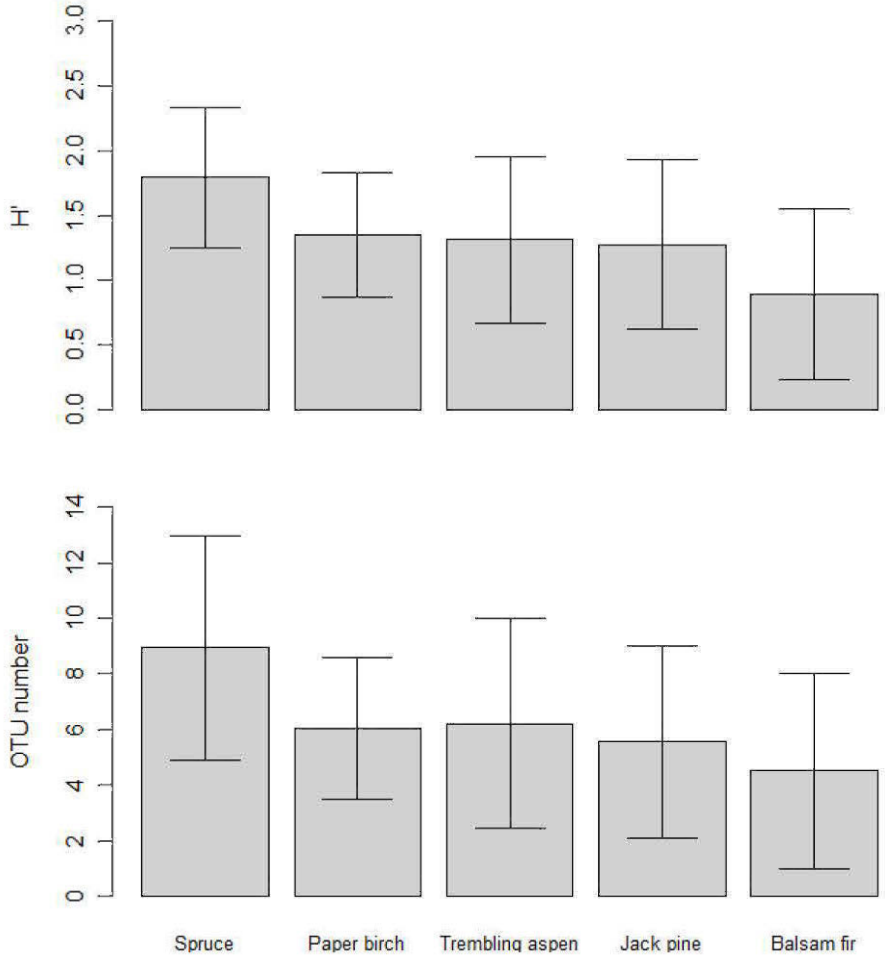

Figure 1.2 OTU number and Shannon diversity index (H′) in relation to log species

We cloned and identified 13 of the 33 OTUs (Table 1.4), corresponding to the highest band intensity observed on the DGGE gels. Other bands were too weak for cloning and with less success at the sequencing steps. The distribution of OTUs was highly variable across log species on the left of figure 1.3 whereas OTUs on the right are more similar between log species. OTU 22 (98% similar to *Bjerkandera adusta*), was present in half of the sampled logs. Sequences similar to *Phlebia centrifuga* (OTU 21), *Athelia* sp. (OTU 20), *Ascocoryne* sp. (OTU 18) and *Phellinus cinereus* (OTU 17) were also common. OTUs 28 and 29 were related to ectomycorrhizal fungi, the latter being rare: only 6.9% of all logs were colonized by these OTUs.

Table 1.4 Sequence analysis of bands excised from DGGE gels

OTU	Most closely related fungal sequence	Similarity (%)	Accession no. of related sequence	Environment where related sequence have been isolated	Occurrence [a]
5	*Leptodontidium elatius* isolate A39WD232	97	FJ903294.1	Stump of *Picea abies* (Latvia)	7.8%
7	Uncultured *Mortierella*	99	FJ553782.1	Forest soil (Canada)	11.8%
8	*Calocera cornea*	99	AY789083	Culture collection	7.8%
11	*Resinicium bicolor* strain JLL13731[b]	99	DQ826535	Isolated on aspen (Canada, Ontario)	17.7%
15	*Ascocoryne cylichnium* isolate N31	99	FJ903373	Stump of *Picea abies* (Latvia)	25.5%
17	*Phellinus cinereus* [c]	99	AY340049	Strain isolated from fruiting body	36.3%
18	*Ascocoryne* sp. isolate E2	97	FJ903331	Decayed wood of *Picea abies* (Latvia)	40.2%
20	*Athelia neuhoffii*	95	U85798.1	Culture collection	39.2%
21	*Phlebia centrifuga*	99	L43380.1	*Pinus ponderosa* (Arizona)	41.2%
22	*Bjerkandera adusta*	98	FJ903353	Decayed wood of *Picea abies* (Latvia)	51.0%
25	Uncultured fungus clone Singleton_24-2804_2353	86	FJ758813	Phyllosphere of *Quercus macrocarpa*	26.5%
28	Uncultured fungus	99	FM999613	Sporocarp of ectomycorrhiza isolated from a mature beech maple forest	18.6%
29	Uncultured fungus clone Singleton_(159-1104_0519)	83	FJ778188	Ectomycorrhizosphere of *Quercus* spp.	6.9%

Most similar Genbank accessions number and percent sequence similarities for the OTUs used in the multivariate analyses.

[a]: Proportion of logs on which this OTU was found

[b]: same similarity to FJ554463 (Uncultured Agaricomycetes clone LTSP_EUKA_P6P23)

[c]: same similarity to *Phellinus nigricans* (AF200239)

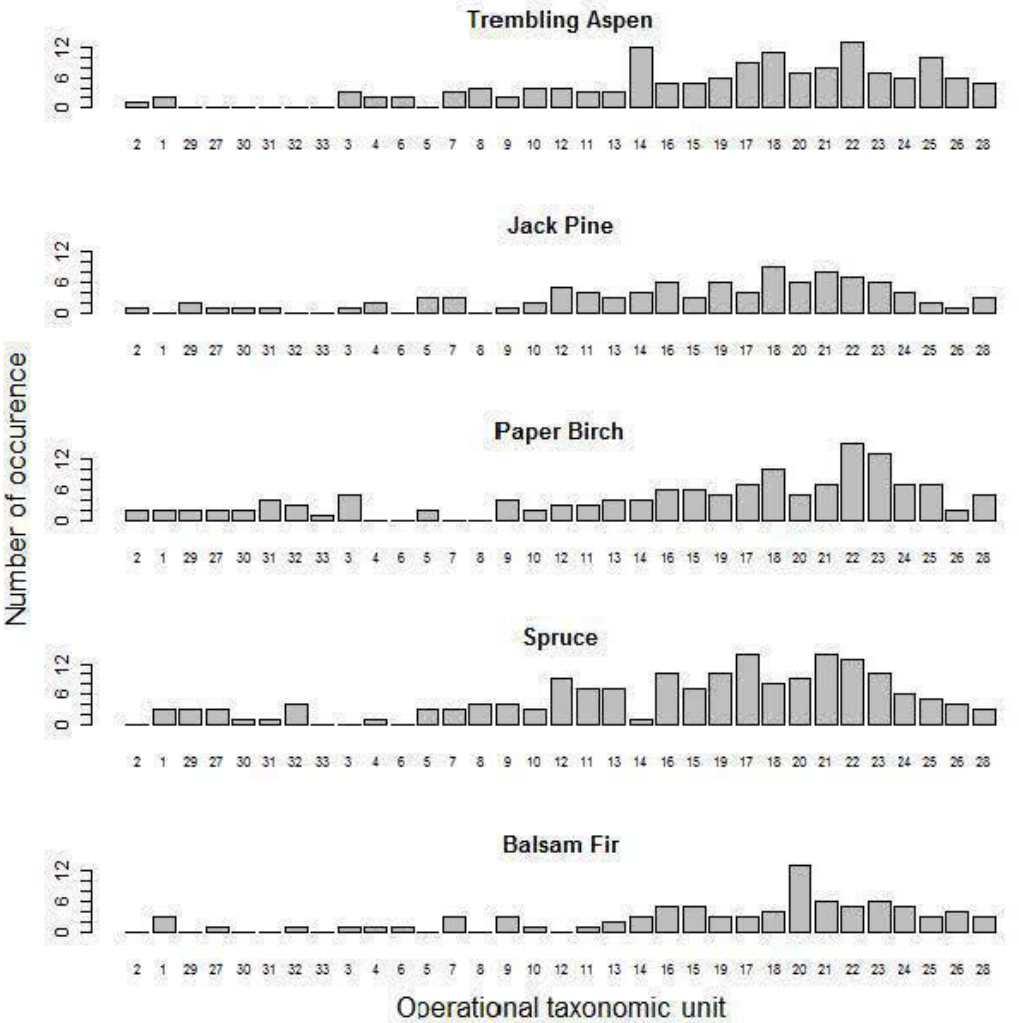

Figure 1.3 Histograms showing the number of occurrences of the 33 OTUs distinguished in DGGE profiles in relation to log species.

For fungal species richness, model 1 had the highest AICc weight (0.99) (Table 1.5), indicating that the model based solely on log species was the most plausible model (i.e., model with lowest AIC) among the set of tested models (Table 1.2). The Akaike weight for model 1 was strong enough (i.e $w_i > 0.9$) to compute model estimates based solely on this model (Table 1.6). Spruce logs had the highest OTU number (species richness). We found no differences among balsam fir, jack pine, trembling aspen and paper birch in terms of richness. Neither log volume, stand CWD volume, deciduous or coniferous basal area, decomposition class or wood density were good predictors of the number of OTUs per log.

Table 1.5 Akaike's Information Criterion (AICc) rank and weights (AICcWt) of models relating species richness (OTU number) and Shannon diversity index to stand and log characteristics.

Models	K[a]	AICc	ΔAICc	w_i[b]	Cum.Wt[c]
	OTU number				
Model 1: log species	9	557.56	0.00	0.99	0.99
Model 3: log chemical features	7	568.31	10.75	0.00	1.00
Model 5: global model	18	570.40	12.85	0.00	1.00
Model 4: stand features	8	570.43	12.87	0.00	1.00
Model 2: log physical features	9	572.31	14.75	0.00	1.00
	Shannon diversity index (H')				
Model 1: log species	9	197.30	0.00	0.99	0.99
Model 5: global model	18	206.87	9.57	0.01	1.00
Model 4: stand features	8	212.47	15.17	0.00	1.00
Model 3: log chemical features	7	212.62	15.32	0.00	1.00
Model 2: log physical features	9	215.63	18.32	0.00	1.00

Models are listed from best to worst based on AICcWt.
[a] K = estimable number of parameters in the model
[b] Akaike weights, also known as model probabilities. These measures indicate the level of support in favor of any given model being the most parsimonious (i.e the best explanatory model) among the candidate model set (Mazerolle, 2009).
[c] Cumulative Akaike weights

For the Shannon diversity index, model 1 (log species only) was also the most probable model with an Akaike weight higher than 0.9 (Table 1.5). Model estimates were computed based solely on this model (Table 1.6). The Shannon diversity index varied strongly among log species, being lowest in balsam fir and highest in spruce. Jack pine, trembling aspen and paper birch presented similar diversity indices. Hierarchical

classification between log species based on H´ is shown in (Fig. 1.2). Other measured characteristics (included in other models) - CWD volume, decomposition class, log volume, deciduous or coniferous basal area and wood log density - were poor predictor of logs Shannon diversity index.

Table 1.6 Model estimates and standard error obtained from linear mixed model 1 relating species richness (OTUs number) and Shannon diversity index to log species (n=102 logs). Significance values for each linear mixed effect are indicated considering spruce as reference level.

	Estimated value	Standard error	Df[1]	*P*
	OTU number			
Balsam fir	-4.421	1.131	57	< 0.001
Paper birch	-2.904	1.081	57	0.009
Trembling aspen	-2.730	1.081	57	0.014
Jack pine	-3.392	1.147	57	0.005
	Shannon diversity index (H')			
Balsam fir	-0.896	0.194	57	< 0.001
Paper birch	-0.442	0.185	57	0.020
Trembling aspen	-0.479	0.185	57	0.012
Jack pine	-0.518	0.196	57	0.011

[1]degrees of freedom

1.4.4 Saproxylic fungal community composition

We assessed the relationship between the composition of saproxylic fungi assemblages and selected environmental factors using canonical correspondence analysis (CCA) (Ter Braak & Prentice, 1988). Variables included were deciduous and coniferous basal area, stand CWD volume and percent lignin and hemicellulose in individual logs. Log species and decomposition category were also included as dummy variables (depicted as centroids on the CCA diagram). Collinearity between selected environmental variables was low (VIF<10). The CCA produced an ordination in which the first axis was significant (p=0.046) and the test of significance for all canonical axes was also significant (p=0.039) (assessed by permutation testing). The eigenvalues for the two first axes were 0.11 and 0.08 and the species-environment correlations for the two first axes were 0.66 and 0.63

respectively. Overall variance explained by environmental variables was 12.44%. The first three axes of the ordination explain 52.1% of this: 23.0% by the first axis and another 15.8% by the second axis.

The first axis was positively correlated with the deciduous basal area and CWD volume and negatively correlated with coniferous basal area (Fig. 1.4 and 1.5) (intraset correlation =0.14, 0.25 and -0.24 respectively). The first axis was also positively correlated with trembling aspen and balsam fir logs but negatively correlated with spruce logs (intraset correlation =0.35, 0.18 and -0.16 respectively).

The second axis was positively correlated with percent lignin and negatively correlated with percent hemicellulose in individual logs and deciduous basal area (Fig. 1.4 and 1.5) (intraset correlations of 0.33 -0.39, -0.38). Hence, axis 2, which accounts for an important part of the variation, appears to represent the influence of both wood chemical composition and stand composition.

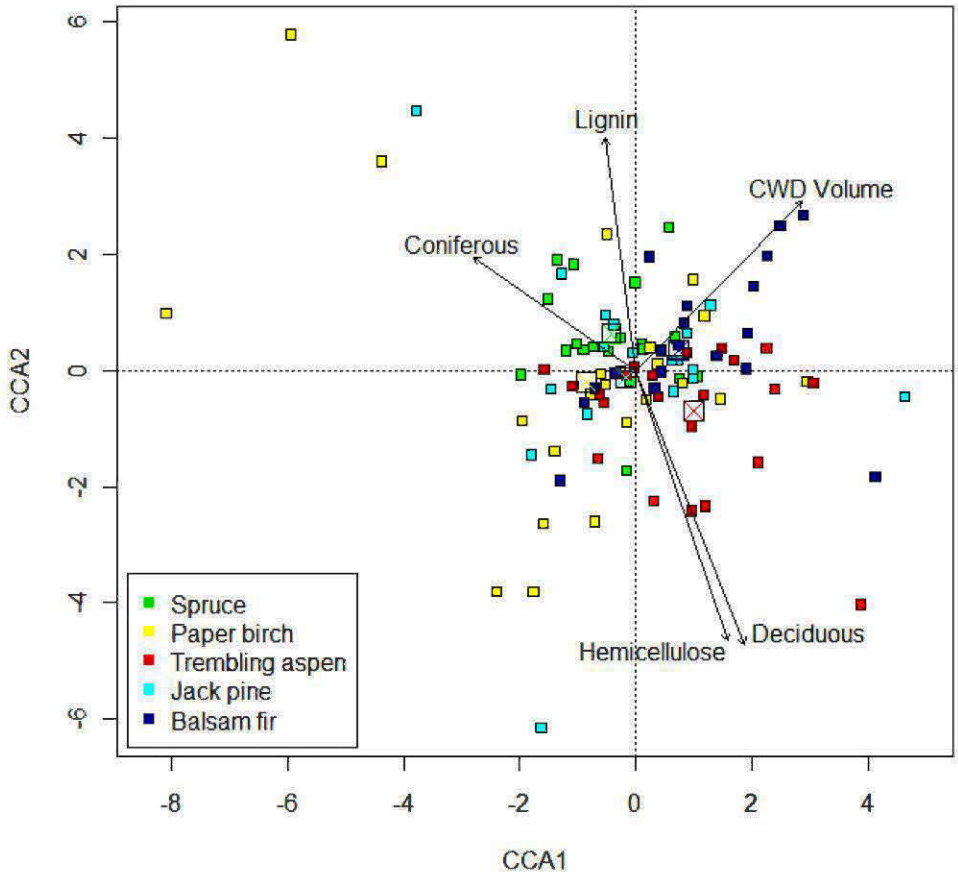

Figure 1.4 Canonical correspondence analysis of the saproxylic fungal communities (based on the DGGE profiling) from 102 logs. ☒ centroids of each log species.

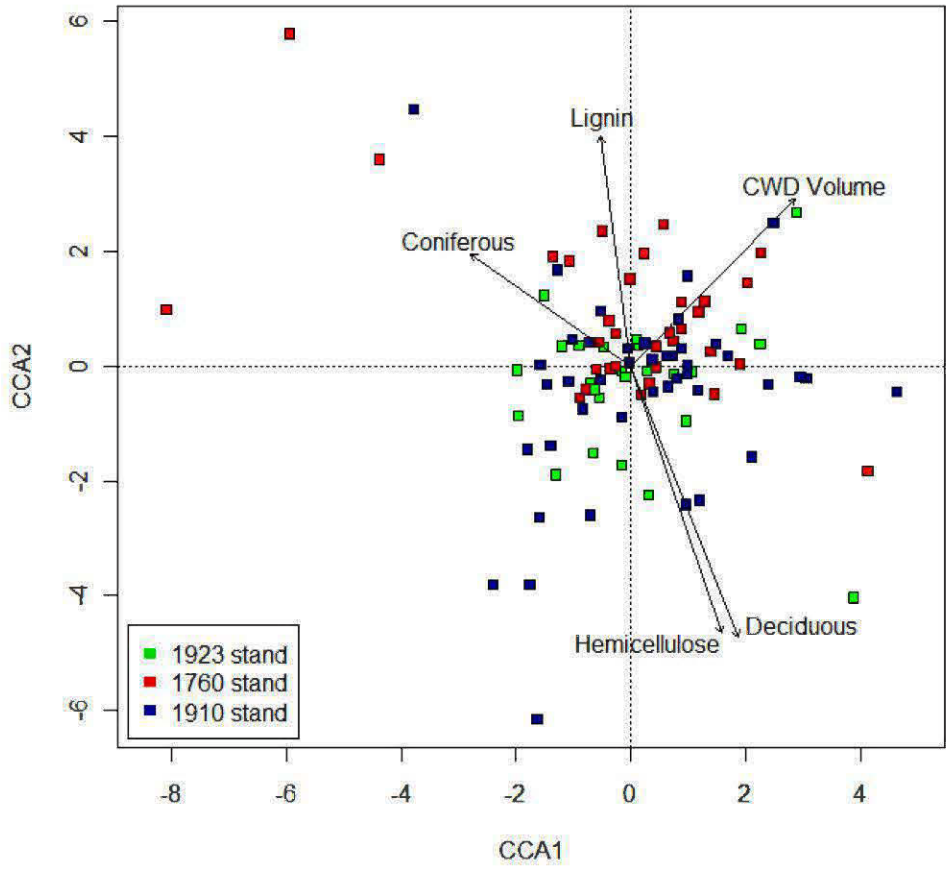

Figure 1.5 Canonical correspondence analysis of the saproxylic fungal communities (based on the DGGE profiling) from 102 logs. Sites are displayed according to stand type.

1.4.5 Indicator species

We found three species characteristic of particular habitats: OTU 14 on trembling aspen logs (indicator value: 0.32, $P < 0.05$), OTU 20 (sequence 95% similar to the Basidiomycete *Athelia* sp.) on balsam fir logs (indicator value: 0.40, $P < 0.05$) and OTU 8 (sequence 99% similar to Dacrymycete *Calocera cornea*), indicative of the stand originating from the 1923 fire (indicator value: 0.19, $P < 0.10$) (Table 1.4 and Fig. 1.3). OTU 14 could not be re-amplified or cloned and therefore was not identified by sequencing.

1.5 Discussion

Although most dominant species (or OTUs) found in this study appeared to be generalists - being found on a large proportion of the sampled logs - our results indicate that log species was the main factor affecting the diversity, richness and community composition of dominant fungal species at the log level. The influence of host tree species on saproxylic fungal communities is in agreement with other studies based on observations of fructifications (Küffer & Senn-Irlet, 2005 ; Yamashita, Hattori & Abe, 2010) and on culture dependant methods (Lumley, Gignac & Currah, 2001). Several authors have also identified decomposition stage as an important factor influencing saproxylic fungal community composition or species richness (Heilmann-Clausen & Christensen, 2003 ; Lumley, Gignac & Currah, 2001). Although we did not find direct evidence for this relationship, we did find that the composition of the saproxylic fungal community was related to log chemical composition, which, in turn is related to both log species and decay stage (Table 1.3, Strukelj-Humphery-personal communication, (Moorhead & Sinsabaugh, 2000)). Wood chemical composition was assessed from wood samples taken as close as possible to the material extracted for DNA analyses while our assessment of decomposition stage was based on the entire log. The lignin and hemicelluloses concentrations may be better indicators of decomposition than decay class estimated at the scale sampled because conditions within a single log are likely to be quite heterogeneous (Pyle & Brown, 1999). It is possible that differences detected between log species may have been influenced to some degree by variations in colonization patterns within individual logs (intra-log variability), which may have resulted in underestimates of fungal diversity for highly variable logs.

We also hypothesized that saproxylic fungal diversity would be highest in large logs. However we found no influence of individual log volume on the fungal community composition or diversity. Our final hypothesis was that fungal composition would be influenced by stand conditions such as age and CWD volume. The influence of deadwood volume on saproxylic communities has been shown previously in Scandinavian boreal forests (Penttilä, Siitonen & Kuusinen, 2004 ; Sippola, Mönkkönen & Renvall, 2005) where variation in fungal species richness was best explained the number and volume of dead trees (Penttilä, Siitonen & Kuusinen, 2004). In our study, the amount of CWD differed between the three successional stages (1760, 1910 and 1923 fires) (Table 1.1). Therefore, stands may support somewhat different fungal communities due to the amount and tree species composition of CWD. This trend was also reported by Kubartová *et al.* (2007), who noted that the dominant tree species had no significant influence on the richness of decomposer fungi but instead influences community composition. In our study, the proportion of coniferous trees (basal area) was correlated to the first axis of the CCA (Fig. 1.4 and 1.5) and was found to be higher in the 1760 stands than for the other successional stages (Table 1.1). From these results, we can infer that the oldest stand (1760) supports a specific saproxylic fungal composition due to differences in CWD amount and the proportion of coniferous trees. However, results may change with the fungal species involved. Jönsson, Edman et Jonsson (2008) found that different communities were affected differently by environmental variables. Early colonizers were influenced by the stage of decomposition, whereas secondary colonizers were influenced by other variables such as log diameter or connectivity. By analyzing the whole community without this distinction, we may have missed some differences among functional groups. Moreover, saproxylic fungal community patterns may be influenced by variables acting at a smaller scale. For example, De Bellis, Kernaghan et Widden (2007) found that most of the variation in a soil saprophytic microfungal composition was not correlated with overstory tree species, but with the composition of the understory herb layer. In the same way, microclimatic regime could have also been measured (Heilmann-Clausen & Christensen, 2003).

OTU 21 was 99% similar to *Phlebia centrifuga* and found on 41.2% of all logs (Table 1.4). *P. centrifuga* prefers unmanaged habitats and is characteristic of old-growth

spruce forests (Franzén *et al.*, 2007). All our sampling sites were within natural stands and a third of our sites were old growth stand originated from a 1760 fire. The closest sequence match to OTU 28 was that of an ectomycorrhizal fungus. The presence of mycorrhizal roots is not uncommon in decomposing logs (Tedersoo *et al.*, 2008 ; Vasiliauskas *et al.*, 2007) and highlights the importance of deadwood for forest nutrition. The closest match for OTU 17 was *Phellinus cinereus*. This white rot fungus plays a role in CWD cycling and forest turnover, as it is saprophytic to weakly parasitic fungus, causing trunk rot (Boulet, 2003). *Phellinus cinereus* is nearly exclusively found on *Betula* in the northern hemisphere (Fischer & Binder, 2004). However, in our study, this species was found relatively frequently (Table 1.4) and seems to be a generalist without specific preference for log species. OTU 22 had the broadest distribution in our study, with the closest match being *Bjerkandera adusta* - a white rot fungus with high ligninolytic enzymatic activity (Meysami & Baheri, 2003) and a preference for aspen logs in northeastern America (Boulet, 2003). However, we found it to be ubiquitous on our sites (Fig. 1.3). OTU 8 (closest species match *Calocera cornea*) - an indicator for the youngest stands (1923 fire origin) – was found preferentially on highly exposed logs (Lindhe, Åsenblad & Toresson, 2004), a condition typical of our youngest and open stands (1923). The white rot fungus *Resinicum bicolor* (closest match for OTU 11) is a weakly parasitic fungus. Although *Resinicum bicolor* has preference for *Abies* and *Pinus* in northeastern North America (Boulet, 2003), it was isolated on trembling aspen in Ontario, Canada (Table 1.4) and we found *R. bicolor* on all log species. The fact that molecular techniques allow us to detect fungal hyphae rather than only fructifications may explain differences between our observations on fungal host preferences and those of based on fruiting bodies. These studies may underestimate species distribution as some fungi may be non-host specific, but fruit preferentially on particular log species. Similar asymmetries between direct molecular studies and fruiting occurrence have been widely reported for ectomycorrhizal fungi (Gardes & Bruns, 1996).

Sample size may be an issue when using molecular tools (Allmér *et al.*, 2006). A large log can be up 21 meters long and have a volume of up to 2.13 m^3, while our samples for DNA analysis consist of only 100 mg (wet weight) of wood. Many more samples per log would have been needed to obtain a more complete picture of the fungal communities.

However that would have been at the cost of a reduction in the total number of logs sampled. The fungal diversity reported here is consistent with previous studies using PCR-TGGE method on wood (Kulhánkova *et al.*, 2006) or PCR-Restriction Fragment Length Polymorphism (Johannesson & Stenlid, 1999). With respect to other identification methods, species richness found in our study is higher than some boreal studies based on fungi fructification (Berglund, Edman & Ericson, 2005) but lower than others (Kruys & Jonsson, 1999). However, comparison is difficult due to differences in the number of logs sampled and the sampling period. For example, more fungal species (i.e 238) were found in a temperate Swiss forest (Küffer & Senn-Irlet, 2005) but 3,339 pieces of dead woody debris were sampled for fruit bodies of wood-inhabiting basidiomycetes, whereas we sampled 102 logs in our study. However, the focus of our study was on the variation in diversity between logs and not on total fungal diversity and any sampling biases remains constant between all of our samples.

The ecological significance of our results is valuable in the context of forest management. The rate and pattern of decomposition depends on fungal community composition and functioning of specific organisms under particular environmental conditions (Boddy, 2001). Because log species harbor different fungal communities, a change in tree diversity would lead to a change in the diversity of log species, thereby influencing important aspects of the decomposition process essential to forest productivity. In the same way, silvicultural practices decrease the quantity of coarse woody debris (Brais *et al.*, 2004 ; Siitonen *et al.*, 2000). Hence, saproxylic fungal community composition will be modified. To better understand the consequence of this change, studies are needed which link fungal community composition and ecological function (e.g. decomposition).

1.6 Acknowledgements

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CHAPITRE II

PATTERNS OF SAPROXYLIC FUNGAL COLONIZATION DURING WOOD DECOMPOSITION ALONG A GRADIENT OF FOREST DISTURBANCE

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2.1 Abstract

This research aims to understand how communities of pioneer saproxylic fungi organize themselves according to disturbance and stand composition. *Abies balsamea* and *Populus tremuloides* wood blocks were deposited on the forest floor and harvested every six months. At each sampling time, respiration and wood chemistry were assessed and saproxylic fungal diversity measured using a culture-independent molecular fingerprinting approach. For balsam fir wood, the fungal community changed over time and was related to disturbance type. For trembling aspen wood however, the fungal community was not affected by disturbance, although the variance in community composition decreased with time becoming significantly lower after 30 months. Trembling aspen and balsam fir were colonized by different assemblages of fungi and the colonization pattern was species specific. On both wood species, fungal richness and diversity remained fairly stable over time, although there was a decrease on balsam fir wood in cut sites. Decomposition proceeded more efficiently in aspen, as indicated by the strong relationship between time, wood density, lignin / (lignin + cellulose) ratio and respiration observed only in aspen. Although respiration increased with time for both wood species, the increase may have resulted from different processes; competition or decomposition, depending on wood species.

2.2 Introduction

Saproxylic fungi play a critical role in forest ecosystems, accessing the nutrients contained in wood and returning them to the soil (Boddy & Watkinson, 1995). Fungi are unique in their ability to decompose wood, as they possess the enzymatic systems needed to degrade the recalcitrant lignin component (Baldrian, 2008). Patterns in coarse woody debris (CWD) colonization, and resulting decomposition, are affected not only by the fungal species available (Fukami *et al.*, 2010 ; Rayner & Boddy, 1988), but also by the wood host species (Větrovský *et al.*, 2011) and a range of abiotic factors, including temperature and moisture (Harmon *et al.*, 1986 ; Progar *et al.*, 2000). As these factors are in turn related to the structure of the surrounding forest, wood decomposition is likely indirectly influenced by factors at the stand level, such as tree species composition, successional stage, and the level of disturbance by forest harvest practices (Hagemann *et al.*, 2010).

Despite an increasing interest in relationships between biodiversity and ecosystem functions (Coleman & Whitman, 2005 ; Fitter *et al.*, 2005 ; Gray *et al.*, 2003 ; Hooper, 2002 ; Naeem, 2002 ; O'Connor & Crowe, 2005), few studies have examined the relationship between microbial diversity and decomposition (Hättenschwiler, Tiunov & Scheu, 2005). However, there is no consensus on how loss of diversity might affect decomposition, as controversy exists in the literature and results are often context dependent (Mikola & Setälä, 1998 ; Müller *et al.*, 2002 ; Setäla & McLean, 2004 ; Wardle *et al.*, 1999). The majority of studies on microbial diversity - ecosystem function relationships have been concerned with soil bacteria while processes involving fungal diversity and wood decomposition are relatively unstudied even though saproxylic fungi are the main decomposers of woody material (Moore *et al.*, 2004), especially the lignin component (Martínez *et al.*, 2005).

In general, forest harvesting reduces the volume of CWD in managed stands (Brais *et al.*, 2004 ; Brassard & Chen, 2008 ; Harvey & Brais, 2007 ; Siitonen *et al.*, 2000) as well as the diversity of saproxylic fungal communities (Bader, Jansson & Jonsson, 1995 ; Penttilä, Siitonen & Kuusinen, 2004). The present study was conducted in the SAFE ("Sylviculture et Aménagement Forestier Ecosystémique") project (Brais *et al.*, 2004 ; Haeussler *et al.*, 2007 ; Harvey & Brais, 2007), a series of forest stand level experiments testing an ecosystem-based

approach in the boreal forests (Bergeron & Harvey, 1997 ; Harvey *et al.*, 2002). Our main purpose was to assess the influence of stand successional status, species composition, and harvesting induced disturbances on the diversity and activity (respiration, decomposition) of saproxylic fungal communities.

We placed blocks of balsam fir (*Abies balsamea*) and trembling aspen (*Populus tremuloides*) wood on the forest floor in stands of differing composition and disturbance and tracked the colonization of saproxylic fungi over time, using the culture-independent approach PCR-denaturing gradient gel electrophoresis (PCR-DGGE) (Anderson, Campbell & Prosser, 2003 ; Kubartová *et al.*, 2007 ; Kubartová *et al.*, 2009). We expected to find different patterns of decomposition between three intensities of stand disturbance (silvicultural treatment) and also between the two wood species (deciduous and coniferous), due to differences in chemical composition such as flavonoids or terpenoids for example (Harmon *et al.*, 1986) or lignin complex (Strukelj *et al.*, 2011). We also expected the diversity and activity (respiration) of saproxylic fungi to parallel changes in stands structure (remaining basal area and woody debris volume on the ground) brought about by natural succession and harvesting (Müller, Engel & Blaschke, 2007 ; Sippola *et al.*, 2004). Finally, we expected a positive correlation between fungal diversity and respiration, due to the complementary roles of different fungal species (Setäla & McLean, 2004 ; Tiunov & Scheu, 2005).

2.3 Materials and methods

2.3.1 Site description

The study is located within the Lake Duparquet Research and Teaching Forest (Harvey, 1999) in the Abitibi region of northwestern Québec (48°86′N–48°32′N, 79°19′W–79°30′W). Climate is humid continental, (Köppen classification) with a mean annual temperature of 0.8 °C and annual precipitation is 890 mm (Environment Canada; Canadian climatic normals 1971-2000). The Lake Duparquet Research and Teaching Forest is situated in the mixedwood zone of the boreal shield.

This study is set in natural stands growing on rich mesic clay sites representing a gradient of forest composition typical of the natural succession. The studied stands originated

from forest fires dating from 1923 (ASPEN stands), 1910 (MIXED stands) and 1760 (OLD stands). Trembling aspen represented 92% of the basal area (40 m^2 ha^{-1}) of the youngest stand type (ASPEN) and 81% of the second youngest (MIXED). White spruce and balsam fir accounted for 18% of the total basal area (37 m^2 ha^{-1}) of the latter stand. The oldest stand type (OLD) was affected by the 1970-1987 outbreak of spruce budworm (*Choristoneura fumiferana*) (Morin, Laprise & Bergeron, 1993), resulting in a mixed composition of white birch, white spruce, and balsam fir representing respectively 50%, 32% and 13% of the stand basal area (19 m^2 ha^{-1}). In 1999, three harvesting treatments were applied to the aspen stands removing 33 to 61% (partial cut) and 100% (clear-cut) of stand basal area (Brais *et al.*, 2004). The same year, a prescribed burn treatment was conducted in the clear-cut units (Belleau, Brais & Paré, 2006). In 2001, partial cut treatments were applied in the mixed stands that removed 42 to 47% of basal area, according to two different spatial patterns of tree removal, a regular dispersed pattern and one removing trees within small gaps. In all, eight different stand conditions representing a range of tree basal area and composition were utilized. The oldest undisturbed stands and the controlled burn represented two opposing extremes with respect to disturbance. All harvesting treatments were replicated three times for a total of 24 experimental units. Treatments were described in terms of disturbance (uncut, cut and controlled burn), softwood and hardwood residual basal area, and CWD volume.

2.3.2 Field methods

In the 24 experimental units of 1 to 2.5 ha (8 treatments x 3 replications), five 400 m^2 permanent, circular sampling plots were established between 2006 and 2008 and all trees greater than 5.0 cm diameter at breast height were identified and measured for basal area estimation. For each experimental unit, the volume of downed wood was estimated along a triangular-transect with 30 m sides (Van Wagner, 1982). Along each transect, the frequency of downed wood was recorded. In the fall of 2005, five 5x5x10 cm blocks of balsam fir wood and five of trembling aspen wood (without bark) were set on the forest floor within two sampling plots of each experimental unit, for a total of 480 blocks (240 of each wood type). The blocks were placed such that 50 cm^2 (20%) of their surface was in contact with the ground. Over the following 30 months, blocks were collected twice per year (spring and fall) for a total of 5 sampling times.

2.3.3 Laboratory analyses

Immediately upon collecting, each block was cut in half, and one of the halves cut again for a total of three pieces. One quarter of each whole block was used for microbial diversity determination, and was drilled through four of its faces with an electric drill fitted with a flat bit (12.7 mm) to produce wood chips. Between blocks, drill bits were cleaned, rinsed, soaked in 95% ethanol and flame sterilized to prevent cross-contamination of samples. All samples were kept frozen at -20°C until analysed.

2.3.4 Wood physical and chemical characteristics

The other quarter of the whole block was used for measurement of dry weight and for wood density, determined by water displacement after immersing samples in hot paraffin. The remaining piece of each wood block was processed for subsequent respiration measurements (see below) then dried to assess water content and finally ground with a cutting mill (Retsch, SM2000) for spectroscopy analyses. Total N was measured by dry combustion using a Leco CNS 2000 analyzer (LECO Corporation, St. Joseph, MI). Lignin and cellulose were determined by near infrared spectroscopy (Foley *et al.*, 1998). To calculate the content of cellulose and lignin, we measured the reflectance of 546 samples (480 ground wood blocks + 66 additional wood blocks that had been placed in the field for 6 months) at wavelengths ranging from 400 to 2500 nm at 2 nm intervals using a Foss NIRSystems 6500 spectrometer (Laurel MD, USA). Calibration was done by the ADF/NDF (acid detergent fiber/neutral detergent fiber) method of Goering and Van Soest (1970) and lignin was measured with the ADF-L (acid detergent fiber-lignin) method according to Brinkmann *et al.* (2002) on 100 samples selected by the spectrometer WinISI software (Foss NIRSystems, Silver Spring, USA) based on PCA scores of the different spectra. Results from chemical analyses were regressed against the absorbance spectra using the Standard Normal Variate and Detrending method (Barnes, Dhanoa & Lister, 1989). We calculated the calibration (number of blocks=70; 74; 77) and validation (n=25; 20; 20), for ADF-L (ADF-Lignin), ADF and NDF respectively, of the regressions between NIR spectra and wood block composition using WinISI software.

2.3.5 DNA extraction and PCR amplification of fungal-specific genes

Wood samples were lyophilized for 48 hours before disruption in a Qiagen TissueLyser (QIAGEN, Mississauga, ON, Canada) for 2 min runs at 26 Hz until the wood was reduced to a fine powder. Samples were put on ice between runs. DNA was extracted with the DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions. DNA was eluted in 100 µL elution buffer and stored at -20°C.

The Internal Transcribed Spacer region (ITS1) of the fungal rDNA was PCR-amplified using the fungal specific primers ITS1-F (CTT GGT CAT TTA GAG GAA GTA A) (Gardes & Bruns, 1993 ; Jasalavich, Ostrofsky & Jellison, 2000) and ITS2 (GCT GCG TTC TTC ATC GAT GC) (White *et al.*, 1990) to obtain a sequence of approximately 280-bp. A GC clamp (CGC CCG CCG CGC CCC GCG CCC GGC CCG CCG CCC CCG CCC CC) was added to the 5' end of the ITS1-F primer to avoid complete separation of DNA strands during the subsequent denaturing electrophoresis. Polymerase chain reactions were performed using 50-µL of PCR assays containing 2 µL of template, 5 µL of PCR reaction buffer (ThermoPol, New England Biolabs, Ipswich, MA), 1 µl dNTP (10 mM), 1 µL of each primer (50 µM), 0.2 µL of Taq polymerase (5 U· μL^{-1}, New England Biolabs, Ipswich, MA). Cycling parameters were an initial denaturation cycle of 3 min at 95 °C followed by 35 cycles of denaturation at 94 °C for 45s, annealing at 55 °C for 45 s, and extension at 72 °C for 1 min 15 s, ending with a final elongation at 72 °C for 8 min (Kubartová *et al.*, 2007). Reactions were performed with negative controls (containing no DNA) and positive controls (fungal DNA from pure culture) in each PCR run. All amplification products were analysed by electrophoresis with 1% (w/v) agarose gels in TAE (40 mM Tris-acetate, 1 mM EDTA), stained with Gelgreen (Biotium, Hayward, CA), and visualized under UV light.

2.3.6 Separation of fungal ITS amplicons by DGGE

Electrophoresis was performed according to the protocol described by Kebli *et al.* (2011). We used a DCode universal mutation detection system (Bio-Rad Laboratories, Hercules, CA) and an acrylamide gel (8% [wt/vol] acrylamide–bis-acrylamide, 37.5:1) with a linear vertical gradient of 20–55% denaturing agents (100% denaturant corresponding to 7 M

urea and 40% [v/v] deionized formamide) and a stacking gel (4% [w/v] acrylamide–bis-acrylamide, 37.5:1) on top. Approximately 400 ng of each PCR product was loaded and electrophoresis was performed in TAE buffer (40 mM Tris-acetate, 1 mM EDTA) at 75 V and 60 °C for 16h. Gels were stained for 15 min with SYBR gold (Invitrogen, Carlsbad, CA), visualized under UV illumination, and digitized using a ChemiDoc XRS System molecular imager (Bio-Rad Laboratories, Hercules, CA). Amplicons that generated prominent DGGE bands were selected for cloning and sequencing according to Kebli *et al.* (2011).

2.3.7 Gel analysis

The software package GelCompar II (version 5.0, Applied Maths, Belgium) was used to analyse ITS DGGE banding patterns. In order to minimize migration differences and to normalize for distortions between gels, we aligned the gels using an external reference pattern comprised of mixed ITS amplicons from five different fungi. A band-matching process was used to obtain a presence-absence matrix for statistical analyses. A 5% band intensity threshold was set for the band selection process. Individual bands were grouped into classes based on melting behaviour (position in the gels). Each band class was then considered to be an operational taxonomic unit (OTU), allowing for the calculation of their frequency among wood block samples. We also calculated the relative intensity of each band, applying a value between 0 and 1 by dividing the intensity of the band by the sum of the intensity of all the bands within the lane, thus eliminating the variation in band intensity due to difference in amplification and amount of DNA loaded on the DGGE gel. A Shannon diversity index was calculated according to Eichner *et al.* (1999).

2.3.8 Cloning and sequencing of excised DGGE bands

Amplicons which generated prominent DGGE bands were selected for cloning and sequencing. Bands were excised from DGGE gels and the DNA eluted in 20 µL of sterile deionized water and subsequently used as a template for PCR using the primers ITS1-F and ITS2 as described above. Amplicons (280 bp) obtained with the fungal specific primers were purified on agarose gels using the Geneclean Turbo kit as recommended by the manufacturer (Q-bio gene, USA). The purified DNA was cloned by ligation to the pGEM-T Easy vector system (Promega, Madison, WI) and transformed into competent *Escherichia coli* cells

(JM109) according to the manufacturer's instructions. Positive clones were selected on appropriate LB agar plates and their plasmids isolated with the Wizard Plus SVMinipreps system (Promega). Sequencing reactions were performed with a BigDye Terminator cycle sequencing kit 3.1 with a genetic analyzer (3130XL; Applied Biosystems, Foster City, CA) by the Plate-forme d'Analyses Biomoléculaires (Université Laval, Québec, Canada). All clones were sequenced in both directions using Sp6 and T7 universal primers. Sequences were aligned using Bioedit software (Hall, 1999) and vector plasmid related sequence were removed. Sequence identity searches were performed using BLASTn against the GenBank database of the National Center for Biotechnology Information (NCBI).

2.3.9 Respiration

Activity of wood decay microorganisms was assessed by measuring respiration with the soda lime method (Edwards, 1982). Respired CO_2 was assessed according to Keith & Wong (2006) from the following equation:

Wood blocks CO_2 efflux ($gC\ g^{-1}\ day^{-1}$) = [(sample weight gain (g)-mean blank weight gain (g)) × 1.69] / wood block dry weight (g) × 24 (h) / time of exposure (h) × 12/44

CO_2 efflux was calculated as the quantity (g) of carbon produced per gram of wood dry matter and per day. Briefly, we incubated wood blocks during 24 hours in closed plastic bags with soda lime. Hence the CO_2 efflux was reflected in weight gain of granules. This weight is measured on oven-dried granules so that differences in water content of the initial batch of soda lime, and water absorption during exposure do not interfere with measured weight gain of CO_2 (Keith & Wong, 2006).

2.3.10 Statistical analysis

The data were analyzed with a linear mixed-effects model. We used general linear mixed models for the response variables "*S*" (species richness calculated as OTU number per sample), "H"(Shannon diversity index), "respiration" (natural log transformed CO_2 efflux), "density" (wood density) and the "LCI" (lignocellulose index = lignin/(lignin + cellulose). The explanatory variables included time of wood block incubation on the forest floor,

disturbance intensity (uncut; cut; controlled burn), the basal area of deciduous and coniferous trees, and coarse woody debris volume. Random factors were stand type, experimental unit and sampling plot (each one nested in the former). We included a within-group correlation structure (autocorrelation structure of order 1) to take into account the repeated measures over time. In models assessing H', S and respiration, we included the binary categorical variable "season" (spring and fall) to take into account a potential seasonal effect (low winter temperatures and snow cover vs. high summer temperatures) (Table 2.1). We estimated the parameters of the linear mixed effect models using the "nlme" package (Linear and Nonlinear Mixed Effects Models, R package version 3.1-90, (R Development Core Team, 2010)). The presence of a simple linear relationship between diversity and respiration was also assessed using a mixed-effects model. Models were compared on the basis of Akaike's information criteria (AIC) (Burnham & Anderson, 2004). The ''best'' model is the model with the lowest AIC score and the highest Akaike weight. Akaike weights (w_i) indicate the level of support in favor of any given model being the most parsimonious and most probable among candidate models (Mazerolle, 2006). For model selection and multimodel averaging, when no model had a $w_i > 0.90$, we used "AICcmodavg" (R package version 1.01; AICcmodavg). Model selection and multimodel inference were based on (Q)AIC(c) (http://CRAN.R-project.org/package=AICcmodavg). Matrices of OTU relative abundance were obtained for trembling aspen and balsam fir wood separately, as wood species has a strong influence on fungal community structure (Kebli *et al.*, 2011).

In order to conduct fungal community composition analyses, we tested variation in OTUs composition among treatments for significance using the adonis function in vegan (R package version 1.15-2; vegan: Community Ecology Package, http://CRAN.R-project.org/package=vegan). The adonis function is an analysis of variance using permutations that partition the species assemblage matrix as the response variable (relative intensity of DGGE bands) among sources of variation; in this case "disturbance", "time" or "wood species". The number of permutations was set at 999. The adonis function is also analogous to redundancy analysis (Legendre & Andersson, 1999). A subsequent test for differences in between-sample distances (i.e. dispersion) was conducted. Multivariate homogeneity of group dispersions (Anderson, 2006) determine if the variance within a group

differed from another group within a biological community. This test is a multivariate analogue of a Levene's test to test for community dispersion (i.e. variability). The analysis was performed in R with the betadisper function in the vegan package.

Table 2.1 General linear mixed models relating response variables (Shannon diversity index (H'), species richness (*S*), respiration, density and LCI) to explanatory variables during the first stages of wood decomposition.

Model	Tested hypothesis	Explanatory variables
1	Effect of disturbance level	Disturbance
2	Effect of time	Time
3	Effect of interaction between time and disturbance	Time + disturbance + time:disturbance
4	Effect of stand composition	Deciduous basal area + coniferous basal area
5	Effect of total deadwood volume	CWD volume
6	Global model (all variables)	Time + Deciduous basal area + coniferous basal area + CWD Volume + disturbance + time:disturbance

The binary categorical variable "season" was included in all models for response variable H', *S* and respiration.

Within each model, each response variable was assessed separately.

2.4 Results

2.4.1 Stands structure

Stands represented a gradient of basal area (tree > 5 cm diameter at breast height) ranging from 39 m^2 ha^{-1} in the uncut ASPEN stand to 0 in the controlled burn treatment (Fig. 2.1a). Both deciduous (mostly trembling aspen) and coniferous basal areas reflected the intensity of harvesting as well as stand successional status. The OLD stand had equal proportions of coniferous and deciduous species and the lowest total basal area of all undisturbed stands. Coarse woody debris volume ranged from 140 m^3 ha^{-1} in the gap harvested MIXED stands to 57 m^3 ha^{-1} in controlled burns (Fig. 2.1b).

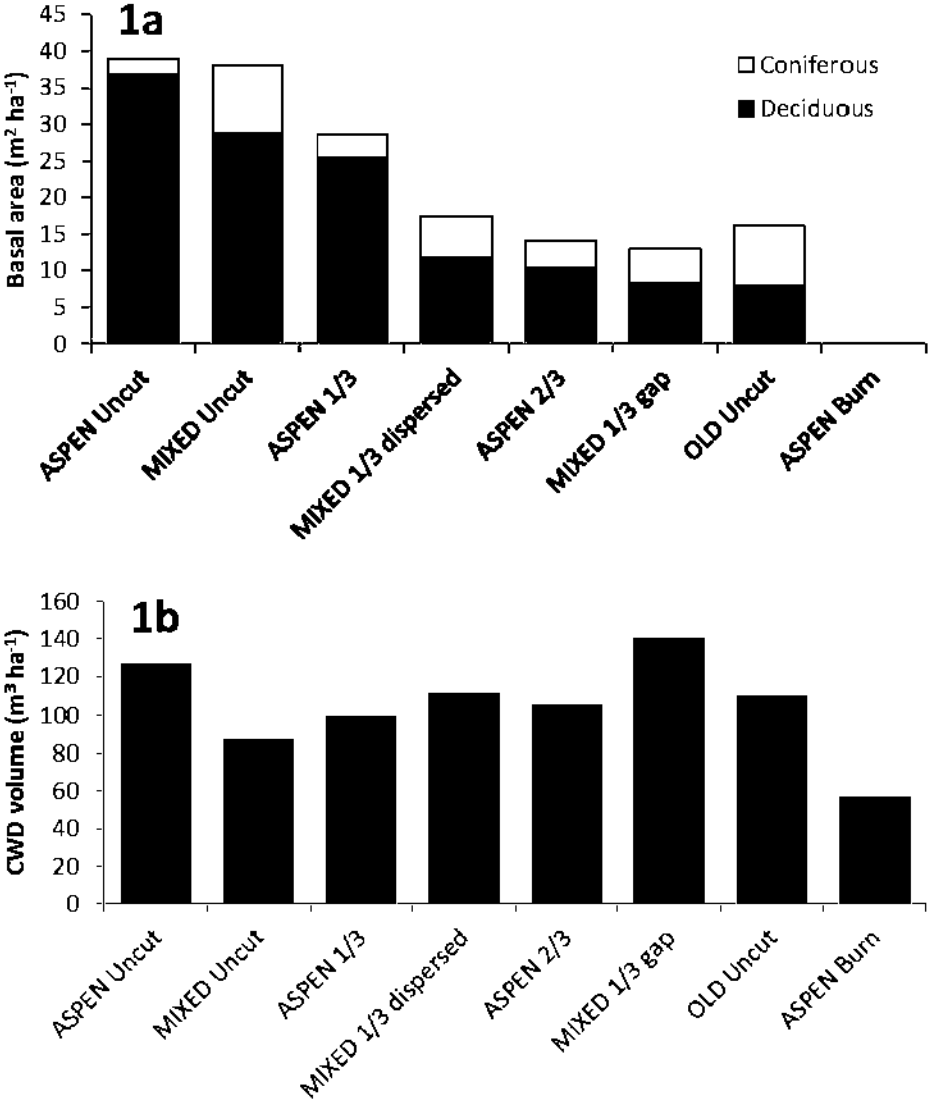

Figure 2.1 (a) Basal area of coniferous and deciduous trees and (b) CWD volume of natural and managed mixedwood stands. ASPEN: stands from 1923 fire; MIXED: stands from 1910 fire and OLD: stands from 1760 fire. Treatments that removed 33 and 61% of ASPEN stand basal area are indicated by 1/3 and 2/3 respectively while 42 to 47% in MIXED stand are indicated by 1/3 and 1/3 gap according to two different spatial patterns of tree removal, a regular disperse pattern and one removing tree within small gaps patterns

2.4.2 Fungal richness and diversity

A total of 325 wood blocks were successfully analyzed for molecular diversity. We found a total of 35 different OTUs, with the mean number of OTUs per block being lower for

balsam fir (mean of 3.9 and maximum of 12 at 12 months in controlled burn stands) than for trembling aspen (mean of 5.1 and maximum of 14 at 12 months in cut stands). All successfully analyzed blocks had at least one OTU.

For both trembling aspen and balsam fir wood blocks, model 2 (time of incubation) had the highest AICc weight for the response variables H' and *S*. However, none of the models had AICcWt > 0.9, meaning that several models could explain the data equally well. We used model averaging in order to compute confidence intervals for the explanatory variables of H' and *S* (Table 2.2). We found no variables affecting the fungal richness (*S*) or the Shannon diversity index of trembling aspen wood blocks (Table 2.2). For balsam fir wood blocks, time of incubation had a negative effect on fungal species diversity and richness but this effect was seen only in cut sites. Every six months, the Shannon diversity index of balsam fir wood blocks decreased by 2.4% (Shannon diversity index mean at 6 months = 1.07) and species richness decreased by 0.24 units, i.e. 2% (mean *S* per balsam fir wood blocks at 6 months = 4.2). Balsam fir species diversity was also related to season, being 8% lower in spring than in fall, with a confidence interval of 94% (Table 2.2). We also found that stand deciduous basal area had clear positive effect on balsam fir *S* (Table 2.2). For each increase of 5 m^2 ha^{-1} of the deciduous basal area, the number of fungal OTUs on balsam fir blocks increased by 1.25% (mean *S* = 3.9).

Table 2.2 Effects of time of incubation, disturbance and stand basal area on Shannon diversity index, species richness, respiration, density and LCI of decaying trembling aspen and balsam fir wood blocks.

Wood species	Explanatory variables	Model averaged estimate	Unconditional SE	Unconditional confidence interval		
				Lower	Upper	%
Trembling aspen	**Shannon index**					
	NA	NA	NA	NA	NA	
	Species richness					
	NA	NA	NA	NA	NA	
	Respiration					
	Time	0.014	0.005	0.005	0.024	95%
	Wood density					
	Time	-0.003	0.001	-0.005	-0.002	95%
	Time*Cut	-0.002	0.001	-0.005	-4×10^{-6}	95%
	LCI					
	Time	0.005	0.0004	0.004	0.006	95%
Balsam fir	**Shannon index**					
	Time	-0.009	0.005	-0.018	-3×10^{-4}	94%
	Deciduous basal area	0.007	0.005	3×10^{-5}	0.015	90%
	Season (spring)	-0.185	0.097	-0.367	-0.002	94%
	Species richness					
	Time	-0.040	0.020	-0.079	-0.001	95%
	Deciduous basal area	0.032	0.016	0.001	0.063	95%
	Respiration					
	Time	0.025	0.007	0.011	0.039	95%
	Wood density					
	Time*Burn	0.004	0.001	0.001	0.007	93%
	Coniferous basal area	-0.003	0.002	-0.005	-1.8×10^{-5}	93%
	LCI					
	Coniferous basal area	-0.002	0.001	-0.003	-5.8×10^{-5}	92%

Levels of significance of unconditional confidence intervals are indicated in parenthesis. "Fall" is the reference level for seasonal effect and "uncut" is the reference level for disturbance. Model averaged estimates and their unconditional standard error were obtained from multimodel inference (see table 2.1 for models specifications) based on AICc. Only variables with confidence interval > 90% are presented (otherwise NA indicates estimates' confidence interval including 0).

2.4.3 Fungal succession

We successfully cloned and identified 22 OTUs observed on the DGGE gels (Table 2.3). OTU 26 was most similar to a *Phialophora* species (accession number FJ903315.1) and OTU 5 was closely related to *Leptodontidium elatius* (FJ903294.1). Both show a seasonal colonizing pattern, with lower abundance after the winter (Fig. 2.2 row 2). Others (OTU 17, 19 and 22) were similar to *Phellinus cinereus* (accession number AY340049), an uncultured species of *Dermateaceae* (accession number FJ554419) and *Bjerkandera adusta* (accession number FJ903353), respectively. OTU 17, 19 and 22 showed variable patterns of colonization (Fig. 2.2 row 3).

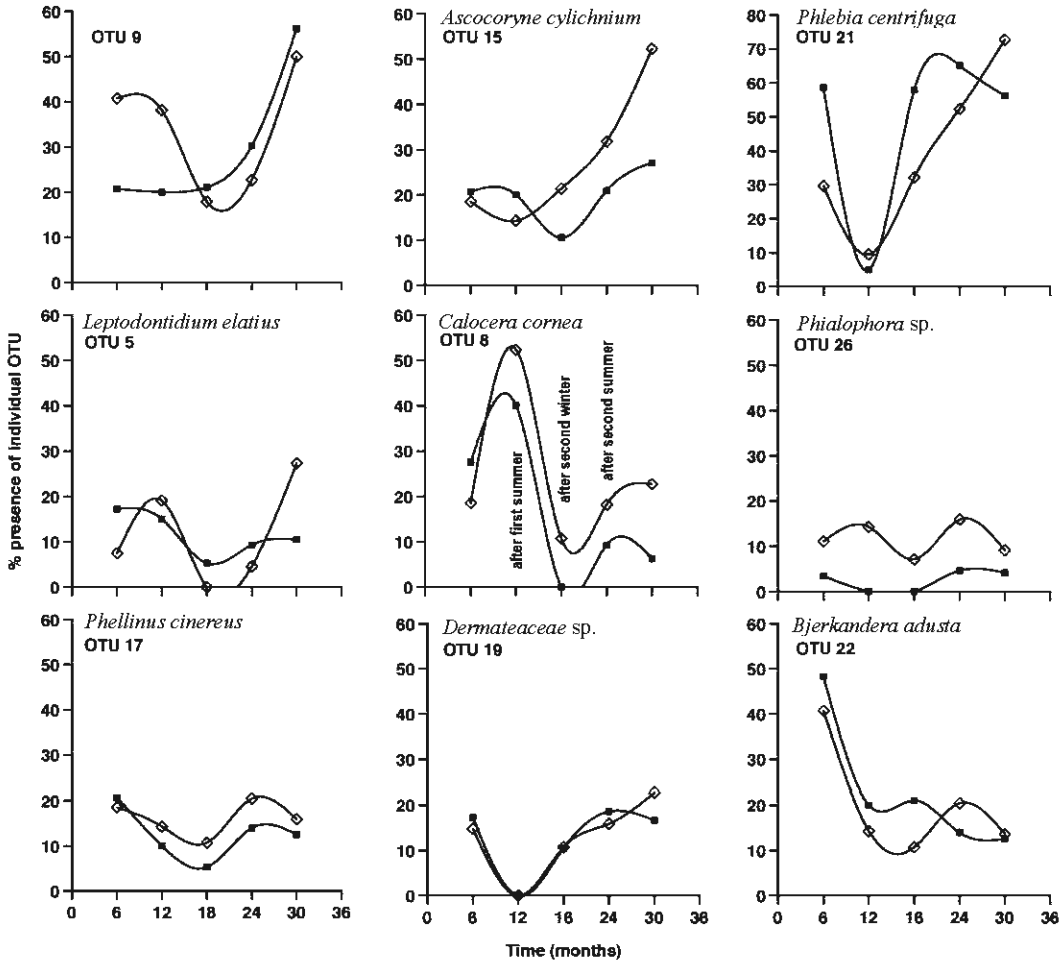

Figure 2.2 Different patterns of blocks colonization over time by 9 different OTUs (upper row: increasing and high colonization; middle row: peak after summers followed by a decrease after winter; bottom row: variable pattern of colonization). All disturbance treatments are pooled. Black points represent balsam fir wood and white points trembling aspen.

Table 2.3 Sequence analysis of bands excised from DGGE gels.

OTU	Most closely related fungal sequence	Similarity (%)	Accession no. of related sequence
4	*Pholiota flavida*	99	JF908576.1
5	*Leptodontidium elatius*	97	FJ903294.1
6	Uncultured *Ascomycota*	95	JF960616.1
7	Uncultured *Mortierella*	99	FJ553782.1
8	*Calocera cornea*	99	AY789083
11	*Resinicium bicolor*[a]	99	DQ826535
15	*Ascocoryne cylichnium*	99	FJ903373
16	*Hyalodendriella betulae*	93	EU040232.1
17	*Phellinus cinereus*[b]	99	AY340049
18	*Ascocoryne* sp. isolate	97	FJ903331
19	*Dermateaceae* sp.	91	FJ554419.1
20	*Athelia neuhoffii*	95	U85798.1
21	*Phlebia centrifuga*	99	L43380.1
22	*Bjerkandera adusta*	98	FJ903353
23	Uncultured fungus isolate DGGE gel band	100	HM015681
24	*Bisporella citrina*	98	AY789386.1
25	Uncultured fungus clone Singleton_24-2804_2353	86	FJ758813
26	*Phialophora* sp.	100	FJ903315.1
27	*Phlebiella christiansenii*	100	EU118659
28	Uncultured fungus	99	FM999613
29	Uncultured fungus	83	FJ778188
30	*Pleurotus ostreatus*	98	AY540325.1

Most similar Genbank accessions number and percent sequence similarities for the OTUs

[a]: same similarity to FJ554463 (Uncultured Agaricomycetes clone LTSP_EUKA_P6P23)
[b]: same similarity to *Phellinus nigricans* (AF200239)

There were significant variations in balsam fir OTU composition among sampling times (Table 2.4, Figs 2.3 and 2.4). The between-sample distances (dispersion) confirmed that the significant effects found for balsam fir blocks were caused by differences in community centroids (i.e. means) rather than community dispersion (i.e. variability). This was not the case for trembling aspen OTU composition. However, the multivariate homogeneity of group dispersions indicated that the variance of OTU community

composition on trembling aspen decreased with time, becoming significantly lower at the end of the incubation period after 30 months (Table 2.4, Fig. 2.5a and 2.5b).

Table 2.4 Effect of time, disturbance and wood block species on assemblage patterns and variability of saproxylic fungi associated with the colonisation of wood blocks.

	Trembling aspen blocks data set		Balsam fir blocks data set		All wood blocks data set	
	Centroid	Variance	Centroid	Variance	Centroid	Variance
Time	R^2=0.029 ***P*=0.001**	F=3.491 ***P*=0.009**	R^2=0.028 ***P*=0.002**	F=0.510 *P*=0.752	R^2=0.023 ***P*=0.001**	F=6.206 ***P*=0.001**
Disturbance	R^2=0.026 *P*=0.385	F=0.437 *P*=0.725	R^2=0.024 ***P*=0.003**	F=1.235 *P*=0.313	R^2=0.010 *P*=0.269	F=1.021 *P*=0.602
Wood block species	NA	NA	NA	NA	R^2=0.007 ***P*=0.008**	F=2.08×10^{-6} *P*=0.898

R^2 values represent the proportion of variation each factor contributes to the total variation in the dataset. "NA" indicates non-applicable. F corresponds to the F value.

OTU composition for both wood species was similar at six months at the stand level (Fig. 2.3), but fungal assemblages began to change over time, with different OTUs exhibiting different dynamics (Fig. 2.2), leading to differences (*P*=0.008) in species assemblages between wood block species (Table 2.4). The following OTU/fungal species are ordered according to their abundance fitness with wood block species. Highest (or lowest) values explain the highest proportion of the variation in fungal community composition between trembling aspen and balsam fir wood due to OTU. Negative values indicate that the fungal species were preferentially found on balsam fir and positive values for trembling aspen. The OTUs are: OTU 9 (-0.0381), *Phlebia centrifuga* (-0.0243), OTU 10 (-0.0203), *Athelia neuhoffii* (0.0145), OTU 13 (0.0122), *Phialophora* sp. (0.0097), *Calocera cornea* (0.0080), *Hyalodendriella betulae* (0.0075), OTU 14 (0.0074) and *Resinicium bicolor* (-0.0062).

Some OTUs were early colonizers, present in a large number of blocks and remained common throughout the course of the experiment (Figs 2.3 and 2.4). Others became less common after the first summer, while still others showed sinusoidal (seasonal) trends (less common after winter) (Fig. 2.2). Two main shifts in fungal assemblages were observed: the first occurred during the first summer (between 6 and 12 months) and the second in the

following winter, between 12 and 18 months (Fig. 2.3). After 18 months, assemblages tended to be more similar between consecutive sampling times. Balsam fir fungal communities reached a relatively stable composition at 18 months, with OTU 9 and OTU 21 (*P. centrifuga*) being clearly dominant (Figs. 2.2 and 2.4). OTUs on balsam fir that were the most influenced (positively or negatively) by time of incubation are: OTU 9 (0.0087), *B. adusta* (-0.0045), *P. centrifuga* (0.0041), *C. cornea* (-0.0028), OTU 10 (0.0025), OTU 12 (-0.0022), *R. bicolor* (-0.0020), uncultured fungus clone (-0.0017), uncultured *Mortierella* (-0.0016), *Ascocoryne cylichnium* (0.0012), OTU 13 (-0.0011). Positive values indicate a positive correlation with time (see Fig. 2.2 for example with OTU 9 and OTU 22 *B. adusta*).

Disturbance affected fungal community composition only on balsam fir wood (Table 2.4, Fig. 2.4). The main effect of disturbance was observed in the controlled burn treatments, with many OTUs absent when compared to uncut and cut treatments. Although some differences in OTU composition are apparent between cut and uncut sites, the assemblages overlapped and some OTUs were common across treatments (e.g. OTU 9 Fig. 2.4). Comparing uncut stands versus cut stands, the following OTUs explained the highest proportion of the variation in fungal community composition: OTU 10 (0.0994), *B. adusta* (-0.0318), OTU 9 (-0.0249), *R. bicolor* (-0.0237), *H. betulae* (0.0179), *Ascocoryne* sp. isolate (0.0150), *C. cornea* (-0.0137), *P. centrifuga* (-0.0124), *A. neuhoffii* (-0.0111), OTU 12 (-0.0103), *Bisporella citrina* (-0.0100). Positive values indicate a positive correlation with cut stands whereas negative values indicate OTU/species related to uncut stands. Comparing uncut stands versus controlled burn stands the following OTUs explained the highest proportion of the variation in fungal community composition: OTU 9 (0.0939), OTU 10 (-0.0580), *R. bicolor* (0.0483), *P. centrifuga* (-0.0381), *C. cornea* (0.0344), *Dermateaceae* sp. (-0.0215), *B. adusta* (0.0195), *Ascocoryne* sp. isolate (-0.0194), *P. cinereus* (-0.0193), Uncultured *Mortierella* (0.0150), OTU 12 (-0.0130). Positive values indicate a positive correlation with controlled burn stands whereas negative values indicate OTU/species related to uncut stands.

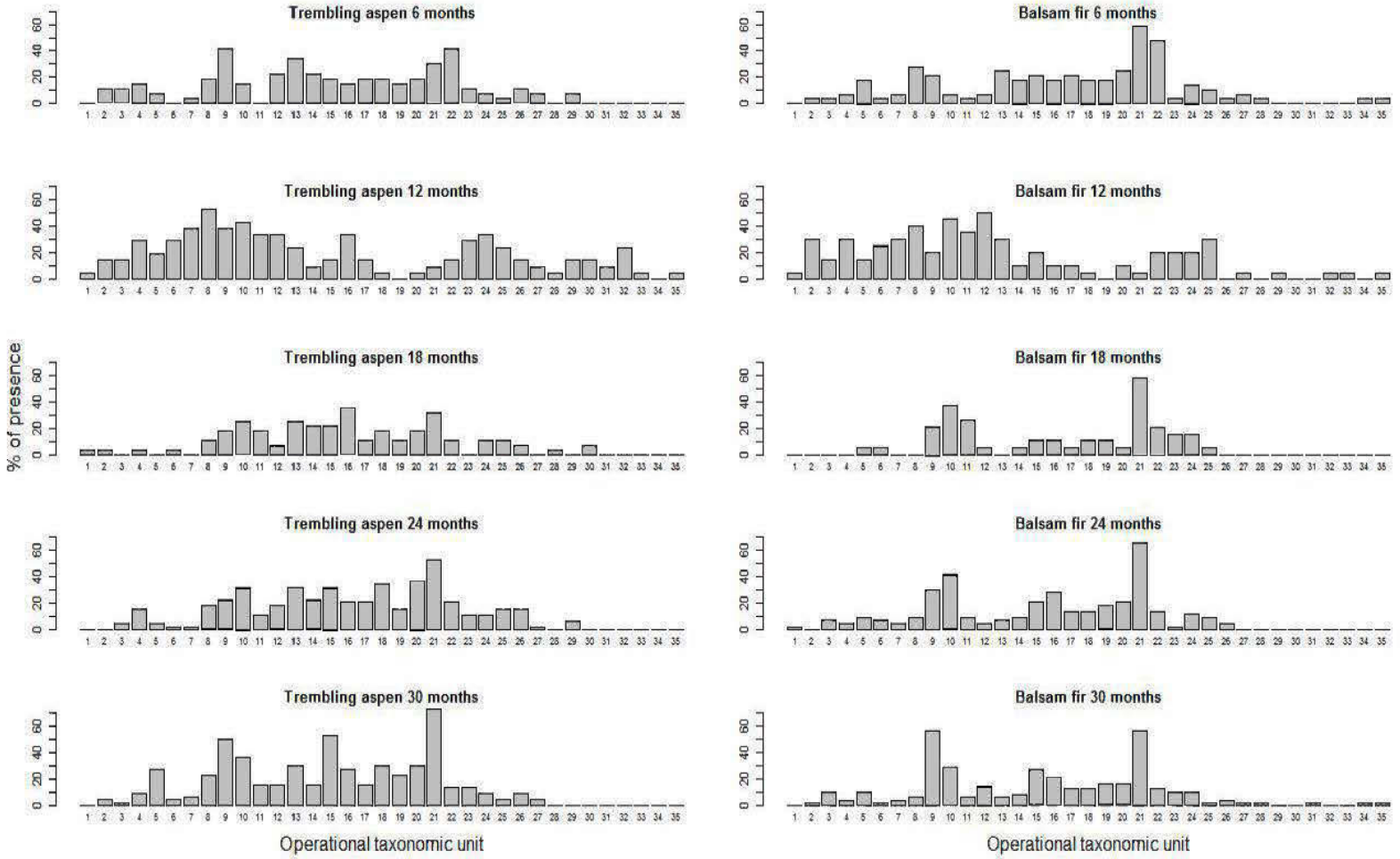

Figure 2.3 Relative frequencies of balsam fir and trembling aspen wood blocks colonization by individual OTUs (OTU 1 to 35) in relation to the time of incubation. OTUs' numbers are the same for both wood species.

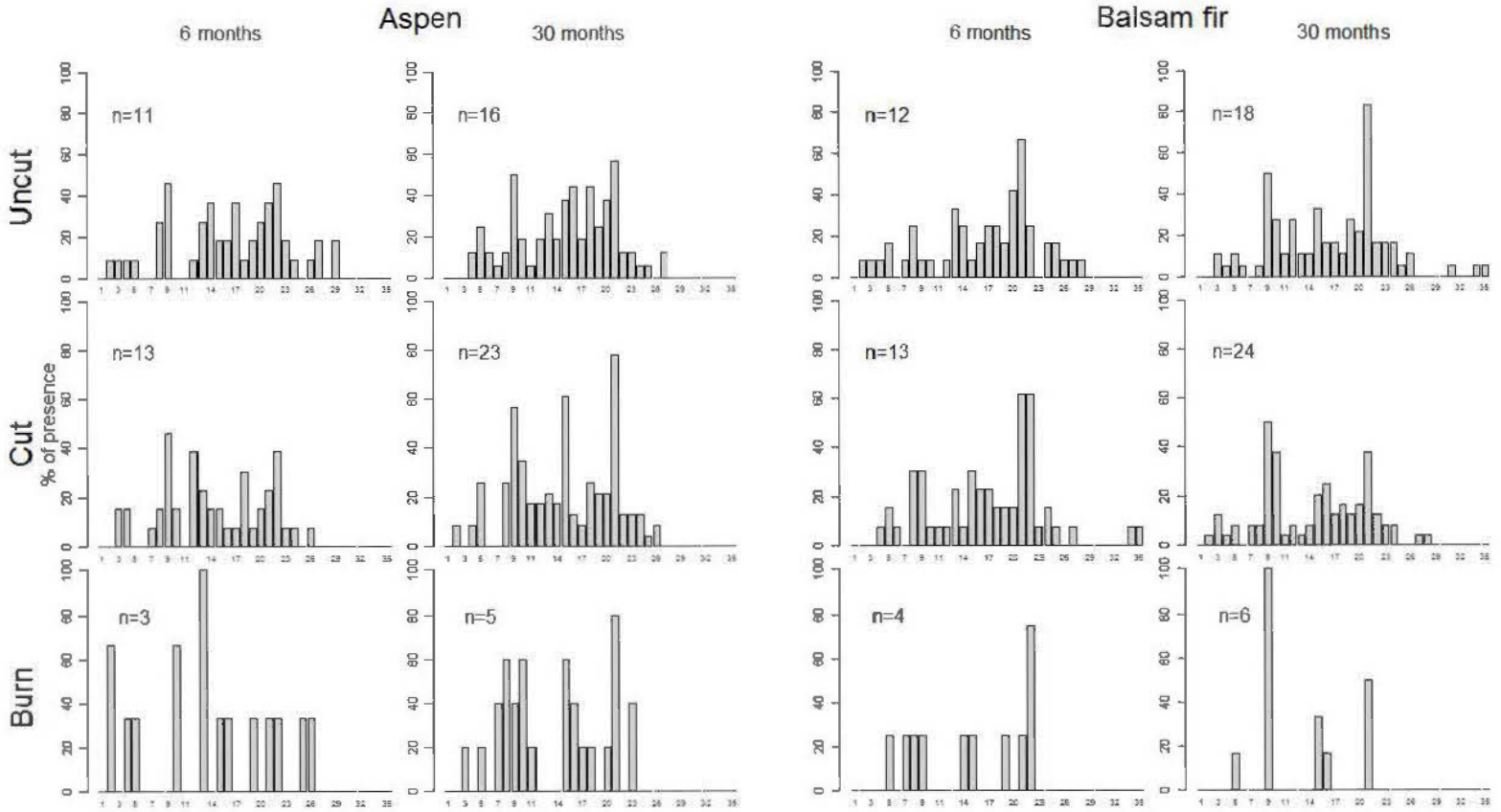

Figure 2.4 Relative frequencies of aspen and balsam fir wood blocks colonization by individual OTUs (OTU 1 to 35) in relation to the type of disturbance following 6 and 30 months of incubation. OTUs' numbers are the same for both wood species.

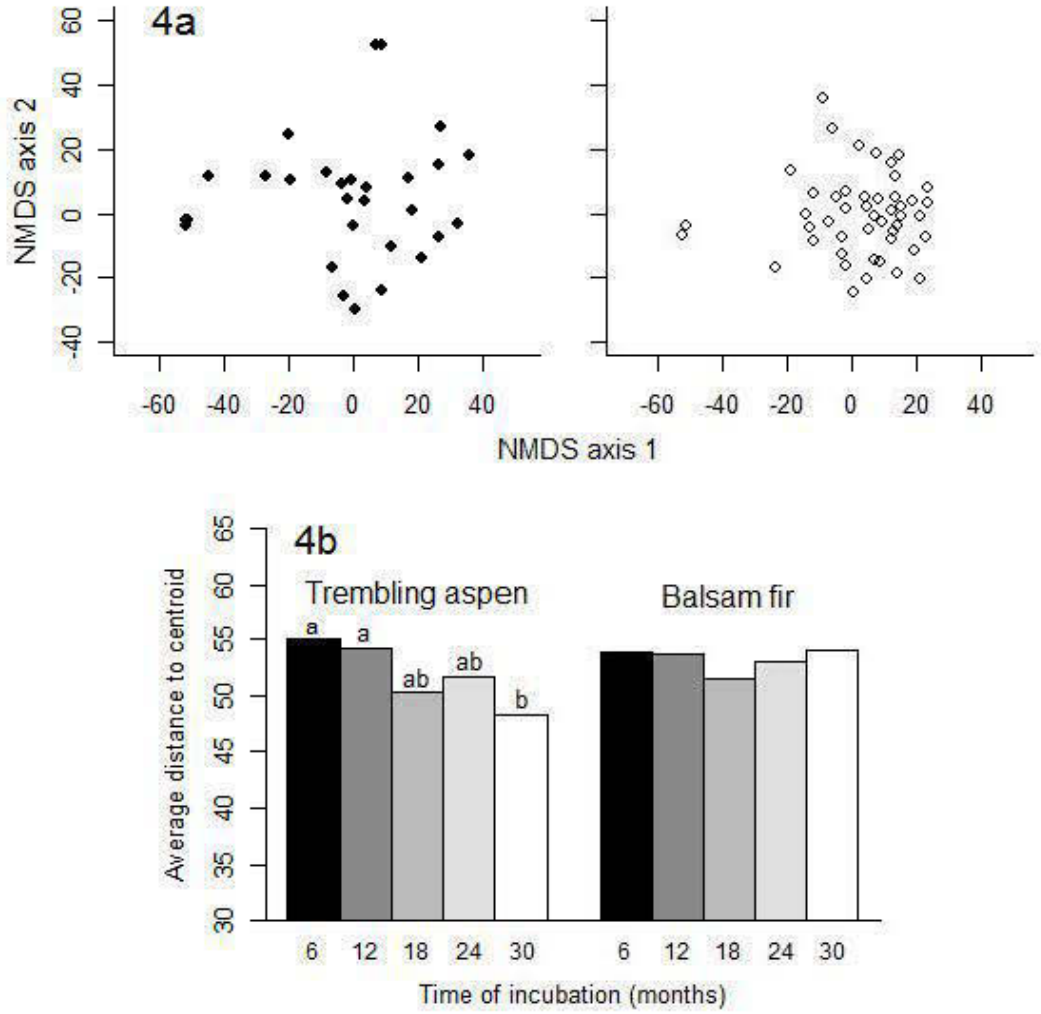

Figure 2.5 (a) Nonmetric Multidimensional Scaling (NMDS) of trembling aspen wood blocks at 6 months (black points) and 30 months (empty circles) based on fungal community composition (OTUs) (b) Multivariate homogeneity of group dispersions (i.e. average distance to centroid) within wood block species. Each bar within a wood species represents the average variance for that sampling time. Significant differences between times within a wood species are indicated by different letters and were only observed in trembling aspen wood blocks.

2.4.4 Respiration

Respiration of trembling aspen and balsam fir wood blocks was best explained by model 2 (effect of time of incubation). Time of incubation had a positive effect on respiration for both species (based on model averaging; Table 2.2). Respiration was higher on trembling aspen wood blocks (Fig. 2.6).

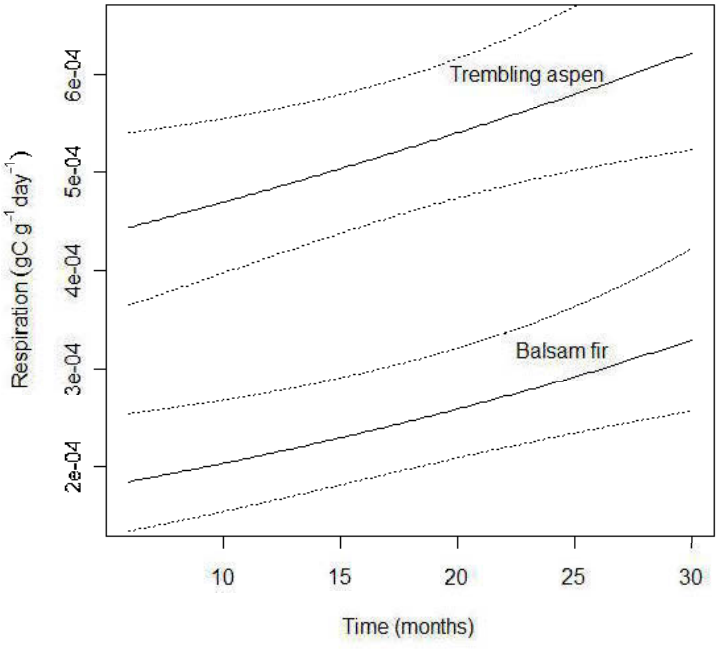

Figure 2.6 Predicted trembling aspen and balsam fir wood blocks respiration in relation to time, based on multimodel averaging of all candidates models. Dash lines showed confidence intervals at 95%.

There was no relationship between respiration and fungal richness or diversity for aspen wood blocks (all sampling times combined). However, there was a negative relationship between diversity (but not richness) and respiration for balsam fir wood blocks (Fig. 2.7).

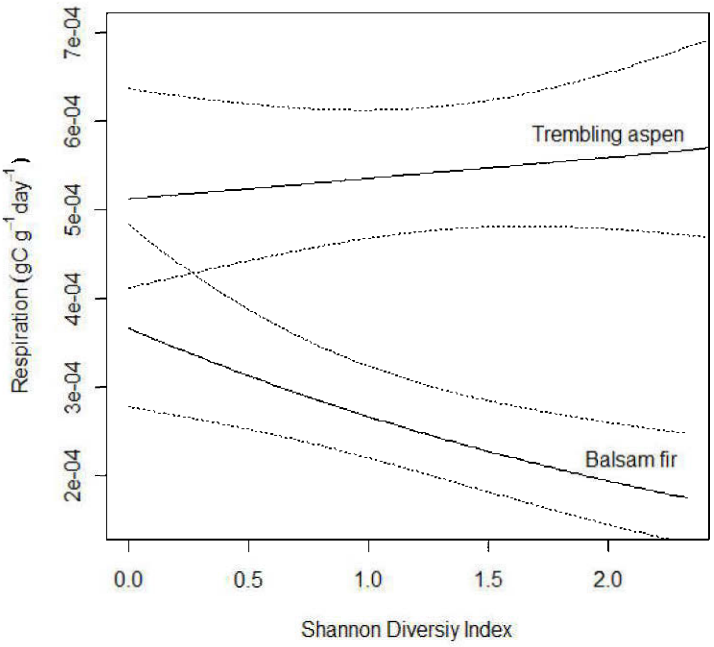

Figure 2.7 Predicted trembling aspen and balsam fir wood blocks respiration in relation to the Shannon diversity index in cut stands, based on a single model between diversity and respiration. Dash lines showed confidence intervals at 95%.

2.4.5 Wood decomposition

Model 2 (time of incubation) best explained the changes in wood density of trembling aspen blocks, with density decreasing at a rate of 4.7% every six months (max density=0.51). Moreover, trembling aspen wood density decreased fastest in the cut stands, followed by the burn and then the uncut stands, although only the difference between the cut and uncut stands was statistically significant. Changes in the lignocellulose index (LCI) of aspen wood blocks were also best explained by model 2 (time of incubation).

No strong trends in wood density and LCI with time were observed for balsam fir wood blocks. Although several models explained wood density equally well, the confidence intervals of the individual explanatory variables were large (93%) (Table 2.2). Balsam fir wood block density remained 1.1% higher in controlled burn than in uncut stands (maximum density=0.47) and decreased with increasing coniferous basal area. Several models could

explain LCI for balsam fir wood. However, model averages were computed, as models based on CWD volume, "time" and stand composition were also plausible. For balsam fir, LCI decreased marginally with increasing coniferous basal area (Table 2.2).

2.5 Discussion

Our results underline the similarities and differences between trembling aspen and balsam fir wood with respect to factors affecting the diversity and activity of early colonizing fungi. Under a wide range of stand conditions, including late successional stands and controlled burn, aspen and balsam fir wood blocks were colonized over time by different assemblages of wood inhabiting fungi with, however, a large overlap in species composition at the stand level. Fungal colonization proceeded rapidly, with the highest species richness observed within the first 12 months of the incubation period. Thereafter, a slow decrease in diversity was observed for balsam fir wood (Table 2.2), while communities became more homogenous between time periods on aspen wood, without significant changes in diversity. For both wood species, fungal activity, as measured by respiration, increased with time. However, while decomposition proceeded rapidly for aspen wood, balsam fir respiration rates were lower and fir wood underwent few physical or chemical changes within the study period.

Our results reported new findings about the ecology of several fungal species according to colonization succession, forest disturbances and host species specificity. We observed three fungal species colonizing balsam fir that decline in time: *B. adusta,* a white rot Basidiomycete that prefer deciduous wood and with high ligninolytic enzymatic activity (Meysami & Baheri, 2003 ; Urcelay & Robledo, 2004); *C. cornea* a Basidiomycete of the Heterobasidiomycete classe, generally known as "jelly fungus" and causing brown rot mainly in deciduous wood (Seifert, 1983); *R. bicolor* a late successional Basidiomycete white rot fungus found on both deciduous and coniferous tree and causing "stringy white rot". Usually white rot fungi (lignin decomposer) are more common at later stage of wood decomposition compared to brown rot (Stenlid, Penttilä & Dahlberg, 2008) although brown rot fungi are also important decayers on conifers at these late stages of decomposition (Niemela, Renvall & Penttila, 1995). However, using molecular methods, our results shown that white rot fungi

can be present during the first step of colonization on both coniferous and deciduous wood. During our assay, several OTUs related to white rot tends to decrease over the 30 months period. Conversely, presence of the OTU related to *P. centrifuga*, a white rot Basidiomycete typically observed on conifers, increased with time. The OTU related to *A. cylichnium,* a saprophytic/endophytic Ascomycete, was also more frequent in time (Hallaksela, 1993). In our study, *R. bicolor* and *P. centrifuga* tend to colonize balsam fir compared to trembling aspen wood. Our results confirm previous observation that *R. bicolor* is commonly found on *Abies* sp. and *Pinus* sp. and that *P. centrifuga* was frequently found on gymnospermous and occasionally angiospermous logs and slash (Boulet, 2003).

Some boreal wood-decaying fungi seem to favour burned areas (Stenlid, Penttilä & Dahlberg, 2008). In our study, we found several OTUs that were strongly associated with controlled burn treatments compared to uncut stands: *R. bicolor*, *C. cornea, B. adusta* and an uncultured *Mortierella. C. cornea* has been previously found on highly sun-exposed logs (Lindhe, Åsenblad & Toresson, 2004) and in open stands (Kebli *et al.*, 2011). Fungi associated with uncut stands were *P. centrifuga, Dermateaceae* sp., *Ascocoryne* sp. and *P. cinereus*. *P. centrifuga* prefers unmanaged habitats and is characteristic of old-growth spruce forests (Franzén *et al.*, 2007). Hence this fungus could be susceptible to high disturbance (controlled burn).

We hypothesized that saproxylic fungal richness and diversity would parallel changes in stand structure brought about by natural succession and harvesting. Fungal community composition on balsam fir was affected by disturbance and species richness increased as basal area of deciduous trees increase. The highest values of deciduous basal areas were observed in unmanaged ASPEN and MIXED stands, whereas the lowest were observed in controlled burn where species assemblages on balsam fir wood represented a subset of those observed in less disturbed stands. It is possible that harsher environmental conditions in controlled burn are interacting with balsam fir wood biochemical properties resulting in fewer species adapted to those conditions and surviving on 30 month-decayed balsam fir wood blocks. Sippola *et al.* (2004) also observed that deciduous species (aspen and birch) contributed significantly to the richness of polypore fungi at the stand level in managed boreal stands while the number of observed fruiting bodies decreased with harvesting

intensity. Contrary to our results, Sippola *et al.* (2004) also observed a positive relationship between species richness and CWD volume. However, CWD volumes observed in our study were much higher than those reported by Sippola *et al.* (2004) (26 to 34 m^3 ha^{-1}) and it is possible that such a relationship may only occur when CWD volumes are more limiting.

While it can be argued that stand successional status may have also affected fungal species composition, Muller *et al.* (2007) have shown that harvesting intensity had a much larger impact on fungal communities than stand age. In natural, unmanaged stands of the region, balsam fir and trembling aspen are both available for fungal colonization, which may increase the fungal diversity at the stand level. Conversely, reducing tree diversity would lead to a decrease in the diversity of pioneer fungi (Kebli *et al.*, 2011 ; Lumley, Gignac & Currah, 2001).

Contrary to balsam fir, fungal community composition, diversity and richness on aspen wood were not affected by disturbance type or stand basal area and showed little changes over time. The high species richness observed at the stand level (Fig. 2.3) and at the wood block level (n=14) at the end of the first year was followed by an increase in similarity in species composition among wood blocks. Rapid initial colonization by pioneer fungi may be followed by a more gradual colonization pattern of later successional species with high competitive abilities. The decrease of the variability in fungal community composition on aspen wood with time may result from the fact that as wood decays, different generalist pioneer fungal communities give way to more specialized assemblages (Boulet, 2003 ; Jönsson, Edman & Jonsson, 2008 ; Rayner & Boddy, 1988).

2.5.1 Decomposition

We expected to find different patterns of decomposition between the different levels of stand disturbance and also between the two wood host species (deciduous and coniferous), due to differences in chemical composition. Aspen decomposition proceeded efficiently as indicated by the strong relationships between time and decomposition parameter (wood density and LCI). This was to be expected, as boreal deciduous species have higher rates of mineralization than coniferous species (Brais, Pare & Lierman, 2006). Aspen decomposition was also highest in harvested stands. On the other hand, balsam fir decomposition proceeded

very slowly as indicated by the lack of significant changes in wood properties over the 30 month period. The accelerated decomposition of aspen on the cut treatment relative to the uncut may have been due to abiotic factors such as increased summer temperatures after the removal of the forest canopy. Hence, decomposer fungi might perform better on trembling aspen wood in open, disturbed environments similar to those encountered during first stages of natural forest succession.

2.5.2 Relationships between fungal diversity and respiration

Although respiration increased over time for both wood species (Table 2.2), we did not find the expected positive correlation between fungal diversity and respiration (Setäla & McLean, 2004). However, for both wood species, respiration and fungal diversity must have increased concomitantly up to some point prior to our first sampling time at six months. The negative relationship we observed between diversity and respiration in balsam fir wood on cut sites may have resulted from competitive species interactions (Boddy, 2000 ; Woodward & Boddy, 2008), which can be metabolically expensive (Wells & Boddy, 2002). The investment of energy into the production of secondary metabolites during interspecific competition, instead of into the synthesis of oxidative enzymes necessary for wood degradation, may result in reduced decomposition rates (Hiscox *et al.*, 2010). Increases in respiration may therefore have resulted from two very different processes; an increase in decomposition in trembling aspen and an increase in fungal competition in balsam fir wood, the latter leading to reduced diversity. Woods *et al.* (2005) found that substrate had a marked effect on interspecific fungal interactions which might be exacerbated by more severe nutrient or energy limitations in balsam fir wood. Aromatic compounds that inhibit decomposition (Ganjegunte *et al.*, 2004), have been found in higher concentrations in balsam fir wood than in aspen (Struckelj *et al.*, unpublished data). This may explain the slow decomposition observed in balsam fir wood (density and LCI were not related to diversity or time). Hence the observed changes in fungal community composition on balsam fir over time (Table 2.3) might reflect species turnover due to competition, as it could be the case for *B. adusta* (Fig. 2.2), reported to be easily replace by parasitic fungal infection (Niemela, Renvall & Penttila, 1995 ; Rayner & Boddy, 1988).

The molecular approach used in this study provides a reasonably broad view of wood-inhabiting fungi community variation according to disturbance gradient. Although it may have underestimate diversity with respect to next-generation sequencing approaches, the focus of our study was on the variation in OTUs community structure between wood blocks not on the total and exhaustive fungal community composition present. Moreover, given the high number of samples, such methods would have led to significantly costs. Any biases (DGGE limitation for the detection of rare species, comigration of amplicons from different species in DGGE gels or the fact that some fungi could have generate more than one ITS fragment) remain constant between all of our samples and sequence analysis of majors DGGE bands discarded the possibility of extensive PCR artifacts. Our results are still valid concerning the dominant population that are detected by DGGE. Moreover our results did not discriminate between metabolically active or inactive fungal species (Rajala *et al.*, 2011). Even if DNA was used instead of cDNA for DGGE, the observed changes in species profiles indicate that the dominant OTUs are from active organisms at time of sampling. A similar study would not be possible by fruiting body identification.

As reviewed by Lonsdale *et al.* (2008), research is needed to elucidate factors that drive the species richness and species composition of wood decay fungi and to determine whether early and late successional tree species are colonized by different functional groups of wood-decay fungi. Although we did not assess functional groups, we have shown that the fungi colonizing the wood of an early successional tree species differed from that of a late successional tree (during 30 months). Wood properties also interacted with disturbance in modulating decomposition efficiency.

The diversity and community composition of primary wood-decaying fungi also have a marked influence on subsequent establishment of later successional fungi, as well as on the rate of wood decomposition (Fukami *et al.*, 2010 ; Heilmann-Clausen & Christensen, 2003 ; Heilmann-Clausen & Boddy, 2005). Information on pioneer saproxylic fungal communities is therefore important for an understanding of the general relationship between fungal diversity and wood decomposition.

Little is known about fungal community composition, diversity and succession on decomposing wood of the Canadian eastern boreal forest and, to our knowledge, no other study has investigated wood decomposition in relation to initial saproxylic fungal diversity and disturbance intensity using a culture-independent molecular fingerprinting approach. Communities of wood decomposing fungi and their activities were modulated by differences in wood quality, forest disturbance and stand composition. Combining molecular approaches with sequencing and field identification of fungi would further improve our understanding of fungal ecology. Moreover, the link between species diversity and community activity and processes need to be investigated in natural environments.

2.6 Acknowledgements

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CHAPITRE III

IMPACT OF HARVESTING INTENSITY ON WOOD-INHABITING FUNGI IN ASPEN BOREAL FORESTS OF EASTERN CANADA

Hedi Kebli, Gavin Kernaghan, Suzanne Brais and Pascal Drouin

3.1 Abstract

Environmental changes, including human disturbances, have a striking impact on the biodiversity of ecosystems. Using a molecular fingerprinting technique we have described fungal community Shannon diversity index, richness and composition variations caused by silvicultural practices on trembling aspen logs and snags. Our study was conducted in the SAFE Project, a series of silvicultural experiments that tests an ecosystem management model based on natural dynamics. We wanted to know how saproxylic communities evolve with varying silvicultural treatments that emulate the natural dynamics on trembling aspen deadwood. We found that large and advanced decayed trembling aspen logs had around 9% more OTU number and around 10% higher Shannon diversity index compared to medium decayed logs independently from diameter size. We also found one indicator species of snags, two indicator OTUs (including *Phlebiella christiansenii*) specific to advanced decayed logs. Two others (*Ascomycota* sp. and *Mortierella* sp.) were selectively found on snags of the one third partial cut stands, and one OTU was preferentially found on freshly dead snag. The effect of log diameter was also strongly dependent on the silvicultural treatment where dead wood was situated. Larger logs carry more fungal species and diversity but only in burned stands compared to uncut stands, hence acting as log refuge. The negative relationship between diversity and fine woody debris volume on logs and snags was related to silvicultural treatment as fine woody debris increased with silvicultural intensity. Our results underline the negative effect of intense silvicultural practice on fungal diversity and species richness by modifying community composition, but they also highlight the benefits of alternative treatments to clear-cut and controlled burn, such as partial cuts which retain coarse woody debris volume.

3.2 Introduction

Deadwood is a key factor in forest biodiversity (Drapeau *et al.*, 2002 ; Siitonen, 2001), especially for wood inhabiting fungi (Lonsdale, Pautasso & Holdenrieder, 2008 ; Sippola & Renvall, 1999). Fungi are the main agents of wood decomposition and are an essential component of forest ecosystem food webs (Moore *et al.*, 2004), influencing nutrient cycling and carbon sequestration (Harmon *et al.*, 1986). By decreasing the amount of deadwood, forestry practices impact the diversity of wood-decay fungi (Bader, Jansson & Jonsson, 1995 ; Penttilä, Siitonen & Kuusinen, 2004). In Scandinavia, intensively managed forests harbor significantly fewer wood-inhabiting fungi than unmanaged forests (Küffer & Senn-Irlet, 2005) and threatened species such as *Phellinus nigrolimitatus*, which are strongly dependent on large diameter, well decomposed woody debris are less common (Stokland & Kauserud, 2004). Some species have also been shown to disappear locally when management intensity exceeded a specific threshold (Sippola *et al.*, 2004).

Post-harvest quantities of coarse wood debris (CWD) are dependent on silvicultural practices (Ranius *et al.*, 2003). CWD is always less abundant in managed than in natural forests while forest thinning leads to an increase in some CWD types (e.g. stumps). However large diameter logs and snags, structures of unmanaged forests, are less abundant following harvesting (Brassard & Chen, 2006 ; Haeussler *et al.*, 2007 ; Montes & Cañellas, 2006). Diversifying harvesting practices, including the use of partial harvesting, has been identified as a key aspect to implementing ecosystemic management in the boreal forest (Bergeron *et al.*, 2002 ; Harvey *et al.*, 2002). Objectives of partial harvesting may include increasing diversity of tree species and size classes, establishment and growth of shade-tolerant species, and altering rotation lengths while also addressing the maintenance of ecosystem functions and biodiversity. However, a broader understanding of keystone processes (Bednarz, Ripper & Radley, 2004) and species associated with the utilization of deadwood is central to the inception and elaboration of forests conservation strategies.

The SAFE Project, a series of silvicultural experiments in the Lake Duparquet Research and Teaching Forest (LDRTF) in the southern part of the eastern Canadian boreal forest, is testing an ecosystem management model (Bergeron *et al.*, 2002 ; Harvey *et al.*,

2002). Briefly, the approach relies on varying silvicultural treatments to more closely reflect aspects of natural dynamics. Clear-cutting or other even-aged silvicultural systems are employed as surrogates for stand reinitiation by fire; partial cutting is used to modify stand composition and structure similar to the process of natural succession from intolerant hardwoods to mixedwoods or conifer-dominated stands and selection cutting is intended to mimic gap dynamics. The first phase of the SAFE project involved natural even-aged aspen (*Populus tremuloides* Michx.) stands (Brais *et al.*, 2004 ; Haeussler *et al.*, 2007). Following stand replacing fires, aspen forms pure or mixed stands that can maintain dominance for over 100 years. In Scandinavia, saproxylic organisms associated with aspen are particularly susceptible to forest management practices and many species are now protected in northern Europe due to their increasing rarity (Siitonen & Martikainen, 1994 ; Sverdrup-Thygeson & Ims, 2002).

Our understanding of fungal diversity in the Canadian boreal forest is incomplete and few studies have addressed the relationship between forest management practices and the diversity of wood-decaying fungi. Denaturing gradient gel electrophoresis (DGGE), a culture-independent molecular technique, was used to provide new insights into the impact of partial harvesting prescriptions on the fungal communities of logs and snags. Our objectives were (i) to assess how saproxylic fungal species richness, diversity and composition vary according to five different silvicultural treatments corresponding to four levels of harvesting intensity and a controlled burn and (ii) to link fungal community structure to physical features of individual logs (size and decomposition stage) and snags (diameter and height) and to the surrounding woody debris volume. We hypothesized that species richness and diversity of saproxylic fungi are negatively correlated with the intensity of harvesting. We also hypothesized that residual downed wood would minimize the effect of harvesting on fungal diversity and richness. We also expected fungal assemblages to show higher similarity when logs and snags are closer (Edman, Kruys & Jonsson, 2004 ; Vasiliauskas *et al.*, 2005). Finally we expected to find higher fungal diversity on larger well decomposed logs or snags (Edman, Kruys & Jonsson, 2004 ; Heilmann-Clausen & Christensen, 2003 ; Nordén *et al.*, 2004).

3.3 Material and Methods

3.3.1 Field sites description

Our study area is located within the Lake Duparquet Research and Teaching Forest (LDRTF) (Harvey, 1999) in the Abitibi region of northwestern Québec, 45 km northwest of Rouyn-Noranda, Québec (48°86′N–48°32′N, 79°19′W–79°30′W). Climate is humid continental (Köppen classification), with a mean annual temperature of 0.8°C and precipitation of 890 mm (Environment Canada; Canadian climatic normals 1971-2000, available online: www.climate.weatheroffice.ec.gc.ca/climate_normals/index_e.html). The region is located in the mixedwood zone of the boreal shield.

The study was conducted in aspen - dominated stands (92% average basal area; 40 $m^2.ha^{-1}$) of fire origin dating from 1923 (Dansereau & Bergeron, 1993). In the winter of 1998-99, four levels of forest harvesting, including uncut sites where no trees were harvested and one clear-cut treatment, were applied according to a complete block design with three replications of each treatment (1 to 2.5 ha/experimental unit). In August of the same year, controlled burns were conducted over clear-cut experimental units (Belleau, Brais & Paré, 2006). The two partial harvesting treatments removed either 33 % (1/3 partial cut) or 61 % (2/3 partial cut) of stand basal area (see Brais *et al.* (2004) for a complete description of harvested stands).

3.3.2 Field methods

In each experimental plot, the volume of downed wood was estimated using two triangular-transects (30 m per side) (Van Wagner, 1982) per experimental unit. Along each transect line, the frequency of downed wood was recorded by diameter class (fine woody debris, 2.5–7.6 cm; medium size woody debris, 7.6–12.5 cm; and large woody debris greater than 12.5 cm) and two decomposition classes (3 and 4-5) (Daniels *et al.*, 1997) without distinguishing wood species. In 2007 and in each of 15 experimental units (5 treatments x 3 replications), a total of 191 trembling aspen logs were identified, sampled for fungal DNA extraction and classified according to their decomposition classes (medium decay ; class 3 and well decayed; classes 4 - 5). Forty two logs were sampled in uncut stands, 41 in the 1/3

partial cuts, 42 in the 2/3 partial cuts, 39 in the clear cuts and 27 in the controlled burn sites. Forty-eight trembling aspen snags were also located and sampled; 12 in the 1/3 partial cuts, 21 in 2/3 partial cuts and 15 in uncut stands. Positions of each log and each snag was recorded by GPS and also manually recorded when logs/snags were too close for GPS precision. Distances between logs or snags were estimated from the GPS data by geographic information system techniques using ArcView 3.2 (ESRI Inc., New York, USA).

Wood chips for DNA extraction was collected by drilling one hole in each log (in the selected decomposition and diameter class) using a flat drill bit (12.7 mm). The bark and the uppermost layer of wood were first removed and precautions taken to prevent cross-contamination of samples; drill bits were cleaned, rinsed with sterile water, soaked in 95% ethanol and flame sterilized between samples. Snags were sampled the same way except that sampling was performed at a height of 137 cm. All samples were transported on ice to the laboratory and frozen at −20°C until analyzed.

3.3.3 DNA extraction and PCR amplification of fungal-specific genes

Wood samples were lyophilized for 48 hours before disruption in a Qiagen TissueLyser (QIAGEN, Canada) and run for 2 min at 26 Hz, or until the wood was reduce to a fine powder. Samples were put on ice between runs. DNA was extracted with the DNeasy Plant Mini Kit (QIAGEN) according to the manufacturer's instructions. DNA was eluted in 100 µL of elution buffer and stored at -20°C. The Internal Transcribed Spacer (ITS) region of the fungal rDNA was PCR-amplified using the fungal specific primers ITS1-F (Gardes & Bruns, 1993 ; Jasalavich, Ostrofsky & Jellison, 2000) and ITS2 (White *et al.*, 1990) to obtain a 280 bp amplicon. A GC clamp (CGC CCG CCG CGC CCC GCG CCC GGC CCG CCG CCC CCG CCC CC) was added to the 5' end of the ITS1-F primer to avoid complete separation of DNA strands during the subsequent denaturing electrophoresis. Polymerase chain reactions were performed using 50-µL of PCR assays containing 2 µL of template, 5 µL of PCR reaction buffer (ThermoPol, New England Biolabs), 1 µl dNTP (10 mM of each dATP, dCTP, dGTP and dTTP), 1 µL of each primer (50 µM), 0.2 µL of Taq polymerase (5 $U.\mu L^{-1}$, New England Biolabs). Cycling parameters used were an initial denaturation cycle of 3 min at 95 °C followed by 35 cycles of denaturation at 94 °C for 45s, annealing at 55 °C

for 45 s, and extension at 72 °C for 1 min 15 s, ending by a final elongation at 72 °C for 8 min (Kubartová *et al.*, 2007). Negative controls (containing no DNA) and positive controls (fungal DNA from pure culture) were included with each PCR batch. All amplification products were analysed by electrophoresis with 1 % (w/v) agarose gels in TAE (40 mM Tris-acetate, 1 mM EDTA), stained with Gelgreen (Biotium) and visualized under UV light.

3.3.4 Separation of fungal ITS amplicons by DGGE

Electrophoresis was performed according to a slight modification from the protocol of Kebli *et al.* (2011). We used the DCode universal mutation detection system (Bio-Rad Laboratories, Hercules, CA) and an acrylamide gel (8% [wt/vol] acrylamide–bis-acrylamide, 37.5:1) with a linear vertical gradient of 20–55 % denaturing agents (100% denaturant corresponding to 7 M urea and 40 % [v/v] deionized formamide), increasing in the direction of the electrophoretic run with a stacking gel (4% [w/v] acrylamide–bis-acrylamide, 37.5:1) on top. Approximately 400 ng of each PCR product was loaded and electrophoresis was performed in TAE buffer (40 mM Tris-acetate, 1 mM EDTA) at 75 V and 60 °C for 16h. Gels were stained for 15 min with SYBR gold (Invitrogen, Carlsbad, CA), visualized under UV illumination, and digitized using a ChemiDoc XRS System molecular imager (Bio-Rad Laboratories, Hercules, CA). Amplicons that generated prominent DGGE bands were selected for cloning and sequencing according to Kebli *et al.* (2011).

3.3.5 Gel analysis

The software package GelCompar II (version 5.0, Applied Maths, Belgium) was used to analyse ITS DGGE banding patterns. In order to minimize migration differences and to normalize for distortions between gels, we aligned the gels using an external reference pattern comprised of mixed ITS amplicons from five different fungi. A band-matching process was used to obtain a presence-absence matrix for statistical analyses. A 5% band intensity threshold was set for the band selection process. Individual bands were grouped into classes based on melting behaviour (positions in the gels). Each band class was then considered to be an operational taxonomic unit (OTU), allowing for the calculation of their frequency among log samples. We also calculated the relative intensity of each band, applying a value between 0 and 1 by dividing the intensity of the band by the sum of the

intensity of all the bands within the lane, thus eliminating the variation in band intensity due to difference in amplification and amount of DNA loaded on the DGGE gel.

3.3.6 Statistical analysis

Matrices of relative abundance and presence/absence were obtained for fungal species on logs and on snags. Species richness (S) and Shannon diversity indices (H') were calculated for each log or snag (Eichner *et al.*, 1999). In order to assess the strength of the relationship between richness, diversity and salient explanatory variables, data were analyzed by means of a linear mixed-effects model using the "nlme" package (R package version 3.1-90; nlme: linear and nonlinear mixed effects models) from R software (R Development Core Team, 2010). Five models for logs and five models for snags were tested based upon our hypotheses (Table 3.1). Each model corresponded to a different hypothesis. Explanatory factors for logs were: silvicultural treatments (uncut; 1/3 partial cut; 2/3 partial cut; clear cut; controlled burn), woody debris volume of the stands (by decomposition and diameter classes), and individual log diameter and decay classes (3 levels: < 10 cm medium decayed log; > 10 cm medium decayed log and > 10 cm advanced decayed log). In order to assess whether species richness varies between small and large logs according to silvicultural treatments, we applied a single model to richness with silviculture treatment, log diameter and the interaction between the two factors as explanatory variables. This model considered only fresh logs as no small, well decomposed logs were sampled. Explanatory factors for snags were: silvicultural treatments (uncut; 1/3 partial cut; 2/3 partial cut), woody debris volume (by decomposition and diameter classes), snag diameter (at breast height) and snag height.

All explanatory variables were entered as fixed factors whereas blocks and experimental units were considered random factors, with experimental units nested within blocks. Models were compared on the basis of Akaike's information criteria (AIC) (Burnham & Anderson, 2004). The ''best'' model, was the model with the lowest AIC and the highest Akaike weight (w_i). Akaike weights indicate the level of support in favor of any given model being the most parsimonious and most probable among candidate models (Mazerolle, 2006). For model selection (and multimodel averaging if no model had a $w_i > 0.90$), we used

"AICcmodavg" R package (R package version 1.01; AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c), http://CRAN.R-project.org/package=AICcmodavg). Comparison between deadwood volumes among silvicultural treatment were conducted by means of a simple linear mixed model with blocks treated as random factors. Multiple comparisons of means used the Tukey contrasts algorithm.

In order to conduct fungal community composition analyses, we tested variation in OTUs composition among treatments for significance using the adonis function in vegan package (R package version 1.15-2; vegan: Community Ecology Package, http://CRAN.R-project.org/package=vegan). The adonis function is an analysis of variance using permutations that partition the species assemblage matrix as the response variable (relative intensity of DGGE bands) among sources of variation. The number of permutations was set at 999. The adonis function is also analogous to redundancy analysis (Legendre & Andersson, 1999). A subsequent test for differences in between-sample distances (i.e. dispersion) was conducted. Multivariate homogeneity of group dispersions (Anderson, 2006) determine if the variance within a group differed from the other group within a biological community. This analysis is a multivariate analogue of a Levene's test and was used to validate community dispersion (i.e. variability). The analysis was performed in R with the betadisper function in the vegan package (continuous variables were converted into categorical class of variable before analysis).

We also use Mantel test (Mantel, 1967) to compare a species dissimilarity matrix (based on DGGE bands and the Jaccard coefficient) with a dissimilarity matrix based on geographical distances (spatial distance between logs and snags). The Jaccard coefficient was calculated as following: $N_{AB}/(N_A+N_B-N_{AB})$, where N_{AB} = number of DGGE bands in common between gel lanes A and B; N_A = the total number of bands in the gel lane A; and N_B = the total number of bands in gel lane B. The relationships between molecular and geographic distance matrices have previously been used in microbial ecology (reviewed by Ramette (2007)). Finally, indicator species analysis was carried out with the duleg function of the "labdsv" package (R package version 1.3-1; labdsv: Ordination and Multivariate Analysis for

Ecology, http://ecology.msu.montana.edu/labdsv/R). A Holm correction (Holm, 1979) was applied to these probabilities.

Table 3.1 General linear mixed models relating species richness (OTU number) and Shannon diversity index to stand, log/snag physical characteristics and woody debris (WD) volumes.

Model	Tested hypothesis	Explanatory variables
	Logs richness and diversity	
L1	Effect of silvicultural treatment	Silvicultural treatment
L2	Effect of physical characteristics of individual log	Log decomposition and diameter class
L3	Effect of stand WD volumes	WD volume (well decayed) + FWD + Medium size WD + Large WD
L4	Effect of log characteristics and WD volumes	Model L2 + Model L3
L5	Global model	Model L1 + Model L2
	Snags richness and diversity	
S1	Effect of silvicultural treatment	Silvicultural treatment
S2	Effect of snags physical characteristics	Snag diameter class + Snag height
S3	Effect of stand WD volumes	WD volume (well decayed) + FWD + Medium size WD + Large WD
S4	Effect of snag diameter according to silvicultural treatment	Model S2 + snag diameter class*Silvicultural treatment
S5	Effect of snag characteristics and WD volumes	Model S2 + Model S3

3.4 Results

3.4.1 Stand characteristics

Total volumes of downed wood ranged from 130 m^3 ha^{-1} in uncut stands to 61 and 57 m^3 ha^{-1} in the clear-cut and controlled burn treatments respectively. All inventoried logs were either from class 3 (medium decay) or classes 4 and 5 (well decomposed). Deadwood volume decreased with harvesting intensity. However, the highest volume of fine woody debris (2.5-7.6 cm) was observed in the clear-cuts and the lowest in the uncut stands (Fig. 3.1). Well

decayed downed wood volumes were significantly higher in the uncut treatment than in the 1/3 partial harvesting while similar amounts were observed in the 2/3 partial harvesting, the clear-cuts and the controlled burn treatments.

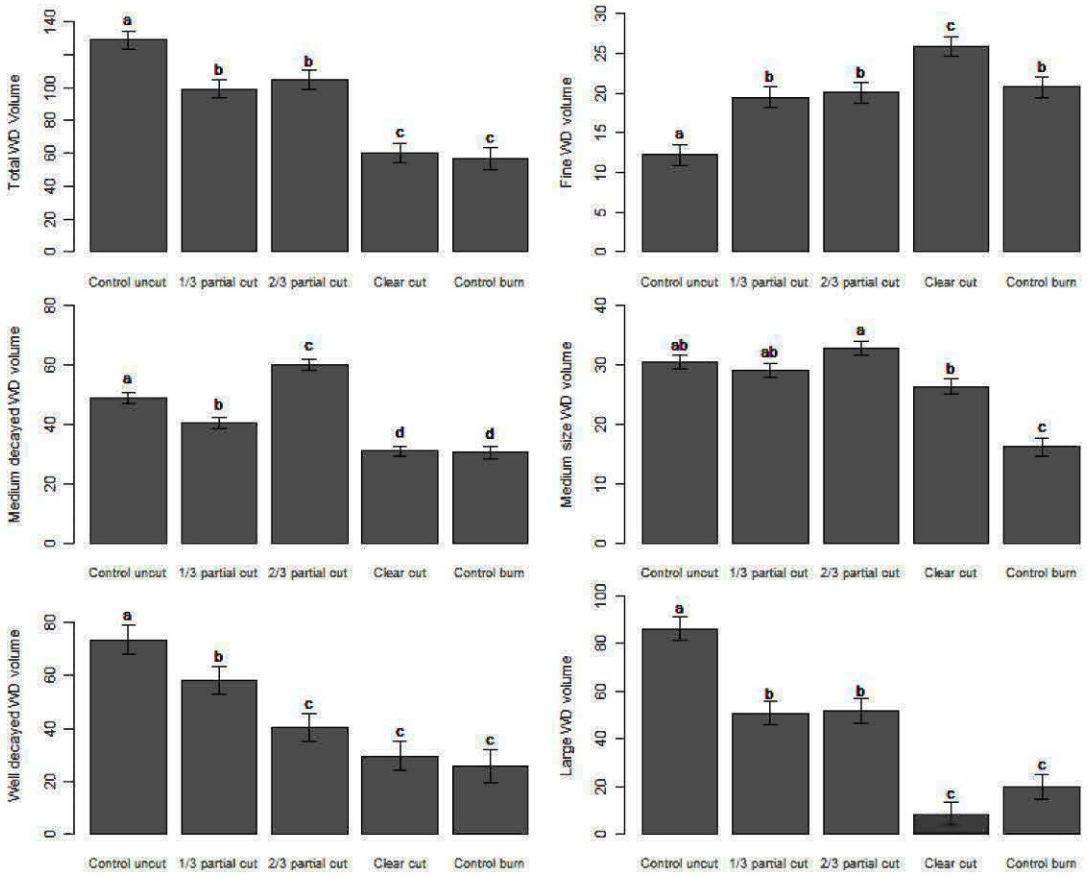


Figure 3.1 Predicted woody debris (WD) volumes (and standard error) according to harvesting treatments (m^3 ha^{-1}). Different letters above the bars indicate a significant difference between categories (Tukey's test at $P \leq 0.05$).

3.4.1 Fungal richness and diversity

A total of 191 trembling aspen logs and 48 snags were successfully analyzed for species richness (*S*) and species diversity (H') by DGGE. We found a total of 35 different DGGE bands (or OTUs) on logs and 31 on snags. The mean number of DGGE bands per log

was 5.5 (minimum of 1 and maximum of 20) and 5.4 (minimum of 1 and maximum of 12) for snags.

For logs, model L2 (log physical variables) had the highest AICc weights for both S and H' (Table 3.2). For H' the model had an insufficient Akaike weight (i.e AICcWt < 0.9), meaning that more than one model could explain the data equally well, and model averaging was used. However, species richness could be explained by model L2 only and model estimates were computed only for variables included in this model (Table 3.3). Our results indicate that, independently from harvesting intensity, large well-decayed logs (> 10 cm of decomposition classes 4 and 5) had higher richness and diversity. These logs had 11.8% higher diversity than small class 3 logs and 9.3% higher than large class 3 logs (Table 3.3). Richness was also higher in large decomposed logs with an increase of nearly 2 OTUs (compared to small, medium decayed wood) and 1.5 OTUs compared to large, medium decayed wood, corresponding to increases of 10% and 7.5%, respectively.

Table 3.2 Akaike's Information Criterion (AICc) rank and weights (w_i) of models relating species richness (OTU number) and Shannon diversity index to stand and logs/snags characteristics.

Type of CWD	Models	K[a]	AICc	ΔAICc	w_i[b]	Cum.Wt[c]
		OTU number				
Logs	L2: Effect of physical characteristics of individual log	6	1016.66	0.00	**0.92**	0.92
	L4: Effect of log characteristics and WD volumes	10	1022.19	5.53	**0.06**	0.98
	L5: Global model	10	1024.58	7.92	**0.02**	1.00
		Shannon diversity index (H')				
	L2: Effect of physical characteristics of individual logs	6	376.78	0.00	**0.86**	0.86
	L4: Effect of log characteristics and WD volumes	10	380.87	4.08	**0.11**	0.97
	L5: Global model	10	384.52	7.74	**0.02**	0.99
		OTU number				
Snags	S1: Effect of silvicultural treatment	6	253.61	0.00	**0.37**	0.37
	S2: Effect of snags physical characteristics	6	253.94	0.33	**0.31**	0.68
	S3: Effect of stand WD volumes	8	254.11	0.50	**0.29**	0.96
		Shannon diversity index (H')				
	S1: Effect of silvicultural treatment	6	95.79	0.00	**0.48**	0.48
	S3: Effect of stand WD volumes	8	96.92	1.12	**0.28**	0.76
	S2: Effect of snags physical characteristics	6	97.37	1.58	**0.22**	0.98

Models are listed from best to worst based on w_i.

[a] K = estimable number of parameters in the model

[b] Akaike weights, also known as model probabilities. These measures indicate the level of support in favor of any given model being the most parsimonious (i.e the best explanatory model) among the candidate model set (Mazerolle, 2009).

[c] Cumulative Akaike weights

Table 3.3 Effect of stand and logs characteristics on Shannon diversity index and OTU number. Model averaged estimates and unconditional standard errors were obtained from linear mixed multimodel inference (see table 3.1 for models specifications). Only variables with confidence interval > 95% are presented.

Type of CWD	Explanatory variable	Model averaged estimate	Unconditional SE	95% unconditional confidence interval	
				Lower	Upper
Logs		OTU number (*S*)			
	Small fresh logs	-1.94	0.60	-3.10	-0.77
	Large fresh logs	-1.46	0.60	-2.64	-0.27
		Shannon diversity index (H')			
	Small fresh logs	-0.33	0.11	-0.55	-0.11
	Large fresh logs	-0.26	0.11	-0.48	-0.04
	Fine woody debris volume	-0.02	0.01	-0.05	-0.001
Snags		OTU number (*S*)			
	Fine woody debris volume	-0.29	0.11	-0.5	-0.08
		Shannon diversity index (H')			
	Fine woody debris volume	-0.05	0.02	-0.09	-0.01

"Uncut" was the reference level for silvicultural treatment and "large (> 10 cm) decomposed log" is the reference level for diameter and decay class.

Assessing the interaction between treatment and log size by comparing diversity and richness between large logs and small logs located in the same treatment units, species diversity increased by 33.7% (estimate = 0.80 ± 0.36; p=0.03) and richness by 27.9% (i.e. 4.2 OTUs; estimate = 4.20 ± 1.73; p=0.02) in large logs but the difference was only significant between controlled burn and uncut treatments (Fig. 3.2).

Finally, we also found fungal diversity on logs to decrease with increasing fine woody debris (FWD) volume (i.e. woody debris with diameter < 5 cm). For each increase of 1 m^3 ha^{-1} in FWD, fungal diversity decreased by 0.7%.

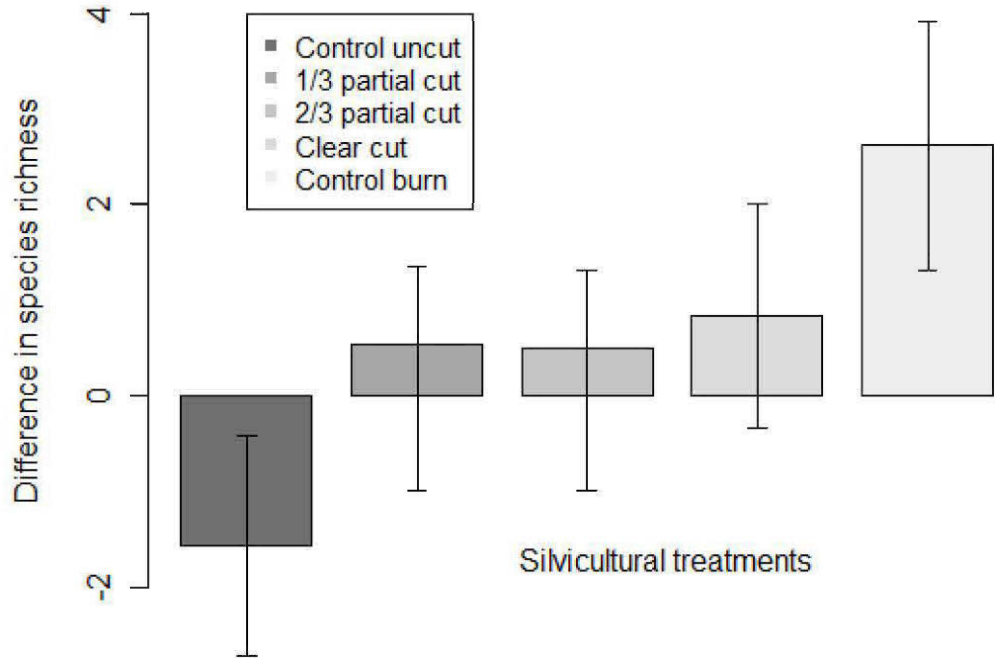


Figure 3.2 Fungal species richness mean differences and standard errors between large and small logs in five silvicultural treatments (based on predicted data).

For snags, model 3 (snag physical variables) has the highest AICc weight for both H' and *S* (Table 3.2). However, the Akaike weight is not sufficient (i.e AICcWt < 0.9) so we used model averaging to determine which variables influence fungal richness and diversity on snags (Table 3.3). We found that trembling aspen snags had a slightly lower fungal diversity when fine woody debris volume increased. Increasing the FWD volume (i.e woody debris with diameter < 5cm) of 1 m^3 ha^{-1} lead to a decrease of 2.4% in fungal species richness and a decrease of 2.2% in fungal diversity (Table 3.3).

3.4.2 Indicator species

Following the indicator species analysis, we found two OTUs that exhibited preference for logs at advanced stages of decay (classes 4 and 5): OTU #27 (most similar to *Phlebiella christiansenii*, accession number EU118659) and OTU #31 (indicator value: 0.30 and 0.10 respectively, $P < 0.05$).

OTU #13 is found mainly on snags. Two other OTUs, #6 (*Ascomycota*) and #7 (*Mortierella* sp.), occurred mainly on snags of the one third partial cut stands (indicator value: 0.08, $P < 0.05$ for both). Also, OTU #25 was preferentially found on snags which had recently died (indicator value 0.75, $P < 0.10$).

3.4.1 Relationships between fungal communities and spatial distribution of snags and logs

We used the Mantel statistic based on Pearson's product-moment correlation to compare the fungal community composition with a dissimilarity matrix based on the spatial distance between dead wood. We did not find any correlation between OTUs fungal community dissimilarity matrix (based on DGGE bands and the Jaccard coefficient) and geographic distance matrix of logs and snags. Including both log and snag data in the same analyses give a Mantel statistic r of -0.0009 and a significance of 0.491. Data with logs only gave an r value of -0.0168, with significance of 0.769. Finally data including only snags gave an r value of 0.0539, with a significance of 0.129.

3.4.3 Community composition

We successfully cloned and identified 22 OTUs (35 OTUs total) observed on the DGGE gels (Table 3.4).

Table 3.4 Sequence analysis of bands excised from DGGE gels.

OTU	Most closely related fungal sequence	Similarity (%)	Accession no. of related sequence
4	*Pholiota flavida*	99	JF908576.1
5	*Leptodontidium elatius*	97	FJ903294.1
6	Uncultured *Ascomycota*	95	JF960616.1
7	Uncultured *Mortierella*	99	FJ553782.1
8	*Calocera cornea*	99	AY789083
11	*Resinicium bicolor*[a]	99	DQ826535
15	*Ascocoryne cylichnium*	99	FJ903373
16	*Hyalodendriella betulae*	93	EU040232.1
17	*Phellinus cinereus*[b]	99	AY340049
18	*Ascocoryne* sp. isolate	97	FJ903331
19	*Dermateaceae* sp.	91	FJ554419.1
20	*Athelia neuhoffii*	95	U85798.1
21	*Phlebia centrifuga*	99	L43380.1
22	*Bjerkandera adusta*	98	FJ903353
23	Uncultured fungus isolate DGGE gel band	100	HM015681
24	*Bisporella citrina*	98	AY789386.1
25	Uncultured fungus clone Singleton_24-2804_2353	86	FJ758813
26	*Phialophora* sp.	100	FJ903315.1
27	*Phlebiella christiansenii*	100	EU118659
28	Uncultured fungus	99	FM999613
29	Uncultured fungus	83	FJ778188
30	*Pleurotus ostreatus*	98	AY540325.1

Most similar Genbank accessions number and percent sequence similarities for the OTUs

[a]: same similarity to FJ554463 (Uncultured Agaricomycetes clone LTSP_EUKA_P6P23)
[b]: same similarity to *Phellinus nigricans* (AF200239)

Logs and snags were colonized by different assemblage of fungal communities (Table 3.5) with snag communities representing a subset of that found on logs (Fig. 3.3). The between-sample distances (or dispersion variance) resulting from the community analysis confirmed that all significant effects in community composition were caused by differences in community centroids (i.e. means) rather than community dispersion (i.e. variability).

Table 3.5 Effect of harvesting treatment, decomposition class, diameter, snag height, woody debris volume and type of WD (snag/log) on species assemblage and variability of fungi associated with dead wood.

	Logs dataset		Snags dataset		All type of wood dataset	
	Centroid	Variance	Centroid	Variance	Centroid	Variance
Treatment	R^2=0.020 *P*=0.578	F=0.49 *P*=0.745	R^2=0.043 *P*= 0.365	F=1.169 *P*=0.3194	R^2=0.014 *P*=0.769	F=0.990 *P*=0.4137
Log diameter and decomposition class	R^2=0.016 ***P*=0.05**	F= 0.116 *P*=0.734	NA	NA	NA	NA
Snag height	NA	NA	R^2=0.042 ***P*=0.025**	F=0.239 *P*=0.6273	NA	NA
Snag diameter	NA	NA	R^2=0.007 *P*=0.940	F=0.0578 *P*=0.811	NA	NA
Medium decayed WD volume	R^2=0.005 *P*=0.603	F= 0.046 *P*=0.955	R^2= 0.010 *P*=0.780	F=0.239 *P*=0.789	R^2=0.003 *P*=0.843	F=0.122 *P*=0.885
FWD volume	R^2=0.003 *P*=0.807	F= 0.1041 *P*=0.901	R^2= 0.050 ***P*=0.008**	F=0.096 *P*=0.909	R^2=0.007 ***P*=0.093**	F=0.164 *P*=0.849
Medium size WD volume	R^2=0.012 ***P*=0.006**	F= 1.880 *P*=0.155	R^2=0.034 *P*=0.120	F=0.263 *P*=0.610	R^2=0.005 *P*=0.397	F=2.275 *P*=0.105
Large WD volume	R^2=0.010 ***P*=0.044**	F= 0.874 *P*=0.419	R^2= 0.015 *P*=0.529	F=0.356 *P*=0.702	R^2=0.003 *P*=0.757	F=0.116 *P*=0.317
Type of WD	NA	NA	NA	NA	R^2=0.012 ***P*=0.004**	F=2.801 *P*=0.096

R^2 values represent the proportion of variation each factor contributes to the total variation in the dataset. "NA" indicates non-applicable. F corresponds to the F value. Each variable for the centroid analyses was tested marginally (type III test).

The following OTUs explained the greatest proportion of the variation in fungal community composition (based on multivariate analyses) and are ordered according to their fitness with dead wood type (log or snag). Highest (or lowest) values explain the highest proportion of the variation in fungal community composition between logs and snags due to OTU. Positive values indicated that the fungal species is preferentially found on logs and negative values for OTUs found on snags. The OTUs are: OTU 13 (-0.0964), *Resinicium bicolor* (-0.0824), OTU 14 (-0.0688), *Athelia neuhoffii* (0.0613), OTU 29 (-0.0542), *Phellinus cinereus* (0.0513), OTU 10 (-0.0480), *Calocera cornea* (0.0471), *Phialophora* sp. (-0.0456) and OTU 28 (-0.0428).

Fungal communities on snags differed according to overall snag height and variation in fungal assemblages was also related to the volume of surrounding fine downed wood. Snag height and volume of fine woody debris explained the highest proportion of variation in community composition (Table 3.5). OTUs on snags that were the most influenced (positively or negatively) by snag height are: *P. cinereus* (0.0266), *Bisporella citrina* (0.0199), *Phialophora* sp. (-0.0180), *Dermateaceae* sp. (0.0175), OTU 9 (-0.0167), OTU 10 (-0.0162), *Phlebiella christiansenii* (-0.0133), OTU 14 (-0.0131), *A. neuhoffii* (0.0125) and OTU 12 (-0.0111). The following OTUs on snags were the most strongly influenced (positively or negatively) by fine woody debris volume: *Phlebia centrifuga* (0.0651), OTU 14 (-0.0624), *Ascocoryne cylichnium* (-0.0615), *Dermateaceae* sp. (-0.0606), OTU 23 (-0.0591), OTU 25 (-0.0561), OTU 29 (-0.0480), OTU 28 (-0.0464), *R. bicolor* (-0.0447) and OTU 10 (-0.0412).

Fungal communities colonizing logs differed according to the diameter and decomposition class of the sampled log (Table 3.5). Diameter and decay class explained the highest proportion of the variation in composition (Table 3.5). Harvesting treatments had no significant direct effects on log communities (Fig. 3.3, Table 3.5). In comparing large well-decomposed logs with small and large fresh logs, the following OTUs explained the highest proportion of the variation in fungal community composition (positive values indicate that the fungal species was preferentially found on small and large fresh logs while those with negative values were found on large and well decomposed logs): OTU 23 (0.0232), *P. christiansenii* (-0.0181), *P. centrifuga* (0.0134), *Phialophora* sp. (-0.0105), *A. neuhoffii* (0.0099), *Ascocoryne* sp. (-0.0087), OTU 25 (-0.0067), *Bjerkandera adusta* (0.0051), OTU 32 (-0.0047) and *B. citrina* (-0.0037).

Large and medium size class (7.6-12.5 cm diameter) woody debris volumes affected fungal community composition on logs. Well-decomposed woody debris volume was not included in the analysis due to its high correlation with total CWD volume (data not shown). OTUs on logs that were the most influenced (positively or negatively) by medium size woody debris volume are: *P. centrifuga* (-0.0097), *B. citrina* (0.0059), *Phialophora* sp. (0.0042), *A. neuhoffii* (-0.0030), *Hyalodendriella betulae* (0.0029), *B. adusta* (-0.0025), *P. christiansenii* (0.0024), *Dermateaceae* sp. (0.0023), *P. cinereus* (-0.0021) and OTU 23 (-

0.0015). OTUs on logs that were the most influenced (positively or negatively) by large size woody debris volume are: *Phialophora* sp. (-0.0018), *B. citrina* (-0.0014), OTU 23 (0.0013), *B. adusta* (-0.0013), *P. centrifuga* (0.0012), OTU 25 (0.0009), OTU 29 (0.0007), *Dermateaceae* sp. (-0.0006), *P. cinereus* (0.0006) and OTU 14 (0.0005).

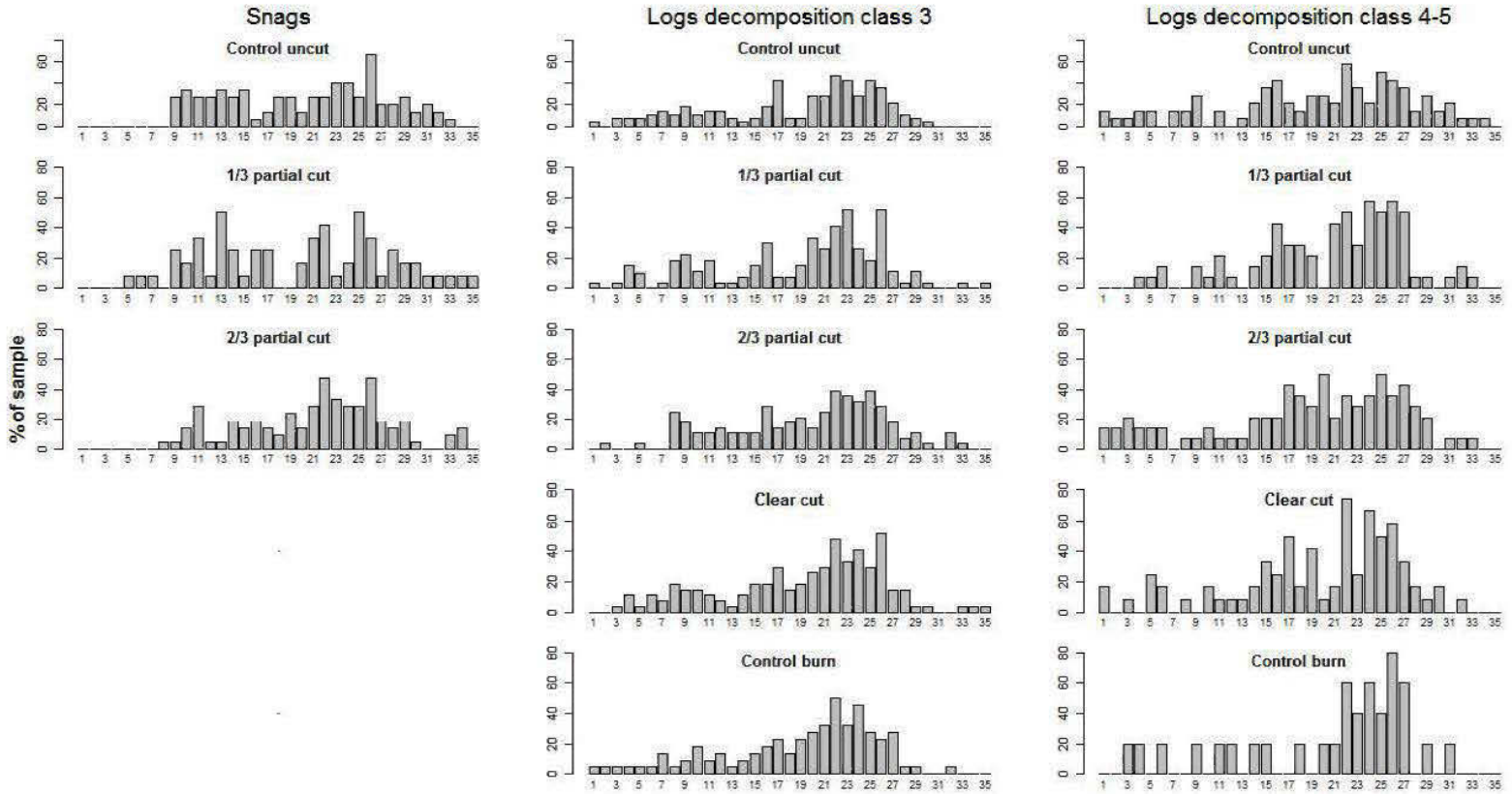


Figure 3.3 Histograms showing the proportion of samples (abundance) colonized by the 35 OTUs distinguished by DGGE profiling in relation to silvicultural treatment for trembling aspen logs (decomposition classes 3 and 4-5) and snags.

3.5 Discussion

Environmental changes, including human disturbances, have a striking impact on the biodiversity of ecosystems. Using molecular techniques, we have described variation in saproxylic fungal communities related to silvicultural practices that mimic canopy succession, gradual stand break-up and clear-cutting to reinitiate even-aged stands (Harvey *et al.*, 2002 ; Harvey & Brais, 2007). Our results confirm the negative effects of intense silvicultural practices on saproxylic fungal communities (Müller, Engel & Blaschke, 2007 ; Müller, Hothorn & Pretzsch, 2007) but they also highlight the benefits of alternative treatments to clear-cut, such as partial cuts. At the stand level, it is assumed that retaining forest structures in managed stands through practices such as partial harvesting and green tree retention should allow for the maintenance of ecosystem functions and biodiversity at the stand level (Sippola, Lehesvirta & Renvall, 2001). Sippola *et al.* (2004) and Muller *et al.* (2007) found that by maintaining higher substrate availability, partial harvesting mitigated the negative effects of harvesting on saproxylic fungal species richness and composition. Our results represent a further contribution toward constructing a framework for the conservation of saproxylic fungi in these systems.

We had hypothesized that species richness and diversity of saproxylic fungi would decrease with the intensity of harvesting in aspen stands. Although we did not find evidence for a direct relationship between treatments and fungal richness or diversity, we did find that fungal diversity on logs and snags was negatively related to the volume of FWD, which in turn, increased with stand harvesting intensity and reached maximum values in clear-cuts and control burns (Fig. 3.1). Sippola *et al.* (2004) found that increased logging intensity decreased the number of polypore observations in four forest site types dominated by *Pinus sylvestris* or *Picea abies*, indicating reduced substrate availability and while species richness was not directly affected by logging intensity, no virgin forest fungal species or threatened species were found in the most disturbed sites. As clear-cuts contained higher FWD volumes than controlled burn, partially harvested or unharvested stands, we might expect that clear-cutting is the most disruptive treatment with respect to the conservation of saproxylic fungal diversity. We can infer from our results that fungal diversity decreases with harvesting intensity, despite larger volumes of residual small diameter logs left on the ground indicating

that small logs may not be sufficient to mitigate the effect of harvesting on saproxylic communities diversity. Heilmann-Clausen and Christensen (2004) found that FWD harboured more species per volume than did larger diameter class of deadwood (based on sporocarps in unmanaged forest). Molecular techniques allowed us to detect fungal hyphae without fructifications and that may explain differences between our observations and those based on fruiting bodies. In Heilmann-Clausen and Christensen (2004) study, fungi might preferentially produce fruit bodies on fine woody debris but be present on larger logs as well. Similar asymmetries between direct molecular studies and fruiting occurrence have been widely reported for ectomycorrhizal fungi (Gardes & Bruns, 1996).

We observed that logs and snags had different community composition (Table 3.5 & Fig. 3.3). *Resinicium bicolor* (Basidiomycete) and *Phialophora* sp. (Ascomycete) tended to be associated with snags while *Athelia neuhoffii*, *Phellinus cinereus* (white rot usually found on *Betula* sp. and *Populus* sp.) and *Calocera cornea* (brown rot found on deciduous wood) were preferentially found on logs. Snags and logs fungal communities reacted differently to the residual volume of deadwood remaining on sites. Snags fungal communities were affected by the volume of FWD while logs fungal communities were affected by the volume of medium and large woody debris. Compared to unharvested stands, FWD volume was highest in clear-cut stands, while medium and large size woody debris volume were lowest in the clear-cut and controlled burn stands. These two treatments would most greatly change fungal community composition. Fungal species on snags that were negatively influenced by high volume of fine woody debris volume were: *Ascocoryne cylichnium* (Ascomycete endophyte), *Dermateaceae* sp. (Ascomycete) and *R. bicolor* (Basidiomycete). On logs, reducing the volume of medium size woody debris would decrease the abundance of *Bisporella citrina* (Ascomycete), *Phialophora* sp. (Ascomycete), *Hyalodendriella betulae* (Ascomycete), *Phlebiella christiansenii* (Basidiomycete) and *Dermateaceae* sp. (Ascomycete). Similarly, reducing the volume of large size woody debris would decrease the abundance of *P. centrifuga* and *P. cinereus*, both of which are white rot Basidiomycetes.

Woody debris volume in the stand was least affected in partial cut treatments, especially medium size woody debris volume, which remained constant between partial cut and unharvested stands. For large woody debris, their volumes in 1/3 partial cut and 2/3

partial cut were lower than those found in unharvested stands but higher compared to clear-cut and controlled burn stands. Hence, partial cut treatments, which more closely simulate the natural forest succession in the absence of stand replacing disturbance, would lead to less severe changes in fungal community (Lõhmus, 2011). Nordén *et al.* (2008) also found that species richness of *Basidiomycota* and *Ascomycota* declined significantly in partial cut stands. In their study, total species richness was also significantly reduced on fine woody debris, but not on coarse woody debris. However they compared fungal richness before and after the partial cut without reference to more intense silvicultural treatments (clear-cuts for example).

The negative relationship between silvicultural intensity and saproxylic fungal diversity can be seen in larger, well decomposed logs, as these tended to support high fungal species richness and diversity and are more common in uncut stands (Fig. 3.1). Large well decomposed logs were more frequently colonized by *P. christiansenii* (Basidiomycete), *Phialophora* sp. (Ascomycete), Ascocoryne sp. (Ascomycete) and *B. citrina* (Ascomycete). The most striking difference between small diameter and large diameter logs was observed when comparing controlled burn stands with uncut stands (Fig. 3.2). In the highly disturbed control burns, large logs may have a buffering effect with respect to solar exposition, temperature, and precipitation, which contributes to the stability of these microclimatic conditions. Conversely, the interiors of small logs are subject to greater variation especially in the most open stands (Bader, Jansson & Jonsson, 1995 ; Sippola & Renvall, 1999). One recommendation for biodiversity conservation during forest management would require the retention of large diameter logs in the most disturbed treatments to act as potential refuges for wood decay fungi. Fungal diversity at the stand level could also be increased by conserving a variety of log species, such as spruces, which have been shown to support a higher fungal diversity than other log species (Kebli *et al.*, 2011).

We expected fungal species assemblages to be more similar between logs or snags with high connectivity (in close proximity to one another), but this relationship was not observed in our study. This could be due to the effect of other variables influencing community composition (humidity, temperature) that were not measured, or their effects may have been obscured by our pooling of all variables (decomposition class, silvicultural

treatment, etc.) for this analyses. Otherwise, and for reasons similar to those mentioned by Penttilä *et al.* (2006) with respect to habitat fragmentation, we had a high level of connectivity (i.e the maximum degree of isolation for forest fragments was < 200 meters and polypores and corticoid fungi are not dispersal-limited at the scale of 100-200 meters). Indeed, the distance between our different stand treatments was not sufficiently large to assess spore dispersion. Stand treatments within the FERLD are surrounded by forest, so a continuous presence of deadwood may provide sources from a mixed fungal community to colonize substrate through the soil (mycelium) or by air (spore) (Boddy, 2001). Finally we could also have a chronological continuity of deadwood in our sites as the stands we sampled had never been subjected to anthropogenic disturbance prior to harvesting. Further, coarse woody decomposition dynamics were still under the influence of natural stand dynamics. Under these conditions, some fungi could still be present to colonize freshly fallen wood rapidly and indiscriminately (Edman, Kruys & Jonsson, 2004).

Our results underscore the negative effects that intense silvicultural practices exert on the richness, diversity and species composition of saproxylic fungal communities. In particular, the lack of large woody debris (Jonsson, Kruys & Ranius, 2005) and increases in fine woody debris in managed stands negatively affect saproxylic fungi diversity. However partial harvesting counteracts these effects by maintaining larger well decayed logs compared to clearcut. Our findings agree with those of Lonsdale, Pautasso et Holdenrieder (2008), who recommended adjustment of forest practices that threaten deadwood-dependent fungi to achieve sustainable forest ecosystems. Given their predictive value, our results can lead to the following recommendations with regards to the preservation of fungal biodiversity in the context of ecosystem forest management:

I) Leaving only fine woody debris following harvesting is not sufficient to maintain saproxylic communities and large and well-decayed aspen logs significantly increase the number and diversity of species of saproxylic fungi. Increased abundance of large WD could be achieved if some large fresh logs were left to decompose after harvesting. The refuge effect of large diameter logs is particularly important in the most extreme treatments (clear-cutting followed by prescribed burning). Several fungal species would benefit from the

deployment of these types of logs: *P. christiansenii* (Basidiomycete), *Phialophora* sp. (Ascomycete), *Ascocoryne* sp. (Ascomycete) and *B. citrina* (Ascomycete).

II) Partial-cutting appears to be a reasonable approach to ecosystem management with respect to wood decay fungi compared to even-age silvicultural practices. Our results differ from those for forest floor microbial communities, in which partial harvesting does not appear to have any benefit over clearcut harvesting in boreal forest sites (Hannam, Quideau & Kishchuk, 2006). However, our results indicate that residual woody debris following harvesting has stronger effects on the diversity and composition of saproxylic fungi than harvesting intensity.

CONCLUSION GENERALE

L'objectif général de cette thèse était d'améliorer les connaissances sur l'écologie du bois mort et des champignons décomposeurs qui lui sont associés en évaluant les facteurs qui structurent les communautés fongiques dans les peuplements naturels et sous aménagement. Il s'agissait également de vérifier les relations entre la diversité fongique et l'activité de décomposition; et enfin de documenter les effets des coupes partielles sur les communautés de champignons saproxyliques. Pour cela nous avons utilisés trois approches expérimentales ou plans d'échantillonnage très différents : une chronoséquence de bois mort, une expérience de décomposition et une étude expérimentale de sylviculture sur le terrain. Nous avons nouvellement décrit en forêt boréale mixte canadienne les conditions environnementales et leurs variations (volume de bois, temps, essence forestière,…) auxquelles certaines espèces fongiques étaient plus sensibles ainsi que l'effet de l'aménagement forestier sur ces espèces.

Nous avons effectué ce travail en nous affranchissant des problèmes méthodologiques liés à l'identification ou à la culture des champignons grâce aux méthodes d'écologie moléculaire, encore très peu utilisées dans ce domaine (Rajala *et al.*, 2010). Ces techniques ont permis d'étudier la diversité des hyphes plutôt que d'identifier les fructifications, ce qui expliquerait les différences entre nos observations et celles basées sur les fructifications concernant l'hôte privilégié par une espèce fongique (Boddy, 2001). Ces études pourraient avoir sous-estimées la distribution de l'espèce dans la mesure où le réseau d'hyphes de certains champignons serait peu spécifique à une essence de bois, mais ce champignon pourrait fructifier préférentiellement sur une essence particulière. Les sporocarpes ne révèlent pas la richesse entière présente mais seulement celle des espèces qui ont fructifié au moment de l'échantillonnage et il est reconnu que les études basées sur l'observation des sporocarpes ont des limitations certaines (Boddy, 2001). A la différence des inventaires de fructifications, la DGGE ne sous-estime pas la diversité fongique car elle détecte des espèces fongiques importantes mais discrètes, qui restent sous forme de mycélium et ne fructifient pas (Rajala *et al.*, 2010 ; Stenlid, Penttilä & Dahlberg, 2008). Les inventaires

des fructifications fongiques sont considérés comme « la partie visible de l'iceberg » dans la mesure où la distribution et l'abondance des sporocarpes ne reflètent pas forcement la distribution et l'abondance du mycélium. Ainsi, l'absence de sporocarpes dans l'échantillon ne signifie pas que le champignon est absent. Le chapitre 2 n'aurait pas pu être réalisé sans l'aide de méthodes moléculaires car il n'y avait que très peu de fructifications pendant toute la durée de l'étude. On n'aurait pas pu détecter la succession fongique. De même, pour le chapitre 3, la différence entre nos résultats et ceux de Heilmann-Clausen et Christensen (2004) concernant la richesse fongique sur les débris ligneux fins peut aussi s'expliquer par le fait que nous avons détecté la présence de mycélium. Les différences entre les études basées sur des méthodes moléculaires et sur la présence de fructifications ont été démontrées pour les champignons ectomycorhiziens (Gardes & Bruns, 1996). D'ailleurs dans notre étude, la présence de mycorhizes dans les billes souligne l'importance du bois mort dans le cycle des nutriments des écosystèmes forestiers.

Bien que toute approche ait ses avantages et ses inconvénients, la technique de DGGE utilisée dans cette thèse fournie une vue suffisamment précise des variations des communautés de champignons saproxyliques en fonction des différentes conditions environnementales. Bien que cette technique puisse sous-estimer la diversité par rapport aux approches plus récentes telles que le pyroséquencage par exemple (Ovaskainen *et al.*, 2010), l'objectif de notre étude n'était pas tant de se focaliser sur la composition totale et exhaustive de la communauté fongique mais plus sur les variations de ces communautés entre les échantillons. Par ailleurs, étant donné le nombre très élevé d'échantillons dans notre étude, de telles méthodes auraient conduit à des coûts rédhibitoires et on n'aurait pas pu analyser autant d'échantillons. Bien que réduit au mieux de nos possibilités, tout biais (limitation pour la détection d'espèces rares, comigration de différentes espèces dans les gels ou le fait que certains champignons puissent générer plus d'un seul fragment) reste constant entre l'ensemble de nos échantillons. L'analyse des séquences des principales bandes a écarté la possibilité d'artefacts formés lors de la PCR.

Cette thèse se démarque également des études réalisées en Scandinavie où les forêts ont un long historique d'aménagement intensif. En forêt boréale européenne, Junninen et Komonen (2011) ont postulé qu'un seuil minimum de volume de bois mort de 20 à 40 m^3 ha^{-1}

était requis pour la présence de polypores rares. Cependant les communautés de champignons en Fenno-Scandinavie pourraient déjà avoir été modifiées à cause de l'aménagement forestier. En effet, dans l'étude de Nordén *et al.* (2008) ayant pour objectifs de caractériser l'effet des coupes partielles sur les champignons, le volume de bois mort est faible avant le traitement sylvicole (14 m^3 ha^{-1}). Dans notre étude en peuplement non aménagé, le volume inventorié était d'au moins 87 m^3 ha^{-1}. Donc les espèces fongiques pourraient être déjà modifiées par le volume de bois mort moindre. Dans cette thèse, les peuplements sont naturels et les traitements sylvicoles réalisés sont les premières interventions anthropiques. Nos résultats expliquent donc les variations des communautés fongiques naturelles et natives. Nous avons trouvé que ces communautés étaient affectées par des changements dans le volume de bois mort même si ceux-ci restent à des niveaux élevés (90 à 130 m^3 ha^{-1}) par rapport à ce que l'on retrouve en Europe.

Les communautés fongiques saproxyliques en peuplements naturels

Les principales espèces de champignons décomposeurs que nous avons analysés en conditions naturelles (chapitre 1) semblent être des espèces généralistes dans la mesure où on les retrouvait sur une grande proportion des billes échantillonnées. Cependant, le principal facteur influençant la diversité, la richesse ainsi que la composition des communautés sur les billes, était l'essence même de la bille. En particulier, les billes d'épinettes supportaient le plus grand nombre d'espèces et une plus grande diversité fongique. Cet effet de l'essence de la bille est en accord avec d'autres études basées sur l'observation des fructifications (Kubartová *et al.*, 2007 ; Yamashita, Hattori & Abe, 2010) et sur la culture sur milieu synthétique (Lumley, Gignac & Currah, 2001). Contrairement aux autres études se concentrant sur moins d'essences (probablement parce que la diversité des essences en Scandinavie est plus faible), notre étude se démarque également par le prélèvement d'échantillons à partir de cinq essences différentes, y compris pour des stades très avancés de décomposition. L'identification à l'aide des structures anatomiques observées sur lames minces était alors nécessaire.

Bien que différentes études aient identifié le stade de décomposition des billes comme étant un facteur important influençant la richesse spécifique ou la composition des

communautés fongiques saproxyliques (Heilmann-Clausen & Christensen, 2003 ; Lumley, Gignac & Currah, 2001), nous n'avons pas trouvé de preuve directe d'une telle relation. Cependant, la composition des communautés était reliée à la composition chimique de la bille qui est elle-même dépendante de l'essence de la bille et du stade de décomposition (Moorhead & Sinsabaugh, 2000). Nos résultats ont pu être dû au fait que la composition chimique a été évaluée en prélevant un échantillon de bois aussi près que possible de l'échantillon servant à l'analyse de diversité moléculaire. Au contraire, le stade de décomposition est évalué sur la bille entière ou sur une très grande proportion. Ainsi, la concentration en lignine et en hémicelluloses est un meilleur prédicteur de la décomposition que la classe de décomposition à cause principalement des conditions hétérogènes régnant à l'intérieur de la bille (Pyle & Brown, 1999).

Le stade successionel des peuplements influence également la composition des communautés saproxyliques par le biais des différences entre les volumes de bois mort ainsi que par une composition forestière différente qui mène à des débris ligneux de différentes espèces. En particulier, les vieilles forêts (feu de 1760 dans notre étude) supportent un assemblage de champignons saproxyliques différent à cause du volume de bois mort plus faible et de la proportion de conifères plus élevée. L'influence du volume de bois mort sur les communautés fongiques a été montré dans les forêts boréales scandinaves (Penttilä, Siitonen & Kuusinen, 2004 ; Sippola, Mönkkönen & Renvall, 2005).

Cependant, différentes communautés fongiques ne réagissent pas forcement de la même façon aux variables environnementales. En effet, Jönsson, Edman et Jonsson (2008) ont montré que les colonisateurs fongiques primaires étaient influencés par les premiers stades de décomposition du bois tandis que les utilisateurs secondaires l'étaient plus par le diamètre de la bille ou la connectivité du bois mort. En analysant les communautés dans leur ensemble, on peut manquer de telles différences. C'est pourquoi nous nous sommes concentrés sur les espèces de début de succession dans notre étude sur la relation diversité fongique-fonction de décomposition du bois (chapitre 2).

Colonisation initiale

L'expérience de décomposition sur des blocs de bois frais (chapitre 2) a permis de montrer que dans une variété de peuplements (des vieilles forêts jusqu'aux brulis), le bois mort frais était colonisé par des assemblages d'espèces fongiques pionnières similaires (seulement pour les premiers mois de colonisation). Après un an, les communautés fongiques se différencient entre les deux essences (tremble et sapin). De plus cette colonisation se déroule rapidement car le nombre maximum d'espèces fongiques était retrouvé une année seulement après la mise en place des blocs de bois.

Pour les espèces pionnières, le temps et la surface terrière en feuillu sont les principaux facteurs affectant la diversité et la richesse des champignons (pour le bois de sapin). Dans cette étude, le volume de bois mort n'a que très peu d'impact sur la diversité fongique du bois de tremble et de sapin (chapitre 2) alors que les volumes de bois mort dans tous les traitements étaient relativement élevés (minimum de 56.7 m^3 ha^{-1}). De plus, les peuplements n'ont été soumis qu'à très peu de perturbation anthropique avant notre étude. Ainsi, la dynamique du bois mort serait encore sous l'influence de la dynamique naturelle et dans ces conditions, l'apport de bois mort provenant d'une diversité d'essences d'arbres pourrait servir de réservoir d'espèces de champignons capables de coloniser rapidement le bois mort frais (Edman, Kruys & Jonsson, 2004). Cependant dans l'étude sur la chronoséquence de bois mort (chapitre 1), le volume de bois mort avait un effet sur la composition des communautés fongiques retrouvées sur les billes alors que les volumes de bois mort étaient au minimum de 89.7 m^3 ha^{-1}. Cette seconde étude (chapitre 2) semble donc indiquer que les espèces pionnières sont moins affectées par les variations de volume de bois mort et qu'elles sont également plus aptes à se maintenir dans les peuplements ayant des volumes de bois mort plus faibles. Les espèces arrivant plus tardivement dans la succession fongique seraient ainsi plus fragiles vis-à-vis du volume de bois mort sur le site. Ceci confirme les résultats de Jönsson, Edman et Jonsson (2008) pour lesquels les colonisateurs primaires et secondaires étaient influencés par différentes variables. Enfin, les compositions des communautés pionnières étaient identiques la première année sur le bois de tremble et de sapin puis se différenciaient. Les communautés retrouvées sur les billes à des stades plus avancés de décomposition et donc de succession fongique sont quant à elles clairement

différentes. Les communautés saproxyliques pionnières seraient plus opportunistes ce qui se traduit par une moins grande sensibilité à l'essence de bois mort par rapport aux communautés arrivant plus tardivement dans la succession.

Relation entre diversité fongique et décomposition du bois mort

A notre connaissance, aucune étude ne s'était intéressée à l'activité des décomposeurs du bois en relation avec leur diversité moléculaire le long d'un gradient de perturbation. Nous avons clarifié ces phénomènes, en particulier l'activité pour les espèces pionnières. Les différents taux de décomposition observés entre le tremble et le sapin pourraient être reliés aux interactions compétitrices sous des conditions variables de disponibilité en nutriments. Bien que la respiration augmente au cours du temps pour les deux essences (tremble et sapin) ; cette augmentation proviendrait de deux processus distincts : une augmentation de la compétition chez le sapin et une augmentation de la minéralisation chez le tremble. Nos résultats ont montré des différences notables entre le tremble et le sapin en ce qui concerne les facteurs influençant la diversité et l'activité des colonisateurs primaires dans les peuplements naturels et aménagés. Même si les communautés semblent initialement similaires (paragraphe précédent : colonisateurs), elles divergent par la suite et réagissent différemment aux conditions environnementales (perturbation, temps, composition du peuplement) selon l'essence de bois qu'elles colonisent. Dans le chapitre 1, nous avons vu que l'essence du bois jouait un rôle primordial dans la diversité et la composition des communautés; le chapitre 2 indique que l'essence intervient également dans l'activité (décomposition) des champignons saproxyliques.

Nous avions émis l'hypothèse qu'il existait une corrélation positive entre la diversité et l'activité des champignons dû au rôle complémentaire des différentes espèces fongiques. Cependant, il s'est avéré pour le sapin que la respiration diminuait lorsque la diversité était plus grande, témoignant d'une activité microbienne moins élevée lorsque la diversité fongique est importante. Cette relation négative observée entre la diversité et la respiration sur le sapin pourrait provenir des interactions compétitrices entre les champignons (Boddy, 2000) dans la mesure où elles auraient un coût énergétique notable (Wells & Boddy, 2002). De plus, il a été démontré que la production de métabolites secondaires durant la compétition

interspécifique au lieu d'enzymes responsables de la dégradation du bois peut mener à une réduction du taux de décomposition (Deacon *et al.*, 2006 ; Hiscox *et al.*, 2010). Donc au cours du temps, la diminution de la diversité, l'augmentation de la respiration, la diminution d'une espèce fongique (*Bjerkandera adusta*) connue pour décroitre à cause du parasitisme et le changement de la composition de la communauté alors qu'il n'y a quasiment pas de décomposition; nous permettent de dire que l'augmentation de la respiration avec le temps d'incubation observée chez le sapin proviendrait d'une augmentation de la compétition.

Enfin, l'augmentation de la respiration avec le temps d'incubation observée chez le tremble traduirait une augmentation de la décomposition par une communauté fongique de plus en plus homogène. Les composés aromatiques seraient moins concentrés chez le tremble par rapport au sapin, en particulier concernant la proportion de lignines (Struckelj *et al.* (données non publiées)). Ces composés aromatiques sont en effets connus pour réduire la décomposition (Ganjegunte *et al.*, 2004). Les lignines du sapin sont composées uniquement de phénols vanillyl, tandis que les lignines du tremble sont composées d'unités syringyl et vanillyl. Comme les phénols syringyl sont plus facilement dégradables que les phénols vanillyl, alors cela indique que les lignines du tremble sont plus décomposables que celles du sapin. De plus les espèces feuillues de la forêt boréale (tremble et bouleau blanc) ont un taux de minéralisation supérieur à celui des essences de conifère telles que le pin ou l'épinette (Brais, Pare & Lierman, 2006). Ceci est en accord avec nos résultats indiquant que le sapin se décompose lentement tandis que le tremble présente une tendance à être dégradé rapidement (Table 2.2). Or dans le chapitre 1, la composition chimique était reliée à l'essence de la bille et au stade de dégradation. On peut donc en déduire que l'activité des champignons saproxyliques sera aussi affectée par les différentes essences de bois présentes en forêt boréale ainsi que par le stade de dégradation. Ces différences d'activités soulignent encore plus les différences relevées au niveau de la structure des communautés sur des essences différentes (chapitre 1).

Les champignons décomposeurs et l'aménagement forestier / perturbation

Nous avons évalué l'effet des pratiques sylvicoles et des perturbations sur les champignons décomposeurs par l'intermédiaire de la composition du peuplement, de la surface terrière résiduelle, du volume de bois mort au sol ainsi que de la dimension et du stade de décomposition des billes (ou des chicots). Cela pourrait guider les exploitants forestiers et établir une aide concernant les recommandations de conservation de la biodiversité dans un cadre d'aménagement écosystémique.

Dans le chapitre 1 et en peuplements naturels, nous avons vu que la diversité fongique et la composition des communautés fongiques n'étaient pas influencées par la taille des billes. Cependant dans le chapitre 3, les grosses billes bien décomposées supportaient plus d'espèces ainsi qu'une plus grande diversité fongique. La différence de résultats entre les deux chapitres peut s'expliquer par le fait que dans le chapitre 1, nous avons échantillonné que des billes de plus de 10 cm de diamètre alors que les débris ligneux de faible diamètre supportent une richesse et une abondance fongique non négligeables (Juutilainen *et al.*, 2011). De plus dans le chapitre 3, nous avons échantillonné une seule essence de bille alors que le chapitre 1 portait sur cinq essences. Les différences de diamètre ont donc pu être masquées par l'effet de l'essence de la bille. Ce phénomène pourrait aussi expliquer l'absence d'effet du stade de décomposition dans le chapitre 1 alors que dans le chapitre 3 le stade de dégradation influençait les communautés fongiques. D'autre part, le diamètre des billes est particulièrement important dans les peuplements très perturbés comme les brulis. En effet, l'influence du diamètre de la bille dépend du traitement sylvicole. Donc si on applique des traitements sévères comme des brûlages, alors il vaudrait mieux conserver des grosses billes qui agiraient en tant que refuges.

On s'attendait également à trouver des patrons de décomposition différents selon les trois intensités de perturbation (traitements sylvicoles) et que la diversité et l'activité (respiration) des champignons saproxyliques suivent les changements de structure du peuplement (surface terrière résiduelle et volume de bois mort au sol) causé par le stade successionel du peuplement et par la perturbation. Ni la richesse spécifique, ni la diversité, ni

la respiration des communautés fongiques pionnières n'étaient affectées par la perturbation (chapitre 2). Seule la surface terrière résiduelle en feuillu avait un effet sur la diversité. Cependant dans le chapitre 3, le traitement sylvicole avait un effet négatif sur la diversité et la richesse des communautés fongiques saproxyliques par l'intermédiaire du volume de bois mort résiduel. Ces résultats vont encore dans le sens que les espèces pionnières sont généralistes et moins sensibles aux variations des conditions environnementales (changement du volume de bois mort par exemple) que les espèces fongiques arrivant plus tard dans la succession. Cependant la perturbation (bois de sapin) et le volume de bois mort résiduel (corrélé significativement au traitement sylvicole) affectent la composition des communautés fongiques. Par ailleurs, les conditions environnementales générées suites aux différentes perturbations ont des effets opposés sur les taux de décomposition selon l'essence de bois que l'on considère (chapitre 2). Plus la perturbation est importante et plus la décomposition est ralentie chez le sapin. C'est pourquoi la densité du bois reste plus élevée dans les peuplements les plus perturbés (brulis) (Table 2.2) où l'augmentation de la température du bois suite à l'exposition au soleil peut limiter l'activité des microorganismes. Pour le tremble, la densité du bois diminue (donc la décomposition augmente) plus rapidement dans les traitements moyennement perturbés. Cette différence peut s'expliquer par l'autécologie des essences d'arbres : le tremble est une espèce pionnière tandis que le sapin arrive plus tard dans la succession forestière. Ainsi, les champignons décomposeurs pourraient être plus efficaces sur le tremble dans des conditions de peuplement ouvert et plus perturbé tandis que la décomposition du sapin serait facilitée par des espèces fongiques spécifiques à des habitats qui n'ont subi que de petites perturbations comme ceux retrouvés à des stades plus avancés de la succession forestière.

La signification écologique de nos résultats est remarquable dans un contexte d'aménagement forestier. En effet, notre hypothèse était que la richesse spécifique et la diversité des champignons saproxyliques étaient corrélées négativement avec l'intensité des pratiques sylvicoles (chapitre 3). En fait, la diversité et la richesse diminuaient avec l'augmentation du volume des débris ligneux fins et ces débris sont plus importants lorsque la coupe est sévère (chapitre 3). De plus, les taux de décomposition dépendent de l'essence, mais aussi du fonctionnement des communautés fongiques saproxyliques sous des conditions

environnementales particulières (intensité de perturbation) (chapitre 2). Donc, un changement de la composition forestière dans le peuplement mènerait à un changement des essences de bois mort et ainsi modifierait les taux de décomposition essentiels à la productivité forestière. De la même façon, les pratiques sylvicoles font diminuer la quantité de DLG (chapitre 3). Donc si on enlève certaines essences de billes alors on risque de perdre certaines espèces de champignons en particulier les espèces fongiques sensibles arrivant tardivement sur le bois. L'activité de décomposition pourrait être modifiée par ce biais en plus de l'effet direct du traitement/perturbation. Donc dans le cas de pratiques sylvicoles qui favoriseraient les peuplements équiens, cela entrainerait :

- une diminution de la diversité des communautés fongiques, en particulier s'il y a moins de billes d'épinette (chapitre 1);
- un changement dans le processus de décomposition (chapitre 2) : la décomposition du bois serait ralentie sur le sapin.
- une diminution de la diversité et de la richesse spécifique des communautés fongiques par l'intermédiaire d'une diminution des grosses billes bien décomposées et une augmentation du volume de petits débris ligneux (chapitre 3);
- une modification de la composition des communautés fongiques (chapitre 1; chapitre 2; chapitre 3).

Réaliser des brulages dirigés dans des peuplements non équiens, et notamment sur des peuplements ayant du sapin, entrainerait un ralentissement de la décomposition du bois et donc du cycle des nutriments (Hagemann *et al.*, 2010 ; Palviainen *et al.*, 2010). De plus les peuplements soumis au brulis sont très différents de ceux issus de feux naturels car les niveaux de bois mort après brûlage dirigé sont extrêmement faibles (chapitre 3) par rapport à ce que l'on retrouve après une grande perturbation telle qu'un feu où l'on peut retrouver de 300 à 460 m^3 ha^{-1} 7 ans après un feu (Brassard & Chen, 2008 ; Seedre *et al.*, 2011). Les espèces fongiques arrivant tardivement dans la succession sont plus sensibles aux perturbations. Donc si le volume de bois mort est réduit suite au brulis, alors il n'y aura pas de réserve de champignons non-pionniers pour recoloniser le bois mort restant. Que ce soit pour les communautés se développant en conditions naturelles ou soumises à des pratiques sylvicoles, les deux chapitres ont montré l'importance de la quantité de bois mort. De plus le

volume de bois mort dans les peuplements est dépendant du stade successionel (chapitre 1). Donc comme les traitements sylvicoles affectent aussi ce volume (chapitre 3), alors là encore, il faudrait être prudent afin de choisir dans quel type de peuplement sera appliqué tel traitement au risque sinon, de trop diminuer le volume total de bois mort.

C'est pourquoi l'aménagement écosystémique par l'intermédiaire des coupes partielles pourrait contrebalancer ces effets en conservant un volume de bois mort plus important comparativement aux pratiques sylvicoles plus intensives telles que les coupes totales ou les brûlages dirigés. Plus précisément, le volume de bois mort au sol de diamètre moyen reste constant entre les coupes partielles et les peuplements non coupés. Concernant le bois mort au sol de grand diamètre, les volumes dans les deux coupes partielles sont plus bas que ceux retrouvés dans les peuplements non coupés mais plus haut que ceux restants après une coupe totale ou un brulage dirigé. Donc, les coupes partielles, plus proches de la succession naturelle de la forêt en absence de perturbation majeure, entrainent des modifications moindres dans les communautés fongiques saproxyliques. De plus, ces types de coupe présentent un avantage pour la décomposition du tremble dans la mesure où celle-ci est accélérée comparativement aux traitements sylvicoles intensifs mais aussi par rapport aux peuplements non aménagés pouvant accélérer le cycle des nutriments (chapitre 2).

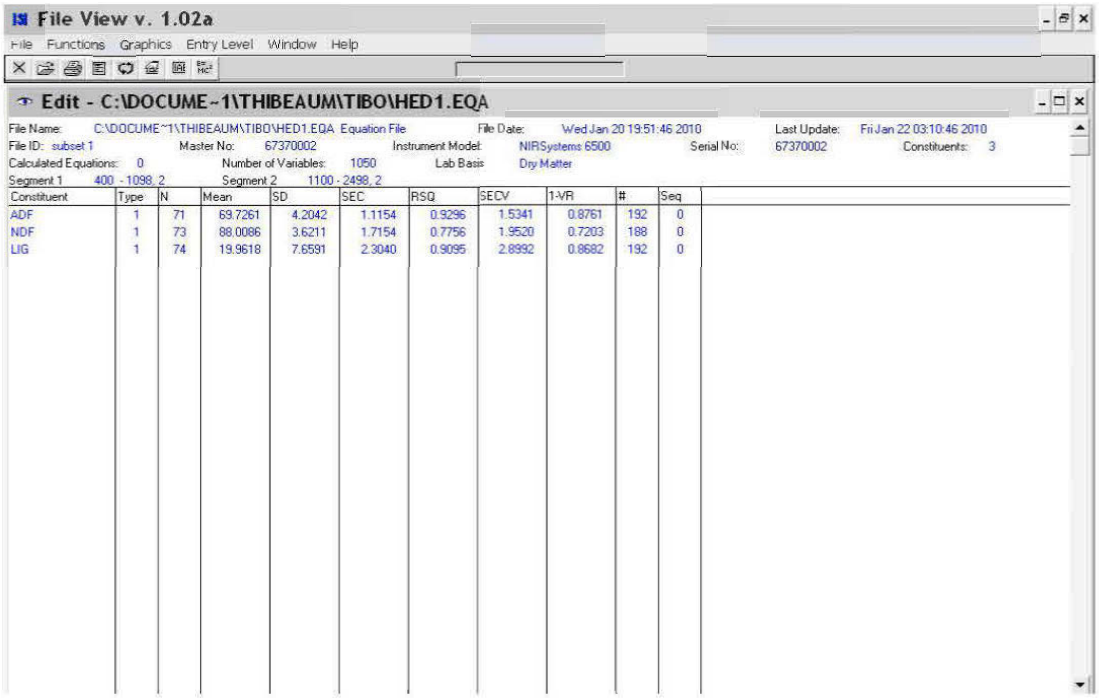
Espèces indicatrices

Malgré le fait que nous n'avons pas été en mesure de séquencer et d'identifier tous les OTUs, nous avons trouvé plusieurs espèces (ou OTUs) indicatrices comme l'OTU « G » (plus proche correspondance avec *Calocera cornea*), indicatrice des peuplements les plus jeunes (1923) et qui est habituellement retrouvée sur les billes les plus exposées (conditions typiques des peuplements naturels non aménagés les plus ouverts et les plus jeunes de notre étude). L'OTU « M » est caractéristique des billes de tremble en peuplements naturels; l'OTU « S » (plus proche correspondance avec le *Basidiomycota Athelia* sp.) est retrouvée préférentiellement sur les billes de sapin et l'OTU « G » (plus proche correspondance avec le Dacrymycete *Calocera cornea*) est indicatrice des peuplements de tremble issus du feu de 1923. Grâce au séquençage, il est possible de continuer l'identification des OTUs et donc d'identifier l'espèce de champignon. D'autre part, dans cette thèse certaines séquences

d'ADN les plus proches accessibles dans les bases de données ne correspondaient que faiblement à nos séquences. C'est le cas des OTUs 25 et 29 qui avaient un pourcentage de correspondance avec des séquences connus que de 86% et 83%. Il pourrait s'agir de nouvelles espèces fongiques inconnues à ce jour (pour lesquelles la séquence n'est pas disponible). Dans le chapitre 2, nous n'avons pas retrouvé d'espèces indicatrices, ce qui souligne encore que les champignons saproxyliques pionniers sont généralistes.

De futures études pourraient évaluer les assemblages des communautés fongiques saproxyliques en mesurant des variables sur des échelles plus petites. En effet, la composition de la communauté végétale de sous-bois pourrait influencer les communautés fongiques de façon plus importante que les variables du peuplement (De Bellis, Kernaghan & Widden, 2007). En effet, dans les brulis la régénération est très faible alors que dans les coupes totales au contraire la densité est très importante surtout en gaulis de tremble (observation personnelle).

ANNEXE A



Constituent	Type	N	Mean	SD	SEC	RSQ	SECV	1-VR	#	Seq
ADF	1	71	69.7261	4.2042	1.1154	0.9296	1.5341	0.8761	192	0
NDF	1	73	88.0086	3.6211	1.7154	0.7756	1.9520	0.7203	188	0
LIG	1	74	19.9618	7.6591	2.3040	0.9095	2.8992	0.8682	192	0

Sortie du logiciel (WinISI software) suite au développement des équations de calibration d'après les analyses chimiques (ADF;NDF;ADF-L) pour prédire la composition chimique des échantillons selon la méthode de régression PLS (« partial least square »).

	Analyse 1			Analyse 2			Coefficient de variation		
Echantillons	ADF	NDF	LIG	ADF	NDF	LIG	ADF	NDF	LIG
1A111S	73,909	87,728	31,252	72,706	87,002	30,182	1,160	0,588	2,464
1A112S	75,866	91,115	31,405	75,494	90,084	29,833	0,348	0,805	3,630
1A1131S	77,35	92,277	31,881	77,618	90,883	30,565	0,244	1,077	2,980
1A114S	77,68	92,914	31,464	77,516	91,222	30,008	0,150	1,300	3,349
1A121S	74,939	89,563	30,898	75,116	89,164	30,903	0,166	0,316	0,011
1A1231P	65,38	84,527	16,715	65,514	85,615	17,905	0,145	0,904	4,859
1A1231S	74,603	88,917	29,481	74,375	88,840	29,421	0,216	0,062	0,145
1A124P	68,613	88,826	14,129	68,601	88,131	13,398	0,013	0,555	3,758
1A124S	73,757	87,748	30,091	73,669	87,920	30,495	0,085	0,138	0,942
1A131P	65,149	85,666	15,069	65,827	85,679	14,883	0,732	0,010	0,879
1A131S	73,669	87,292	28,55	73,285	88,259	29,614	0,369	0,779	2,586
1A132P	67,686	87,738	16,346	67,472	87,114	15,379	0,224	0,505	4,309
1A132S	73,753	87,813	30,352	74,158	88,923	31,044	0,387	0,888	1,594
1A1331P	67,97	87,986	16,559	68,026	88,073	16,168	0,058	0,070	1,692
1A1331S	75,754	90,083	31,921	74,772	89,081	30,180	0,922	0,791	3,965
1A134P	67,223	86,952	16,203	67,987	87,558	16,242	0,799	0,491	0,171
1A134S	76,02	90,602	31,559	74,941	89,466	30,481	1,011	0,892	2,458
1A215P	68,356	88,66	16,111	68,522	88,397	14,961	0,171	0,210	5,235
1A225P	66,281	83,446	16,653	65,949	84,772	17,711	0,355	1,115	4,354
1A225S	73,758	87,012	30,832	73,395	87,837	30,827	0,349	0,667	0,013
1A235P	67,3	87,624	14,915	67,680	87,474	14,695	0,398	0,121	1,051
1A235S	73,014	87,765	28,58	73,321	88,377	29,009	0,296	0,491	1,052
1A3110P	67,153	88,471	14,837	66,830	88,280	13,589	0,341	0,153	6,208
1A3110P	67,025	88,257	14,914	67,379	88,655	14,526	0,372	0,318	1,864
1A3110S	76,424	90,938	30,974	76,233	90,798	30,728	0,177	0,109	0,563
1A317S	77,032	92,596	31,397	77,026	91,409	30,197	0,006	0,913	2,756
1A319S	71,953	81,066	33,727	70,194	83,861	35,191	1,750	2,397	3,005
1A3210P	66,002	85,121	15,773	66,608	85,889	16,326	0,646	0,635	2,434
1A327P	67,313	88,117	13,267	67,163	87,409	13,381	0,158	0,571	0,602
1A327S	75,521	89,989	30,552	74,024	88,551	28,706	1,415	1,139	4,406
1A329S	74,267	84,049	34,169	71,681	85,399	33,200	2,506	1,126	2,034
1A3310P	65,208	81,665	18,535	64,806	82,765	19,948	0,438	0,946	5,194
1A337S	76,35	91,021	31,374	76,395	90,186	30,696	0,042	0,652	1,546
1A339P	65,424	84,439	15,156	64,358	83,660	15,897	1,162	0,655	3,374
1A339S	73,974	88,113	29,313	73,815	88,108	29,528	0,152	0,004	0,516
1D111P	71,266	91,408	12,022	70,552	91,426	11,068	0,713	0,014	5,843
1D111S	75,208	89,358	30,402	75,925	90,219	29,946	0,671	0,678	1,070
1D113-1P	68,592	88,546	13,453	68,762	89,383	13,419	0,175	0,665	0,180
1D113-1S	74,077	88,248	30,045	74,399	89,117	29,756	0,307	0,693	0,683
1D114P	70,776	91,591	12,605	70,162	91,264	12,038	0,616	0,253	3,254
1D114S	74,063	88,5	29,346	74,310	89,168	28,743	0,235	0,532	1,468
1D121P	71,374	92,05	12,153	70,300	90,896	10,920	1,072	0,892	7,559

1D121S	75,636	89,848	30,677	75,810	90,107	30,170	0,162	0,204	1,178
1D122S	74,315	88,261	29,904	75,093	89,627	30,043	0,736	1,086	0,327
1D123-1P	68,542	88,413	13,335	68,333	88,871	13,410	0,216	0,366	0,394
1D123-1S	73,847	87,821	30,103	73,475	88,288	29,532	0,358	0,375	1,355
1D124P	70,956	91,356	12,399	70,435	91,379	11,976	0,521	0,018	2,457
1D124S	74,746	88,872	30,069	74,767	89,704	29,598	0,020	0,659	1,118
1D131S	74,965	88,67	30,677	75,207	88,820	29,391	0,227	0,120	3,028
1D132S	74,485	88,248	30,553	74,319	89,289	30,302	0,158	0,829	0,583
1D133-1S	74,808	88,682	30,626	74,966	89,098	30,406	0,149	0,331	0,510
1D134P	68,777	90,224	12,835	68,674	89,900	12,469	0,106	0,254	2,048
1D134S	74,191	87,938	30,718	74,601	89,366	30,650	0,389	1,139	0,158
1D215P	70,347	90,787	11,669	69,376	91,022	11,112	0,983	0,183	3,458
1D215S	75,455	89,563	30,355	75,550	90,177	30,083	0,089	0,483	0,638
1D225P	67,988	87,896	13,312	68,842	89,351	13,999	0,883	1,161	3,556
1D225S	74,495	88,52	29,242	74,090	89,197	29,047	0,386	0,539	0,473
1D235S	73,126	87,111	30,466	73,369	88,053	30,215	0,234	0,761	0,586
1D3110P	69,278	90,594	12,032	69,579	91,036	12,177	0,306	0,344	0,847
1D3110S	74,411	87,799	30,115	74,354	89,445	30,081	0,055	1,313	0,079
1D317P	70,209	91,721	11,862	69,885	90,833	11,191	0,327	0,688	4,116
1D319P	71,556	91,827	11,871	69,765	91,066	10,880	1,793	0,588	6,162
1D319S	74,683	88,882	30,157	75,002	89,703	29,705	0,301	0,650	1,069
1D3210P	69,932	90,648	12,912	68,923	90,124	12,001	1,028	0,410	5,170
1D3210S	74,331	88,242	29,604	74,826	89,246	29,696	0,469	0,800	0,220
1D327P	72,043	92,768	11,606	70,538	91,443	9,988	1,493	1,017	10,595
1D327S	74,249	88,309	28,74	74,107	89,253	29,233	0,136	0,752	1,201
1D329P	70,757	90,935	12,524	69,787	91,284	12,425	0,976	0,271	0,563
1D329S	73,705	87,193	30,68	73,640	88,490	30,642	0,063	1,044	0,088
1D3310S	74,231	88,322	29,962	74,791	89,339	29,828	0,531	0,809	0,318
1D337P	70,528	90,39	13,095	72,697	92,586	14,010	2,141	1,697	4,775
1D337S	74,975	88,887	30,196	75,290	89,818	30,092	0,297	0,737	0,245
1D339P	70,704	91,25	12,005	69,493	90,890	11,157	1,221	0,280	5,181
1D339S	74,769	87,712	31,613	74,816	88,731	30,761	0,044	0,816	1,933
1M111P	68,801	89,523	14,765	70,086	89,933	13,843	1,308	0,323	4,559
1M111S	74,201	88,78	30,044	74,671	89,394	29,881	0,447	0,487	0,386
1M112S	76,91	92,068	31,612	76,302	90,711	30,041	0,561	1,050	3,605
1M114P	64,935	82,692	18,8	65,821	84,131	20,144	0,958	1,220	4,881
1M114S	77,65	93,436	30,926	77,676	91,903	29,656	0,023	1,170	2,965
1M121P	68,804	90,048	14,728	69,783	92,240	15,263	0,999	1,701	2,522
1M121S	74,851	89,917	31,131	74,633	89,249	30,133	0,206	0,527	2,305
1M122P	65,16	85,462	15,004	65,766	85,368	15,075	0,655	0,078	0,334
1M122S	73,312	87,553	28,533	73,685	88,624	28,887	0,359	0,860	0,871
1M1231P	65,844	86,291	15,519	66,693	87,698	15,128	0,906	1,144	1,803
1M1231S	76,166	91,108	29,969	76,583	90,642	29,693	0,386	0,362	0,655
1M124P	68,215	88,872	14,237	68,527	88,476	13,646	0,322	0,316	2,996

1M124S	75,263	90,021	30,909	75,333	90,186	30,496	0,066	0,129	0,951
1M131S	74,335	88,575	29,401	74,736	89,648	30,112	0,381	0,851	1,688
1M132P	65,388	84,961	16,504	65,839	84,370	16,512	0,486	0,493	0,032
1M132S	75,799	91,168	31,431	76,218	90,627	30,651	0,390	0,421	1,776
1M1331P	66,842	86,48	15,873	66,210	85,810	14,947	0,671	0,550	4,250
1M1331S	75,308	90,448	30,543	76,117	90,933	30,567	0,755	0,378	0,055
1M134P	67,354	87,276	15,812	68,131	87,527	15,452	0,811	0,203	1,627
1M134S	75,601	90,833	31,171	75,104	89,770	29,801	0,467	0,833	3,179
1M215S	76,123	91,036	30,947	75,926	90,423	29,681	0,183	0,477	2,953
1M235P	68,397	88,785	14,945	69,356	88,803	14,514	0,985	0,014	2,070
1M235S	73,475	88,623	30,332	73,616	88,435	29,559	0,136	0,150	1,825
1M3110P	66,775	88,25	14,528	68,982	89,746	14,189	2,299	1,189	1,671
1M317P	66,646	87,521	13,577	67,176	86,922	13,550	0,560	0,486	0,143
1M317S	77,381	92,591	30,983	77,383	91,811	30,279	0,002	0,598	1,625
1M319S	76,888	92,401	30,551	77,080	91,371	29,499	0,176	0,793	2,478
1M3210P	66,946	87,571	13,954	68,358	88,121	13,889	1,476	0,443	0,333
1M3210S	71,198	78,831	36,324	69,585	83,834	38,909	1,620	4,350	4,858
1M327P	69,207	89,654	12,482	69,031	88,950	12,095	0,180	0,558	2,230
1M327S	77,514	92,517	30,235	77,325	91,820	29,006	0,173	0,535	2,933
1M329P	66,958	88,265	14,413	68,619	89,019	14,013	1,732	0,601	1,991
1M329S	75,297	90,421	29,886	75,836	90,265	29,213	0,504	0,122	1,611
1M3310S	75,583	90,422	29,621	75,630	90,499	28,858	0,044	0,060	1,845
1M337P	68,483	89,515	15,095	69,983	91,580	15,292	1,532	1,613	0,915
1M337S	76,994	92,066	30,592	76,740	91,581	29,719	0,234	0,373	2,048
1M339P	67,557	88,456	13,57	68,033	87,907	13,223	0,496	0,440	1,832
1M339S	74,794	89,294	29,742	74,887	89,860	29,457	0,088	0,447	0,681
1P111P	63,202	80,215	19,333	62,860	81,919	20,760	0,384	1,487	5,034
1P111S	73,888	88,261	29,619	73,476	88,120	29,773	0,396	0,113	0,367
1P112S	70,698	83,676	30,031	70,566	84,649	30,604	0,132	0,817	1,337
1P121S	73,875	87,792	28,375	73,803	87,867	28,724	0,069	0,060	0,865
1P122P	68,121	89,682	15,108	68,118	91,087	16,483	0,003	1,099	6,156
1P123-1S	73,955	88,113	30,131	73,876	88,023	29,930	0,076	0,072	0,473
1P124S	75,125	89,514	28,429	74,612	89,748	28,214	0,485	0,185	0,538
1P131P	62,943	80,418	20,099	63,735	81,603	21,384	0,884	1,035	4,381
1P131S	72,853	86,865	28,244	72,906	87,649	29,029	0,051	0,635	1,939
1P132S	76,18	90,514	32,122	76,517	90,428	31,836	0,312	0,067	0,632
1P133-1P	66,271	84,737	18,48	66,346	85,206	18,977	0,080	0,390	1,876
1P133-1S	74,13	88,442	29,503	74,825	88,806	29,798	0,660	0,291	0,703
1P134P	66,352	86,202	16,06	65,999	84,676	14,970	0,377	1,263	4,970
1P134S	74,077	87,122	31,005	73,674	87,895	31,239	0,386	0,625	0,531
1P215S	65,467	84,599	16,888	65,919	84,040	17,202	0,486	0,469	1,301
1P225P	76,622	91,915	31,535	75,965	90,465	30,043	0,609	1,124	3,426
1P225S	66,002	85,637	15,458	65,373	84,276	15,174	0,678	1,133	1,313
1P235P	66,152	80,785	18,755	65,703	85,205	20,396	0,482	3,766	5,927

1P235S	74,527	88,529	30,111	74,814	88,466	30,545	0,271	0,051	1,011
1P3110S	73,996	88,22	28,782	73,768	88,424	29,242	0,219	0,163	1,120
1P319P	67,592	88,874	15,482	67,902	89,761	15,738	0,323	0,702	1,157
1P319S	74,374	88,585	29,368	74,122	88,812	29,354	0,240	0,181	0,033
1P3210S	74,343	87,825	30,751	73,720	88,605	30,572	0,595	0,625	0,413
1P327S	75,588	90,587	29,385	75,371	90,186	28,972	0,203	0,313	1,002
1P329P	66,517	87,071	15,806	67,227	87,696	16,188	0,751	0,505	1,689
1P3310P	64,962	85,083	16,022	65,448	85,031	16,461	0,526	0,044	1,911
1P3310S	73,754	87,763	28,611	73,875	88,596	29,151	0,115	0,668	1,322
1P337S	73,947	88,068	28,297	74,098	88,223	28,323	0,144	0,124	0,066
3A111S	74,663	89,469	30,774	73,830	88,471	28,900	0,793	0,793	4,442
3A112S	73,456	87,563	28,893	72,706	87,986	28,830	0,725	0,341	0,156
3A114P	68,424	88,65	15,713	68,280	90,379	18,396	0,149	1,366	11,124
3A114S	76,559	91,416	31,241	76,998	90,739	30,382	0,404	0,525	1,973
3A121S	74,229	88,597	29,729	73,013	88,007	28,813	1,168	0,472	2,213
3A122S	76,01	90,256	31,996	76,290	90,271	31,762	0,260	0,011	0,518
3A1231P	65,527	83,933	17,059	66,676	84,716	18,513	1,229	0,657	5,780
3A1231S	74,084	88,54	30,832	73,073	87,415	29,561	0,972	0,904	2,976
3A124S	67,878	88,998	15,105	68,689	90,329	15,476	0,839	1,050	1,715
3A131P	64,767	83,084	17,977	66,071	84,728	19,049	1,409	1,385	4,094
3A131P	67,269	88,424	14,551	67,618	88,963	14,323	0,366	0,429	1,117
3A131S	73,669	87,71	30,252	73,965	88,502	30,921	0,284	0,636	1,547
3A132S	76,161	90,52	31,824	76,100	90,394	31,415	0,056	0,099	0,915
3A1331P	67,361	85,274	16,109	66,249	86,203	16,242	1,177	0,766	0,581
3A1331S	74,973	89,11	32,014	74,094	88,360	31,137	0,834	0,597	1,963
3A134P	67,193	85,594	16,131	66,877	86,703	16,559	0,334	0,911	1,851
3A134S	71,239	77,174	37,175	69,558	83,193	40,298	1,689	5,308	5,701
3A215P	66,518	86,615	16,119	67,166	86,226	15,893	0,686	0,318	0,997
3A225P	66,679	85,815	18,261	67,388	86,884	18,713	0,748	0,876	1,728
3A225S	76,921	91,848	30,822	76,606	90,856	30,069	0,290	0,768	1,750
3A235P	66,314	84,732	18,412	65,452	84,305	17,585	0,925	0,357	3,248
3A3110S	73,078	87,272	29,299	73,398	87,966	29,589	0,309	0,560	0,695
3A317S	77,19	92,012	32,66	77,341	91,341	31,406	0,138	0,518	2,769
3A319S	75,69	90,275	30,361	76,159	90,254	30,315	0,436	0,016	0,108
3A329S	77,385	92,15	32,995	75,034	90,249	30,453	1,245	1,474	5,665
3A3310S	73,971	88,044	29,623	73,458	88,641	29,509	0,492	0,478	0,272
3A337P	66,206	85,931	13,331	65,905	86,033	14,247	0,322	0,084	4,699
3A337S	73,803	87,462	29,327	73,505	87,842	29,808	0,286	0,306	1,150
3A339S	74,371	88,443	30,091	73,720	88,740	29,843	0,621	0,237	0,586
3D111S	74,048	87,845	29,56	73,869	88,858	29,742	0,171	0,811	0,434
3D112S	73,978	88	30,463	74,400	89,094	30,352	0,402	0,873	0,258
3D113-1P	69,642	90,202	12,498	69,038	89,783	12,327	0,616	0,329	0,977
3D113-1S	74,091	87,538	30,535	74,108	88,193	30,795	0,016	0,527	0,600
3D114P	70,401	91,111	13,534	70,450	91,473	12,747	0,049	0,281	4,238

3D114S	75,006	89,061	30,066	75,168	89,732	29,871	0,152	0,531	0,461
3D121P	69,134	89,7	12,531	69,274	90,228	13,103	0,143	0,415	3,153
3D121S	74,46	88,892	29,628	74,349	89,169	29,346	0,106	0,220	0,677
3D122P	70,317	90,592	13,379	70,493	90,570	12,914	0,177	0,017	2,500
3D122S	74,295	88,212	30,065	74,660	89,012	30,165	0,346	0,639	0,234
3D123-1P	68,785	88,78	13,26	69,295	89,766	13,385	0,523	0,781	0,661
3D124P	71,479	92,158	12,698	70,929	91,126	12,001	0,546	0,796	3,992
3D124S	74,005	88,073	29,307	74,754	89,589	29,510	0,712	1,207	0,489
3D131P	69,589	90,659	12,873	69,307	90,351	12,383	0,288	0,240	2,742
3D131S	74,376	88,443	30,413	74,465	89,120	29,882	0,085	0,539	1,245
3D132S	75,62	89,541	30,971	76,031	90,245	30,444	0,383	0,554	1,215
3D133-1S	73,274	87,389	28,999	73,233	87,952	28,811	0,040	0,454	0,461
3D134P	70,549	91,142	12,749	70,020	90,916	11,753	0,532	0,176	5,752
3D134S	75,298	89,701	30,678	75,400	89,978	30,324	0,096	0,218	0,821
3D215P	70,955	91,741	12,055	69,714	90,849	10,772	1,248	0,691	7,950
3D215S	74,492	88,287	30,643	74,680	89,015	30,242	0,178	0,581	0,932
3D225P	71,93	92,48	12,804	70,889	91,939	11,993	1,031	0,415	4,624
3D225S	74,132	88,304	29,833	74,783	89,777	29,884	0,618	1,170	0,121
3D235S	75,976	89,917	30,332	76,129	90,349	30,208	0,142	0,339	0,289
3D3110P	69,811	89,873	12,564	69,809	90,879	13,109	0,002	0,787	3,002
3D3110S	74,313	88,352	29,801	74,316	89,189	29,643	0,002	0,667	0,377
3D317P	70,063	89,912	12,933	70,869	91,510	13,646	0,809	1,245	3,792
3D317S	75,102	89,357	30,551	74,994	89,582	29,624	0,102	0,178	2,180
3D319P	69,705	91,14	11,892	69,063	90,428	11,209	0,655	0,554	4,183
3D319S	73,947	88,014	29,229	74,672	89,709	29,693	0,690	1,349	1,112
3D327P	70,196	90,453	12,279	69,181	90,921	12,091	1,030	0,365	1,094
3D327S	74,002	87,888	30,103	74,128	89,119	30,176	0,121	0,984	0,171
3D329P	69,769	89,921	13,165	68,890	90,223	12,918	0,896	0,237	1,339
3D329S	74,048	87,918	30,436	73,926	88,752	30,678	0,117	0,667	0,559
3D3310P	71,384	91,539	11,592	70,561	91,802	11,038	0,820	0,203	3,464
3D3310S	74,863	88,753	29,8	74,937	89,825	29,466	0,070	0,849	0,796
3D337P	71,512	92,569	12,206	70,908	92,237	11,201	0,600	0,254	6,074
3D337S	74,075	88,013	28,658	73,954	89,238	28,739	0,116	0,978	0,200
3D339P	70,136	90,884	12,439	69,715	90,734	12,241	0,426	0,117	1,136
3D339S	74,764	88,608	29,743	74,718	89,491	29,737	0,044	0,701	0,014
3M111P	65,448	84,084	17,945	66,734	86,067	19,644	1,376	1,648	6,391
3M111S	76,657	92,031	30,852	76,271	90,793	29,916	0,357	0,958	2,178
3M112P	65,585	84,659	14,971	65,681	85,652	16,377	0,103	0,824	6,342
3M112S	74,795	89,37	29,583	74,697	89,107	29,639	0,093	0,208	0,133
3M1131P	68,056	88,117	15,777	68,049	88,319	15,402	0,007	0,162	1,701
3M1131S	76,812	91,81	31,291	76,745	91,220	30,870	0,062	0,456	0,957
3M121P	69,561	90,21	14,385	69,669	90,265	13,249	0,109	0,043	5,815
3M121S	73,703	88,678	29,137	74,173	89,220	29,509	0,449	0,431	0,896
3M122P	69,363	89,390	16,018	70,030	89,711	15,188	0,677	0,254	3,761

3M1225	76,4	91,234	30,98	75,899	90,297	30,839	0,465	0,730	0,324
3M1231P	67,219	87,232	15,335	66,980	86,710	15,027	0,252	0,424	1,433
3M1231S	75,691	90,178	31,328	76,406	90,103	31,363	0,664	0,059	0,078
3M124P	68,298	89,421	13,503	68,908	89,249	13,050	0,629	0,136	2,411
3M124S	76,818	91,826	31,33	76,777	90,841	30,458	0,038	0,762	1,996
3M131P	67,027	86,325	16,48	67,915	87,006	15,605	0,930	0,556	3,857
3M131S	76,546	91,790	31,423	76,636	90,833	30,756	0,083	0,741	1,517
3M132P	67,153	87,051	15,511	67,143	87,271	15,273	0,011	0,179	1,093
3M132S	76,69	91,483	31,613	76,860	91,071	31,338	0,156	0,319	0,618
3M1331S	76,326	91,171	31,749	76,556	90,803	31,273	0,213	0,286	1,069
3M134P	68,094	86,869	15,187	67,984	87,546	15,380	0,115	0,549	0,891
3M134S	76,827	91,898	31,494	76,309	90,159	30,061	0,479	1,351	3,292
3M215P	67,591	87,372	16,42	67,460	87,206	16,224	0,137	0,134	0,850
3M215S	77,241	92,527	31,11	77,257	91,192	30,009	0,015	1,027	2,547
3M225P	68,747	89,743	13,85	68,647	88,886	12,737	0,103	0,679	5,919
3M225S	75,672	90,654	30,649	75,291	89,858	29,865	0,357	0,624	1,833
3M235P	67,17	86,824	16,284	66,981	86,072	15,813	0,199	0,615	2,076
3M235S	75,003	89,313	30,796	75,302	89,681	30,591	0,281	0,291	0,472
3M3110P	66,431	87,05	13,85	67,793	86,800	14,670	1,435	0,203	4,067
3M3110S	75,517	90,617	29,814	76,000	90,724	29,422	0,450	0,083	0,937
3M317P	68,604	90,283	14,459	70,411	90,848	13,545	1,839	0,441	4,614
3M317S	77,367	92,846	31,279	77,247	91,626	30,237	0,110	0,935	2,397
3M319S	76,125	91,276	31,642	76,246	90,738	30,514	0,112	0,418	2,566
3M3210S	74,499	88,781	28,633	73,818	88,600	28,524	0,649	0,145	0,270
3M327S	77,564	92,704	30,826	76,925	91,704	29,853	0,585	0,767	2,269
3M329P	66,869	87,54	16,088	68,361	89,314	16,850	1,560	1,418	3,272
3M329S	76,826	92,081	31,003	76,910	91,729	29,746	0,077	0,271	2,926
3M3310P	65,135	85,653	15,116	66,691	86,252	15,245	1,669	0,493	0,601
3M3310S	74,847	89,139	29,372	74,896	89,925	29,631	0,047	0,621	0,621
3M337S	74,373	88,687	29,762	74,772	89,495	29,996	0,379	0,641	0,553
3P111S	75,25	90,237	30,318	75,355	89,800	29,816	0,098	0,343	1,182
3P113-1S	74,619	88,837	30,171	74,677	89,276	29,941	0,054	0,349	0,542
3P114S	77,441	92,174	31,315	77,455	91,337	30,430	0,013	0,645	2,026
3P121S	73,129	88,148	28,321	72,863	87,782	28,299	0,258	0,294	0,056
3P122P	67,516	87,423	15,868	67,352	87,145	15,084	0,172	0,225	3,582
3P122S	75,927	90,35	32,616	75,612	89,096	31,695	0,294	0,989	2,025
3P123-1P	65,438	85,107	15,763	66,125	86,314	17,101	0,738	0,995	5,759
3P123-1S	73,944	88,242	29,482	73,822	88,349	29,856	0,117	0,086	0,892
3P132S	75,113	89,683	31,505	75,180	89,165	30,471	0,063	0,410	2,359
3P133-1P	65,592	80,96	19,172	65,647	85,398	21,246	0,059	3,773	7,255
3P134P	74,272	88,956	30,326	73,598	88,685	29,300	0,645	0,216	2,433
3P215P	74,197	88,118	28,649	73,680	88,162	28,639	0,494	0,035	0,026
3P225P	65,634	85,418	16,012	66,259	85,680	16,052	0,670	0,216	0,174
3P225S	70,53	77,86	36,638	68,696	83,055	39,002	1,863	4,566	4,419

3P235P	74,719	89,152	29,881	74,362	89,331	29,836	0,339	0,142	0,108
3P235S	74,147	88,675	28,933	73,653	88,142	28,567	0,472	0,426	0,901
3P3110S	73,747	87,969	28,909	73,685	88,440	29,428	0,059	0,378	1,257
3P317P	68,085	90,007	14,032	69,236	90,569	13,036	1,185	0,440	5,205
3P317S	76,73	91,994	31,344	77,855	91,523	30,938	1,029	0,363	0,922
3P319P	77,561	93,013	31,344	76,890	90,975	29,886	0,615	1,566	3,368
3P319S	75,648	90,541	30,857	76,424	90,432	30,523	0,721	0,085	0,770
3P327S	74,439	89,014	29,159	74,856	89,401	29,236	0,395	0,307	0,186
3P329S	74,672	88,817	29,997	74,674	88,799	30,275	0,002	0,014	0,652
3P3310P	63,824	81,879	17,461	62,603	81,713	18,511	1,366	0,144	4,128
3P337P	65,565	84,879	13,798	65,497	84,732	14,909	0,073	0,123	5,472
3P337S	74,277	88,606	28,66	73,740	88,550	28,259	0,513	0,045	0,998
B1	67,895	88,554	13,871	67,357	87,367	12,955	0,563	0,954	4,829
B10	67,841	84,106	20,951	65,914	82,863	17,884	2,037	1,053	11,171
B100	73,488	85,681	30,409	71,673	84,217	29,566	1,769	1,218	1,987
B101	66,822	87,604	15,284	67,504	87,071	14,628	0,718	0,431	3,102
B102	73,224	85,191	34,254	71,631	84,790	33,149	1,555	0,334	2,319
B103	63,85	70,324	35,581	59,703	71,632	36,159	4,747	1,303	1,138
B104	69,214	79,156	29,606	67,643	78,760	30,625	1,624	0,355	2,393
B105	66,65	79,069	22,462	64,933	84,068	23,247	1,846	4,334	2,429
B106	73,945	87,538	28,334	71,858	87,078	27,106	2,024	0,373	3,134
B107	65,719	81,764	25,175	65,145	81,434	26,939	0,621	0,286	4,787
B108	68,444	70,688	43,008	64,208	78,393	45,259	4,516	7,309	3,606
B109	72,733	84,355	34,072	71,031	83,387	33,117	1,675	0,816	2,010
B11	67,774	78,02	32,227	66,340	77,848	29,994	1,512	0,156	5,077
B110	75,734	85,477	36,305	73,846	87,016	35,700	1,786	1,262	1,188
B111	71,471	75,114	38,799	69,066	83,249	41,172	2,421	7,265	4,196
B112	74,342	86,511	34,034	73,263	86,149	33,222	1,034	0,297	1,707
B113	65,831	85,208	17,518	65,357	84,088	15,664	0,511	0,936	7,901
B114	70,06	82,011	32,273	68,251	82,009	32,028	1,850	0,002	0,539
B115	73,616	86,519	33,537	72,094	85,347	32,469	1,477	0,965	2,289
B116	65,225	80,655	21,531	62,643	78,632	20,054	2,856	1,796	5,025
B117	69,685	70,85	42,36	66,260	79,777	45,810	3,563	8,381	5,533
B118	69,521	78,699	34,711	66,745	78,234	34,459	2,881	0,419	0,515
B119	67,889	69,68	43,666	64,828	78,498	46,570	3,262	8,416	4,552
B12	68,728	88,818	13,814	67,661	88,027	12,700	1,106	0,633	5,941
B120	69,538	81,468	28,922	67,699	79,527	28,582	1,895	1,705	0,836
B121	72,102	82,118	34,314	70,173	82,457	34,150	1,917	0,291	0,338
B122	71,776	81,496	33,526	71,357	85,254	33,304	0,414	3,187	0,470
B123	66,47	66,547	43,076	63,297	75,911	46,623	3,458	9,296	5,592
B124	77,075	90,852	33,723	75,884	89,575	32,429	1,101	1,001	2,766
B125	70,541	78,872	34,454	67,401	81,409	34,838	3,220	2,239	0,783
B126	74,224	85,618	32,949	71,356	84,923	31,853	2,786	0,577	2,393
B127	68,232	86,742	11,661	65,572	84,958	10,542	2,811	1,469	7,127

B128	76,847	92,029	29,178	75,203	90,193	26,275	1,530	1,425	7,405
B129	72,513	86,472	30,194	71,044	86,055	27,553	1,447	0,342	6,467
B13	67,948	87,999	14,726	68,566	88,562	14,948	0,640	0,451	1,059
B130	65,878	77,265	21,64	65,447	85,460	24,804	0,464	7,122	9,633
B131	73,184	87,027	28,885	71,357	85,599	28,227	1,788	1,170	1,630
B132	74,37	88,75	28,034	73,057	87,106	26,548	1,260	1,322	3,850
B133	76,476	92,194	27,406	75,149	90,677	24,620	1,238	1,173	7,575
B134	64,282	75,359	23,581	61,653	82,563	26,799	2,953	6,451	9,034
B135	71,261	91,59	12,952	69,874	91,167	11,298	1,390	0,327	9,646
B136	73,095	87,291	27,545	71,077	85,561	26,324	1,979	1,416	3,207
B137	68,411	87,245	14,998	67,289	85,886	13,547	1,170	1,110	7,190
B138	74,337	85,617	33,508	73,600	86,254	32,013	0,705	0,524	3,228
B139	61,128	52,251	56,314	55,767	68,050	61,888	6,486	18,572	6,669
B14	70,269	90,973	12,183	68,873	89,203	10,507	1,419	1,390	10,448
B140	72,786	85,792	30,827	70,635	84,996	29,205	2,121	0,659	3,821
B141	71,159	90,108	11,472	67,518	89,134	8,875	3,713	0,769	18,048
B142	73,442	87,976	26,226	71,996	87,847	25,207	1,406	0,104	2,802
B143	74,935	88,453	30,633	73,136	87,776	28,969	1,718	0,543	3,948
B144	74,51	85,841	34,025	73,370	86,660	33,246	1,090	0,671	1,637
B145	73,355	89,582	17,543	70,640	90,374	15,027	2,666	0,622	10,925
B146	70,155	89,843	16,11	69,981	90,291	14,676	0,176	0,351	6,590
B147	75,547	89,236	33,288	73,691	87,648	30,570	1,759	1,269	6,020
B148	58,778	70,944	28,736	48,851	62,530	27,513	13,045	8,915	3,076
B149	64,244	60,071	53,309	59,218	70,784	57,358	5,757	11,578	5,175
B15	65,589	82,026	21,841	65,417	81,608	22,588	0,186	0,362	2,378
B150	76,855	91,253	30,017	74,657	88,932	27,275	2,052	1,821	6,768
B151	74,051	88,644	28,854	72,578	86,876	27,405	1,420	1,425	3,643
B152	71,983	83,971	31,587	70,280	82,760	31,120	1,693	1,027	1,053
B153	72,112	83,72	31,612	69,653	81,901	30,820	2,453	1,554	1,793
B154	78,581	93,26	30,127	76,605	91,160	26,539	1,801	1,611	8,956
B155	66,623	64,108	47,803	61,154	73,059	50,754	6,053	9,228	4,235
B156	64,663	79,661	23,988	63,419	75,195	23,539	1,374	4,078	1,338
B157	75,442	88,823	29,456	72,878	87,186	27,696	2,445	1,315	4,354
B158	66,46	70,394	39,249	63,439	73,803	42,696	3,289	3,343	5,948
B159	68,244	87,436	15,15	66,769	87,357	15,333	1,545	0,064	0,849
B16	69,392	89,354	13,124	68,673	89,096	11,581	0,736	0,205	8,834
B160	69,869	88,137	13,698	67,371	86,139	11,353	2,574	1,621	13,238
B161	66,797	84,857	18,672	64,483	82,160	16,515	2,493	2,283	8,670
B162	73,752	87,161	29,435	71,896	85,316	28,429	1,802	1,513	2,458
B163	68,924	88,318	12,839	67,095	88,176	12,292	1,901	0,114	3,077
B164	73,239	86,986	29,534	71,968	86,213	28,378	1,238	0,631	2,822
B165	73,053	86,171	30,931	71,912	85,177	29,616	1,114	0,821	3,071
B166	74,561	87,125	31,43	73,118	86,395	30,610	1,382	0,595	1,870
B167	74,379	88,318	31,461	73,655	86,218	29,163	0,692	1,702	5,361

B168	74,025	87,671	32,219	72,849	85,920	30,842	1,132	1,427	3,089
B169	68,555	86,817	18,14	67,215	86,925	17,539	1,396	0,088	2,384
B17	67,301	83,493	20,841	63,613	82,942	22,048	3,985	0,468	3,980
B170	71,521	83,421	31,437	69,634	82,634	29,940	1,891	0,670	3,449
B171	68,018	72,439	38,697	64,862	77,543	39,465	3,359	4,812	1,390
B172	68,873	75,701	36,558	66,166	77,824	37,306	2,835	1,956	1,432
B173	69,671	80,514	33,688	68,692	81,086	31,563	1,001	0,501	4,605
B174	74,089	86,506	32,601	72,270	85,371	30,195	1,758	0,934	5,418
B175	73,579	87,013	30,436	72,319	86,092	28,313	1,222	0,752	5,112
B18	71,283	78,611	36,204	68,948	82,846	34,689	2,355	3,710	3,023
B19	70,158	79,866	32,43	68,157	80,148	32,689	2,046	0,249	0,561
B2	71,444	81,565	34,278	69,280	81,652	32,973	2,175	0,075	2,745
B20	71,321	80,05	35,078	70,500	84,204	34,628	0,819	3,577	0,913
B21	73,797	88,602	29,04	73,215	88,451	27,247	0,560	0,121	4,506
B22	67,068	73,311	36,602	61,839	73,670	37,568	5,736	0,345	1,842
B23	68,436	87,721	15,213	67,083	87,288	14,673	1,412	0,350	2,558
B24	66,48	85,438	20,299	65,138	81,926	18,062	1,442	2,968	8,248
B25	67,752	88,781	14,392	67,517	87,978	12,760	0,245	0,642	8,502
B26	73,736	88,606	27,671	72,660	87,102	26,045	1,039	1,211	4,280
B27	72,924	87,08	28,137	70,903	84,057	26,986	1,987	2,498	2,953
B28	68,541	88,115	15,903	68,248	89,256	16,569	0,303	0,910	2,902
B29	71,708	85,41	30,284	69,138	82,264	29,268	2,580	2,654	2,413
B3	60,808	64,082	39,149	56,992	67,130	40,275	4,582	3,286	2,005
B30	67,562	86,158	17,407	66,597	86,217	16,153	1,017	0,048	5,287
B31	64,259	73,896	32,553	64,088	74,841	29,545	0,188	0,899	6,850
B32	61,242	70,112	32,513	60,259	70,975	30,834	1,145	0,865	3,748
B33	69,341	75,835	36,48	64,674	79,537	37,466	4,925	3,370	1,886
B34	69,944	87,902	17,351	68,578	87,957	17,181	1,395	0,044	0,696
B35	62,205	54,165	54,776	56,148	69,549	59,735	7,238	17,586	6,124
B36	67,914	89,409	10,37	67,655	89,457	8,675	0,270	0,038	12,584
B37	62,465	76,626	23,011	59,126	74,288	23,025	3,884	2,191	0,042
B38	70,359	79,685	33,494	69,838	83,851	33,245	0,526	3,603	0,528
B39	69,947	81,129	32,007	68,042	81,300	31,528	1,952	0,149	1,066
B4	72,138	85,782	30,386	71,012	85,245	29,146	1,112	0,444	2,947
B40	68,077	69,347	44,729	65,518	79,555	47,714	2,709	9,695	4,567
B41	67,924	72,641	40,201	66,646	80,781	41,523	1,343	7,503	2,288
B42	67,956	86,755	17,558	67,186	86,064	16,664	0,806	0,566	3,695
B43	68,739	88,199	14,823	68,381	88,765	14,673	0,369	0,453	0,722
B44	70,593	83,304	31,119	69,002	83,743	29,898	1,612	0,372	2,829
B45	71,897	82,31	33,032	69,710	84,440	33,457	2,184	1,807	0,904
B46	70,216	80,129	35,199	68,272	81,005	34,446	1,985	0,769	1,529
B47	69,281	89,887	14,821	70,071	89,830	13,364	0,801	0,045	7,311
B48	63,999	71,292	28,027	62,865	81,558	31,214	1,265	9,498	7,607
B49	69,443	88,458	14,756	69,154	89,560	13,931	0,295	0,875	4,070

B5	72,214	85,4	31,179	70,100	84,246	30,243	2,101	0,962	2,155
B50	62,951	79,28	24,627	60,523	75,842	23,803	2,781	3,134	2,406
B51	71,736	81,221	32,374	71,481	85,090	31,862	0,252	3,290	1,128
B52	65,654	81,955	25,326	63,324	80,690	25,416	2,555	1,100	0,251
B53	68,633	76,809	34,104	65,367	76,425	34,548	3,447	0,354	0,914
B54	61,225	66,128	31,403	61,628	80,520	36,880	0,463	13,879	11,343
B55	71,036	91,933	12,632	69,578	90,501	10,861	1,466	1,110	10,664
B56	70,009	90,004	14,193	68,114	87,130	11,937	1,940	2,295	12,213
B57	65,095	81,835	21,000	63,573	78,411	19,258	1,673	3,021	6,121
B58	70,547	77,517	34,512	65,829	79,553	35,617	4,892	1,833	2,227
B59	61,044	70,425	35,28	54,139	62,005	32,025	8,478	8,992	6,839
B6	73,479	87,343	30,23	72,245	86,361	28,924	1,198	0,800	3,122
B60	68,137	86,535	19,697	66,656	83,008	17,487	1,554	2,942	8,404
B61	70,242	84,956	18,493	66,304	85,151	17,106	4,078	0,162	5,510
B62	66,125	85,898	15,401	65,130	85,204	14,212	1,072	0,574	5,677
B63	67,822	86,355	17,74	66,596	84,629	16,992	1,290	1,428	3,045
B64	66, 105	8 1,44 3	26,397	64,395	80,655	26,814	1,306	0,688	1,107
B65	67,6	85,238	20,271	65,436	83,785	19,431	2, 247	1,216	2,810
B66	68,301	75,946	34,321	65,322	77,402	35,250	2,614	1,342	1,888
B67	74,606	88,031	30,652	73, 039	87,520	29,034	1,501	0,412	3,834
B68	71,137	91,284	13,893	70,015	90,510	11,689	1, 125	0,602	12,134
B69	6S,143	82,074	24, 199	59,482	74,575	24,946	9,597	6,770	2, 149
B7	71,687	91,052	14,703	69,926	90,191	12,406	1,758	0,695	11,983
B70	71,938	82,308	31,789	70,680	84,963	31, 057	1,247	2,249	1,647
B71	73,645	35,197	31,629	71,953	35,120	31,230	1,643	0,064	0,S98
B72	67,194	37,541	15,525	67,647	36, 296	13,001	0,475	1,013	12,516
B73	72,356	36,341	28,933	71,191	85,231	27, 992	1,148	0,873	2,337
B74	74,550	85,331	35,374	72,693	86,373	34,800	1,784	0,863	1,157
B75	77,983	90,65	35,652	77,200	90,963	34,265	0,714	O, 243	2,805
B76	68, 593	79,003	33,4b2	66,070	77, 195	33,675	2,650	1,637	0,469
B77	61,329	68,612	34,17	59, 199	69,354	32,832	2,500	0,760	2,825
B78	66,651	77,379	31,721	65,324	77,924	30,502	1,422	0,496	2,772
B79	72,884	36,100	23,517	71,078	85,877	27,73 1	1,774	0,184	1,350
B8	70,827	90,52 2	13,208	69,247	90, 142	12,143	1,595	0,297	5,915
B80	63,400	62,508	45,796	60,505	73,885	43, 185	3,305	11,796	3,595
B81	72,826	36,249	28,939	70,485	34,236	27,898	2,310	1,670	2, 590
B82	65,635	74,498	32,519	63,473	74,433	31,1/1	2,368	0,062	2,994
B83	71,239	79,863	35,255	69, 144	SO,798	36,716	2,110	0,823	2,370
B84	68,262	90,244	10,470	69,131	90,229	8,963	0,S9S	0,012	10,965
B85	75,215	86,31	34,988	73,054	83,809	32,713	2,061	2,079	4,753
B86	61,090	73,770	36,009	54,320	59,299	30,493	3, 296	15,380	11,719
B87	64,373	79,62 1	73,400	63,736	79,437	22,532	0,705	0,164	2,680
B83	71,811	82,089	36,250	69,202	82,227	35,740	2,617	0,118	1,001
B89	72,238	3 1,567	34, /9	70,331	83,306	35,508	1,892	1,492	1,445

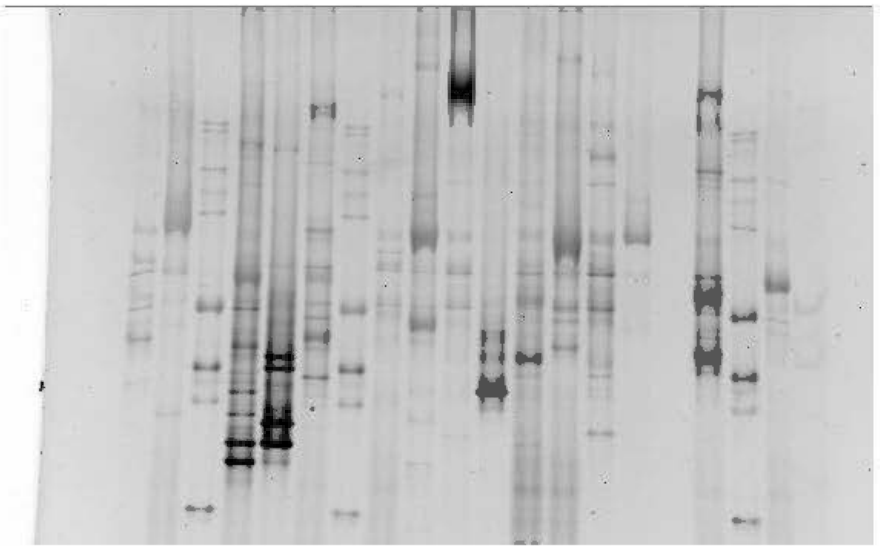
B9	69,589	39, 589	13,651	68,151	89,219	12,582	1,476	0,293	5,762
B90	68,000	70,515	40,442	64,222	77,362	42,520	4,041	7,002	3,543
B91	63,329	87,962	14,587	67,9 14	88,011	13,001	0,431	0,040	8,129
B92	73,4 7 3	85,806	30,410	71, 135	85,533	29,961	2,286	0,221	1,052
B93	72,150	84,17	32,577	70,534	82,869	31,919	1,607	1,101	1,443
B94	63,898	80,322	21,327	63,02 8	79,638	20,969	0,970	0,604	1,197
B95	63,459	75,479	31,69	60,901	73,165	33,495	2,909	2, 202	3,915
B96	74,320	36,229	33,518	72,878	36,226	33,075	1,385	0,002	0,940
B97	71,850	33,569	30,110	69,442	33,163	29,794	2,411	0,344	0,74 6
B98	75,301	88,307	30,676	73,627	87,732	29,45 1	1,589	0,861	2,881
B99	71,897	82,27 1	33,826	69,741	81,471	33,773	2,153	0,691	0,100
J111P	69,914	89,97	13,439	69,179	89,497	13,010	0,748	0,373	2,297
J111S	77,566	92,07	30,954	76,880	91,336	29,970	0,628	0,566	2,284
J112S	77,862	92,341	30,587	77,898	93,101	29,545	0,033	0,580	2,451
J1131P	70,03	90,488	12,651	69,953	90,733	11,755	0,078	0,191	5,193
J1131S	74,926	89,578	30,855	74,319	89,081	29,383	0,576	0,394	3,456
J114P	70,189	90,058	12,033	70,198	91,295	11,728	0,009	0,964	1,818
J114S	77,056	91,871	30,798	76,594	91,998	29,063	0,425	0,098	4,098
J121P	69,095	88,659	13,11	66,980	87,521	10,894	2,198	0,914	13,056
J121S	76,7	90,812	31,413	76,127	91,209	30,968	0,530	0,309	1,010
J122P	69,855	89,868	14,077	68,478	89,347	13,404	1,408	0,411	3,462
J122S	77,716	92,304	31,137	76,722	91,916	29,645	0,911	0,298	3,473
J1231P	70,311	90,541	12,205	69,558	91,034	11,526	0,761	0,384	4,045
J124S	76,248	90,928	31,238	75,861	91,369	30,033	0,360	0,342	2,782
J131P	69,615	89,917	14,227	69,093	89,265	14,142	0,532	0,515	0,425
J131S	75,212	90,157	30,441	75,393	91,076	29,700	0,170	0,717	1,744
J132P	70,609	91,284	12,715	69,743	90,528	10,903	0,873	0,588	10,848
J132S	75,464	90,695	31,213	75,683	91,220	30,785	0,205	0,408	0,976
J1331P	70,32	89,811	13,683	69,278	89,859	12,325	1,055	0,038	7,386
J1331S	76,403	90,4	32,315	75,320	89,741	31,753	1,010	0,518	1,241
J134S	77,375	91,894	31,878	76,383	91,086	30,799	0,912	0,624	2,435
J215P	70,405	90,604	14,012	69,368	89,684	12,160	1,049	0,721	10,007
J215S	77,783	92,63	31,082	77,047	92,264	29,677	0,672	0,280	3,270
J225P	69,998	90,588	12,435	70,041	90,653	11,542	0,043	0,050	5,269
J225S	77,424	92,061	30,758	77,008	92,267	29,309	0,381	0,158	3,411
J235P	70,827	90,578	14,285	68,822	89,297	13,126	2,031	1,007	5,982
J235S	77,063	91,3	31,074	77,200	92,413	30,158	0,125	0,857	2,116
J3110P	69,311	90,056	12,882	68,605	89,778	11,821	0,724	0,218	6,074
J3110S	77,784	92,844	30,716	78,339	93,447	29,955	0,503	0,458	1,774
J317P	69,658	91,644	13,262	70,117	91,363	11,471	0,464	0,217	10,244
J317S	77,987	93,198	30,946	77,649	92,733	29,450	0,307	0,354	3,502
J319P	70,082	91,283	12,902	68,478	89,459	11,383	1,637	1,427	8,847
J3210P	70,083	91,884	13,172	69,964	90,730	11,609	0,120	0,894	8,920
J3210S	77,239	91,64	31,459	77,196	92,195	30,475	0,039	0,427	2,246

J327P	70,715	91,606	13,152	69,927	90,226	11,301	0,793	1,073	10,707
J329P	68,952	89,62	12,868	68,165	88,835	12,164	0,812	0,622	3,977
J3310P	69,339	90,867	12,585	69,558	90,239	11,956	0,223	0,490	3,628
J3310S	77,157	91,792	32,561	75,929	90,222	31,435	1,135	1,220	2,488
J337S	77,911	92,055	31,199	76,730	91,183	30,332	1,080	0,673	1,993
J339S	78,75	93,338	32,262	77,842	91,612	30,870	0,820	1,320	3,119
S1B1P1P	68,032	88,46	13,583	68,019	88,451	13,965	0,014	0,007	1,961
S1B1P1S	75,33	89,48	29,754	74,852	90,356	29,602	0,450	0,689	0,362
S1B1P2P	68,57	89,564	15,175	70,917	91,465	14,697	2,380	1,485	2,264
S1B1P2S	77,115	91,585	30,795	76,897	92,145	30,100	0,201	0,431	1,615
S1B1P31S	76,804	91,682	30,79	75,697	90,380	29,514	1,027	1,011	2,992
S1B1P4P	66,245	85,434	15,249	66,535	86,025	16,379	0,309	0,487	5,051
S1B1P4S	77,022	92,446	31,25	76,613	91,261	29,653	0,376	0,912	3,708
S1B2P1P	68,617	89,04	15,658	69,922	90,564	16,305	1,332	1,200	2,864
S1B2P1S	76,715	90,494	31,293	76,124	91,241	30,661	0,547	0,582	1,444
S1B2P2S	75,472	90,05	30,428	75,658	90,883	30,122	0,174	0,651	0,715
S1B2P31P	68,971	90,058	14,237	69,455	90,009	12,998	0,494	0,038	6,434
S1B2P31S	76,182	90,486	31,327	75,987	91,056	31,016	0,181	0,444	0,706
S1B2P4P	68,765	89,82	14,288	69,292	89,904	13,802	0,540	0,066	2,446
S1B2P4S	75,958	90,152	30,867	75,277	90,528	30,485	0,637	0,295	0,882
S1B3P1S	74,805	89,064	30,07	74,573	90,189	30,236	0,220	0,887	0,390
S1B3P2P	67,726	88,942	13,736	68,392	88,628	12,773	0,692	0,250	5,136
S1B3P31P	69,251	89,164	14,335	68,275	88,933	14,044	1,004	0,184	1,453
S1B3P31S	76,447	90,552	31,714	76,062	90,954	31,251	0,357	0,313	1,041
S1B3P4P	69,706	90,164	14,659	69,460	89,929	13,855	0,250	0,184	3,986
S1B3P4S	76,537	90,412	33,011	75,232	89,409	32,744	1,216	0,789	0,575
S2B1P5S	77,862	92,902	30,999	77,342	91,753	30,075	0,474	0,880	2,139
S2B2P5P	66,559	85,579	15,161	66,334	86,812	15,825	0,240	1,012	3,028
S2B2P5S	75,323	89,039	30,851	74,670	89,919	30,452	0,616	0,696	0,922
S2B3P5P	69,49	90,157	13,655	69,182	89,842	13,136	0,314	0,247	2,741
S2B3P5S	76,438	90,688	31,74	76,372	91,365	30,936	0,062	0,526	1,814
S3B1P10S	76,646	91,679	30,845	75,666	90,927	29,865	0,910	0,582	2,284
S3B1P7P	66,857	86,628	14,311	65,983	86,203	14,496	0,931	0,348	0,909
S3B1P7S	77,683	92,707	31,151	77,101	91,522	30,345	0,532	0,909	1,855
S3B1P9P	66,521	86,626	13,857	65,807	86,299	13,986	0,763	0,267	0,655
S3B1P9S	75,807	88,767	30,218	74,662	90,022	30,513	1,077	0,993	0,686
S3B2P10P	67,029	87,029	15,099	66,414	86,689	14,436	0,652	0,277	3,175
S3B2P10S	74,353	86,128	31,402	73,334	88,131	32,105	0,976	1,626	1,565
S3B2P7P	68,097	88,533	11,837	66,475	87,395	11,029	1,705	0,915	5,001
S3B2P7S	76,584	91,037	30,709	75,824	90,919	29,078	0,706	0,092	3,858
S3B2P9P	67,055	88,186	13,957	67,730	88,709	13,611	0,708	0,418	1,776
S3B2P9S	76,254	90,837	30,798	75,920	90,912	30,145	0,311	0,058	1,516
S3B3P10P	68,039	87,306	15,393	67,966	88,077	15,290	0,076	0,622	0,474
S3B3P10S	76,498	90,99	29,785	75,231	90,944	28,860	1,181	0,035	2,231

S3B3P7P	67,946	89,325	15,085	69,719	90,412	14,582	1,821	0,856	2,399
S383P7S	77,323	91,988	31,256	76,631	91,260	30,277	0,636	0,562	2,250
S3B3P9P	66,847	87,035	13,587	66,535	87,131	13,960	0,331	0,078	1,915
S3B3P9S	76,59	91,162	30,385	76,144	91,533	29,555	0,413	0,287	1,959

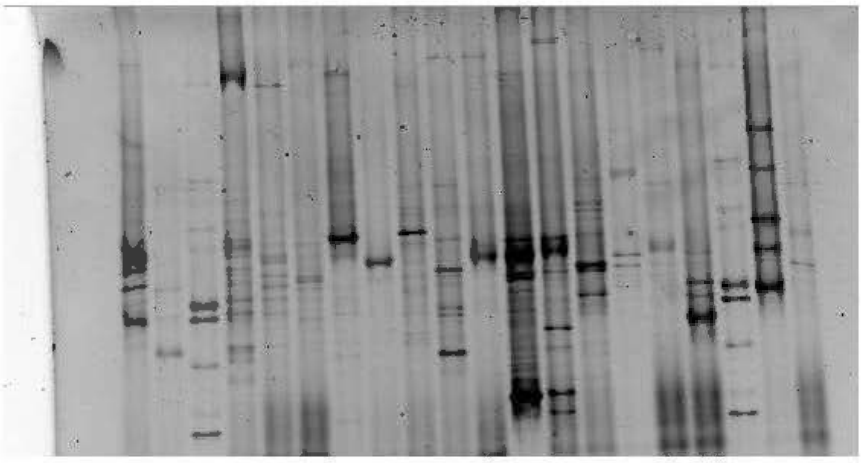
Composition en ADF, NDF et lignine (% de matière sèche) des différents échantillons (chapitre 1 et 2). Deux analyses (répétitions) ont été effectuées sur chaque échantillon et le coefficient de variation calculé.

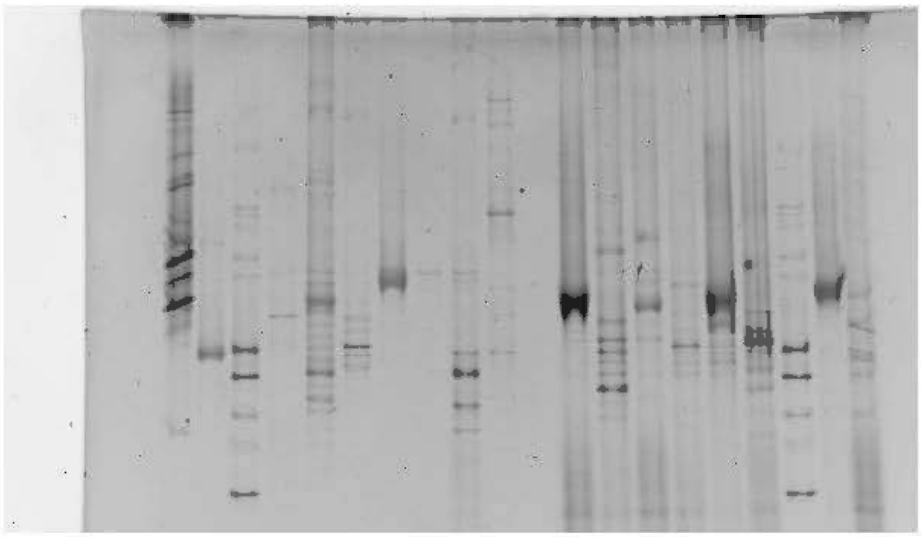
ANNEXE B



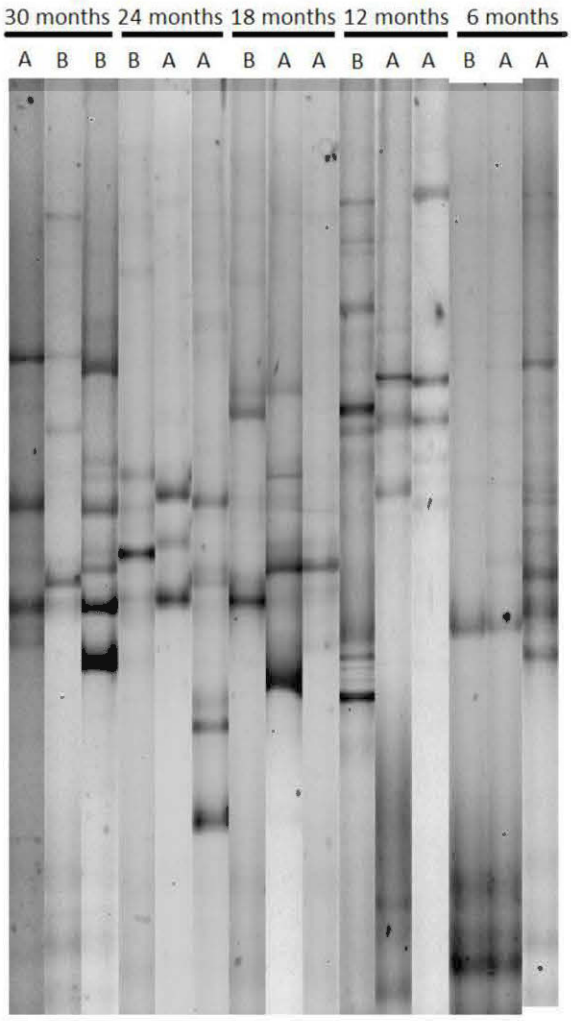
Example of a denaturing gradient gel electrophoresis (DGGE) of rDNA derived fragments PCR amplified by ITS1F/ITS2 primer pairs in chapter 1.

ANNEXE C



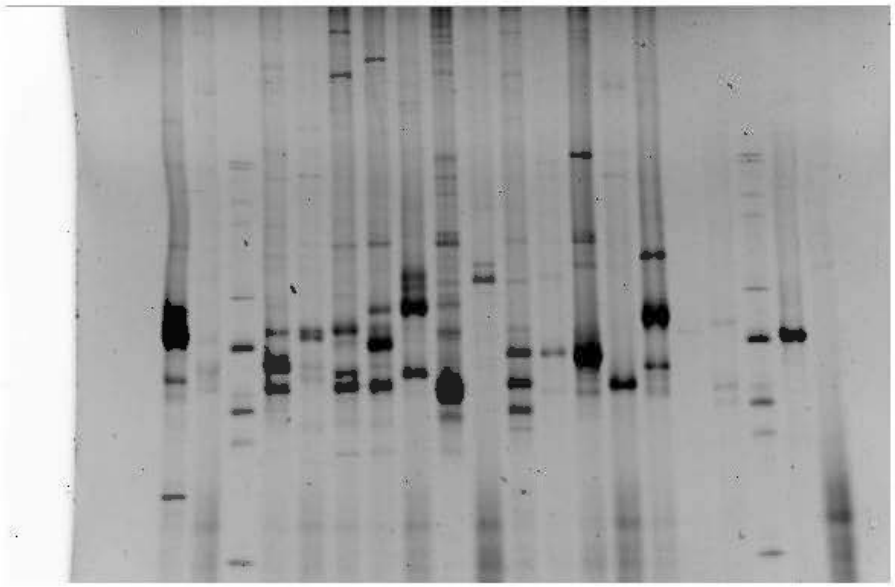


Example of two denaturing gradient gel electrophoresis (DGGE) of rDNA derived fragments PCR amplified by ITS1F/ITS2 primer pairs in chapter 2.

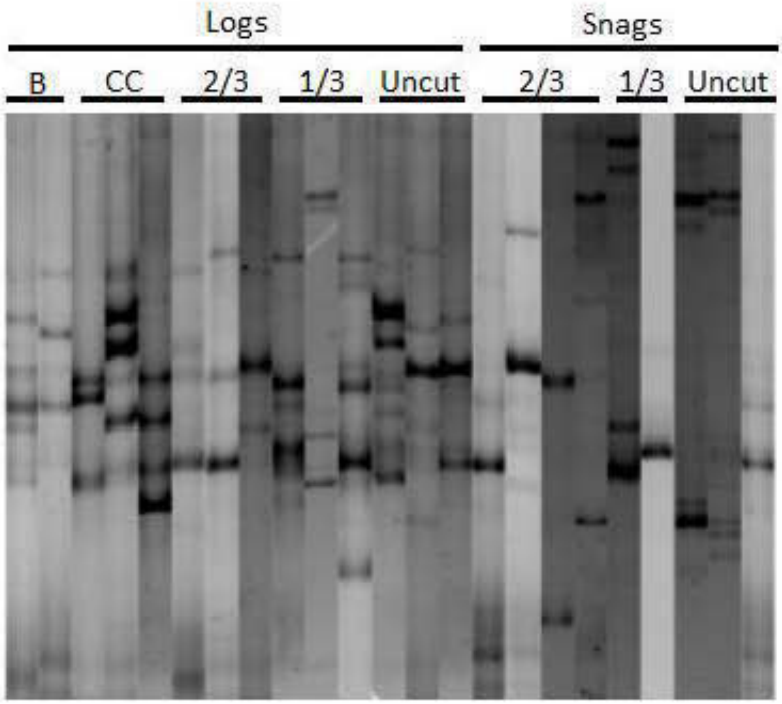


Denaturing gradient gel electrophoresis of rDNA based ITS1 region profiles of fungal communities inhabiting trembling aspen (A) and balsam fir (B) wood blocks, classified from time of incubation (6 to 30 months). Three wood samples from each sampling time are presented including both wood species (chapter 2).

ANNEXE D



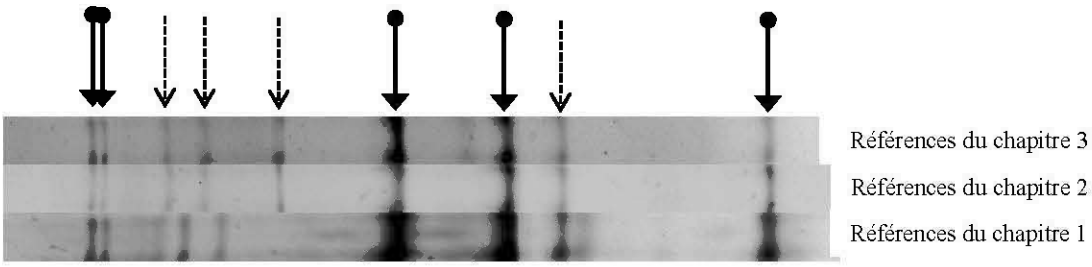
Example of a denaturing gradient gel electrophoresis (DGGE) of rDNA derived fragments PCR amplified by ITS1F/ITS2 primer pairs in chapter 3.



Denaturing gradient gel electrophoresis of rDNA based ITS1 region profiles of fungal communities inhabiting trembling aspen logs and snags, classified from harvesting treatment (B=controlled burn, CC=clear-cut, 2/3=partial cut 2/3, 1/3=partial cut 1/3, Uncut=unharvested stands). Between 2 and 4 wood samples from each harvesting treatments are presented (chapter 3).

ANNEXE E

La comparaison entre les gels des différents chapitres a été possible grâce à des bandes communes dans le marqueur de référence. La même méthode que celle indiquée dans le matériel et méthodes des chapitres a été utilisée. Nous avons utilisé le logiciel GelCompar II (version 5.0, Applied Maths, Belgique) afin d'analyser les patrons des bandes de DGGE (région ITS). Afin de minimiser les différences de migration et de normaliser les distorsions entre les gels d'électrophorèse, nous avons aligné les gels en utilisant une référence externe constituée d'un mélange d'ADN ITS amplifié à partir de 5 espèces différentes de champignons. Cependant, les espèces fongiques de référence utilisées dans le premier chapitre ne sont pas exactement les mêmes que pour les autres chapitres (3 espèces communes). Donc pour les gels d'un même chapitre, toutes les bandes issues de ces 5 espèces ont servi à aligner les gels alors que pour aligner les gels du chapitre 1 avec ceux des chapitres 2 et 3, seulement 5 bandes réparties équitablement sur le gel ont servi de référence.



La figure ci-dessus représente les marqueurs de référence des gels de DGGE entre les trois chapitres. Les flèches pleines indiquent les bandes communes entre les 3 chapitres, tandis que toutes les bandes marquées par des flèches indiquent les bandes pour les chapitres 2 et 3.

REFERENCES

Alkaslassy, E. 2005. «Abundance of plethodontid salamanders in relation to coarse woody debris in a low elevation mixed forest of the western Cascades». *Northwest Science*, vol. 79, no 2-3, p. 156-163.

Allmér, J., R. Vasiliauskas, K. Ihrmark, J. Stenlid & A. Dahlberg. 2006. «Wood-inhabiting fungal communities in woody debris of Norway spruce (*Picea abies* (L.) Karst.), as reflected by sporocarps, mycelial isolations and T-RFLP identification». *FEMS Microbiology Ecology*, vol. 55, no 1, p. 57-67.

Ammer, U. 1991. «Consequences from the results of research on dead woody material for the forestry practice». *Forstwissenschaftliches Centralblatt*, vol. 110, no 1, p. 149-157.

Anderson, I. C., C. D. Campbell & J. I. Prosser. 2003. «Diversity of fungi in organic soils under a moorland - Scots pine (*Pinus sylvestris* L.) gradient». *Environmental Microbiology*, vol. 5, no 11, p. 1121-1132.

Anderson, M. J. 2006. «Distance-based tests for homogeneity of multivariate dispersions». *Biometrics*, vol. 62, no 1, p. 245-253.

Bader, P., S. Jansson & B. G. Jonsson. 1995. «Wood-inhabiting fungi and substratum decline in selectively logged boreal spruce forests». *Biological Conservation*, vol. 72, no 3, p. 355-362.

Baldrian, P. 2008. «Chapter 2 Enzymes of saprotrophic basidiomycetes». p. 19-41: British Mycological Society Symposia Series.

Baptist, F., L. Zinger, J. C. Clement, C. Gallet, R. Guillemin, J. M. F. Martins, L. Sage, B. Shahnavaz, Ph Choler & R. Geremia. 2008. «Tannin impacts on microbial diversity and the functioning of alpine soils: A multidisciplinary approach». *Environmental Microbiology*, vol. 10, no 3, p. 799-809.

Barnes, R. J., M. S. Dhanoa & Susan J. Lister. 1989. «Standard normal variate transformation and de-trending of near-infrared diffuse reflectance spectra». *Applied Spectroscopy*, vol. 43, no 5, p. 772-777.

Bednarz, J. C., D. Ripper & P. M. Radley. 2004. «Emerging concepts and research directions in the study of cavity-nesting birds: Keystone ecological processes». *Condor*, vol. 106, no 1, p. 1-4.

Belleau, A., S. Brais & D. Paré. 2006. «Soil nutrient dynamics after harvesting and slash treatments in boreal aspen stands». *Soil Science Society of America Journal*, vol. 70, no 4, p. 1189-1199.

Bergeron, Y., A. Leduc, B. D. Harvey & S. Gauthier. 2002. «Natural fire regime: A guide for sustainable management of the Canadian boreal forest». *Silva Fennica*, vol. 36, no 1, p. 81-95.

Bergeron, Yves, & Michelle Dubuc. 1989. «Succession in the southern part of the Canadian boreal forest». *Plant Ecology*, vol. 79, no 1, p. 51-63.

Bergeron, Yves, & Brian Harvey. 1997. «Basing silviculture on natural ecosystem dynamics: an approach applied to the southern boreal mixedwood forest of Quebec». *Forest Ecology and Management*, vol. 92, no 1-3, p. 235-242.

Berglund, H., M. Edman & L. Ericson. 2005. «Temporal variation of wood-fungi diversity in boreal old-growth forests: Implications for monitoring». *Ecological Applications*, vol. 15, no 3, p. 970-982.

Boddy, L. 1992. «Development and function of fungal communities in decomposing wood». In *The Fungal Community. Its Organization and Role in the Ecosystem,* D.T. Wicklow & C.G. Carroll, p. 749-782. New York: Marcel Dekker.

--------. 2000. «Interspecific combative interactions between wood-decaying basidiomycetes». *FEMS Microbiology Ecology*, vol. 31, no 3, p. 185-194.

--------. 2001. «Fungal community ecology and wood decomposition processes in angiosperms: From standing tree to complete decay of coarse woody debris». *Ecological Bulletins*, vol. 49, p. 43-56.

Boddy, L., & S. C. Watkinson. 1995. «Wood decomposition, higher fungi, and their role in nutrient redistribution». *Canadian Journal of Botany*, vol. 73, no Suppl. 1.

Bonkowski, M., & J. Roy. 2005. «Soil microbial diversity and soil functioning affect competition among grasses in experimental microcosms». *Oecologia*, vol. 143, no 2, p. 232-240.

Bouget, C., & P. Duelli. 2004. «The effects of windthrow on forest insect communities: A literature review». *Biological Conservation*, vol. 118, no 3, p. 281-299.

Boulet, B. 2003. *Les champignons des arbres de l'est de l'Amerique du Nord.*: Les Publications du Québec, Ste-Foy, 356-357 p.

Bowman, J. C., D. Sleep, G. J. Forbes & M. Edwards. 2000. «The association of small mammals with coarse woody debris at log and stand scales». *Forest Ecology and Management*, vol. 129, no 1-3, p. 119-124.

Brais, S., B. D. Harvey, Y. Bergeron, C. Messier, D. Greene, A. Belleau & D. Paré. 2004. «Testing forest ecosystem management in boreal mixedwoods of northwestern Quebec: Initial response of aspen stands to different levels of harvesting». *Canadian Journal of Forest Research*, vol. 34, no 2, p. 431-446.

Brais, S., D. Pare & C. Lierman. 2006. «Tree bole mineralization rates of four species of the Canadian eastern boreal forest: Implications for nutrient dynamics following stand-replacing disturbances». *Canadian Journal of Forest Research*, vol. 36, no 9, p. 2331-2340.

Brais, S., F. Sadi, Y. Bergeron & Y. Grenier. 2005. «Coarse woody debris dynamics in a post-fire jack pine chronosequence and its relation with site productivity». *Forest Ecology and Management*, vol. 220, no 1-3, p. 216-226.

Brassard, B. W., & H. Y. H. Chen. 2006. «Stand structural dynamics of North American boreal forests». *Critical Reviews in Plant Sciences*, vol. 25, no 2, p. 115-137.

--------. 2008. «Effects of forest type and disturbance on diversity of coarse woody debris in boreal forest». *Ecosystems*, vol. 11, no 7, p. 1078-1090.

Bratton, J. H. (2003). Habitat management to conserve fungi: A literature review. Natural Science Report. Countryside Council for Wales.

Brinkmann, K., L. Blaschke & A. Polle. 2002. «Comparison of different methods for lignin determination as a basis for calibration of near-infrared reflectance spectroscopy and implications of lignoproteins». *Journal of Chemical Ecology*, vol. 28, no 12, p. 2483-2501.

Buée, M., P. E. Courty, D. Mignot & J. Garbaye. 2007. «Soil niche effect on species diversity and catabolic activities in an ectomycorrhizal fungal community». *Soil Biology and Biochemistry*, vol. 39, no 8, p. 1947-1955.

Burnham, K. P., & D. R. Anderson. 2004. «Multimodel inference: Understanding AIC and BIC in model selection». *Sociological Methods and Research*, vol. 33, no 2, p. 261-304.

Butler, J., K. Alexander & T. Green. 2002. «Decaying wood: An overview of its status and ecology in the United Kingdom and continental Europe». *Proceedings of the Symposium on the Ecology and Management of Dead Wood in Western Forests*, p. 11-19.

Bütler, R., P. Angelstam, P. Ekelund & R. Schlaepfer. 2004. «Dead wood threshold values for the three-toed woodpecker presence in boreal and sub-Alpine forest». *Biological Conservation*, vol. 119, no 3, p. 305-318.

Butts, S. R., & W. C. McComb. 2000. «Associations of forest-floor vertebrates with coarse woody debris in managed forests of western Oregon». *Journal of Wildlife Management*, vol. 64, no 1, p. 95-104.

Coleman, D. C., & W. B. Whitman. 2005. «Linking species richness, biodiversity and ecosystem function in soil systems». *Pedobiologia*, vol. 49, no 6, p. 479-497.

Coulombe, G. (2004). Rapport final de la Commission d'étude sur la gestion de la forêt publique québécoise.

Crawford, R. H., C. Y. Li & M. Floyd. 1997. «Nitrogen fixation in root-colonized large woody residue of Oregon coastal forests». *Forest Ecology and Management*, vol. 92, no 1-3, p. 229-234.

Daniels, L. D., J. Dobry, K. Klinka & M. C. Feller. 1997. «Determining year of death of logs and snags of *Thuja plicata* in southwestern coastal British Columbia». *Canadian Journal of Forest Research*, vol. 27, no 7, p. 1132-1141.

Dansereau, P. R., & Y. Bergeron. 1993. «Fire history in the southern boreal forest of northwestern Quebec». *Canadian Journal of Forest Research*, vol. 23, no 1, p. 25-32.

Davies, Z. G., C. Tyler, G. B. Stewart & A. S. Pullin. 2008. «Are current management recommendations for saproxylic invertebrates effective? A systematic review». *Biodiversity and Conservation*, vol. 17, no 1, p. 209-234.

De Bellis, T., G. Kernaghan & P. Widden. 2007. «Plant community influences on soil microfungal assemblages in boreal mixed-wood forests». *Mycologia*, vol. 99, no 3, p. 356-367.

De Boer, W., L. B. Folman, R. C. Summerbell & L. Boddy. 2005. «Living in a fungal world: Impact of fungi on soil bacterial niche development». *FEMS Microbiology Reviews*, vol. 29, no 4, p. 795-811.

Deacon, L. J., E. Janie Pryce-Miller, J. C. Frankland, B. W. Bainbridge, P. D. Moore & C. H. Robinson. 2006. «Diversity and function of decomposer fungi from a grassland soil». *Soil Biology and Biochemistry*, vol. 38, no 1, p. 7-20.

Degens, B. P. 1998. «Decreases in microbial functional diversity do not result in corresponding changes in decomposition under different moisture conditions». *Soil Biology and Biochemistry*, vol. 30, no 14, p. 1989-2000.

Dilly, O., J. Bloem, A. Vos & J. C. Munch. 2004. «Bacterial diversity in agricultural soils during litter decomposition». *Applied and Environmental Microbiology*, vol. 70, no 1, p. 468-474.

Drapeau, P., A. Nappi, J. F. Giroux, A. Leduc & J. P. Savard. 2002. «Distribution patterns of birds associated with snags in natural and managed eastern boreal forests». *Proceedings of the Symposium on the Ecology and Management of Dead Wood in Western Forests*, p. 193-205.

Drapeau, P., A. Nappi, L. Imbeau & M. Saint-Germain. 2009. «Standing deadwood for keystone bird species in the eastern boreal forest: Managing for snag dynamics». *Forestry Chronicle*, vol. 85, no 2, p. 227-234.

Edman, M., N. Kruys & B. G. Jonsson. 2004. «Local dispersal sources strongly affect colonization patterns of wood-decaying fungi on spruce logs». *Ecological Applications*, vol. 14, no 3, p. 893-901.

Edwards, N. T. 1982. «The use of soda-lime for measuring respiration rates in terrestrial systems». *Pedobiologia*, vol. 23, p. 321-330.

Ehnström, B. 2001. «Leaving dead wood for insects in boreal forests - Suggestions for the future». *Scandinavian Journal of Forest Research*, vol. 16, no 1, p. 91-98.

Eichner, C. A., R. W. Erb, K. N. Timmis & I. Wagner-Döbler. 1999. «Thermal gradient gel electrophoresis analysis of bioprotection from pollutant shocks in the activated sludge microbial community». *Applied and Environmental Microbiology*, vol. 65, no 1, p. 102-109.

Feller, M. C. 2003. «Coarse woody debris in the old-growth forests of British Columbia». *Environmental Reviews*, vol. 11, no 1 SUPPL.

Fischer, Michael, & Manfred Binder. 2004. «Species recognition, geographic distribution and host-pathogen relationships: a case study in a group of lignicolous basidiomycetes, *Phellinus* s.l.». *Mycologia*, vol. 96, no 4, p. 799-811.

Fitter, A. H., C. A. Gilligan, K. Hollingworth, A. Kleczkowski, R. M. Twyman & J. W. Pitchford. 2005. «Biodiversity and ecosystem function in soil». *Functional Ecology*, vol. 19, no 3, p. 369-377.

Foley, W. J., A. McIlwee, I. Lawler, L. Aragones, A. P. Woolnough & N. Berding. 1998. «Ecological applications of near infrared reflectance spectroscopy - A tool for rapid, cost-effective prediction of the composition of plant and animal tissues and aspects of animal performance». *Oecologia*, vol. 116, no 3, p. 293-305.

Franklin, J. F. 1997. *Alternative silvicultural approaches to timber harvesting: Variable retention harvest systems. Creating a Forestry for the 21st Century*: Island Press, Washington, D.C., 111-139 p.

Franzén, I., R. Vasaitis, R. Penttilä & J. Stenlid. 2007. «Population genetics of the wood-decay fungus *Phlebia centrifuga* P. Karst. in fragmented and continuous habitats». *Molecular Ecology*, vol. 16, no 16, p. 3326-3333.

Frey, S. D., J. Six & E. T. Elliott. 2003. «Reciprocal transfer of carbon and nitrogen by decomposer fungi at the soil-litter interface». *Soil Biology and Biochemistry*, vol. 35, no 7, p. 1001-1004.

Fridman, J., & M. Walheim. 2000. «Amount, structure, and dynamics of dead wood on managed forestland in Sweden». *Forest Ecology and Management*, vol. 131, no 1-3, p. 23-36.

Fromin, N., J. Hamelin, S. Tarnawski, D. Roesti, K. Jourdain-Miserez, N. Forestier, S. Teyssier-Cuvelle, F. Gillet, M. Aragno & P. Rossi. 2002. «Statistical analysis of denaturing gel electrophoresis (DGE) fingerprinting patterns». *Environmental Microbiology*, vol. 4, no 11, p. 634-643.

Fukami, T., I. A. Dickie, J. Paula Wilkie, B. C. Paulus, D. Park, A. Roberts, P. K. Buchanan & R. B. Allen. 2010. «Assembly history dictates ecosystem functioning: Evidence from wood decomposer communities». *Ecology Letters*, vol. 13, no 6, p. 675-684.

Galindo-Leal, C., & F. L. Bunnell. 1995. «Ecosystem management: implications and opportunities of a new paradigm». *Forestry Chronicle*, vol. 71, no 5, p. 601-606.

Ganjegunte, G. K., L. M. Condron, P. W. Clinton, M. R. Davis & N. Mahieu. 2004. «Decomposition and nutrient release from radiata pine (Pinus radiata) coarse woody debris». *Forest Ecology and Management*, vol. 187, no 2-3, p. 197-211.

Gardes, M., & T. D. Bruns. 1993. «ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts». *Molecular Ecology*, vol. 2, no 2, p. 113-118.

--------. 1996. «Community structure of ectomycorrhizal fungi in a Pinus muricata forest: Above- and below-ground views». *Canadian Journal of Botany*, vol. 74, no 10, p. 1572-1583.

Gauthier, S., M.-A. Vaillancourt, A. Leduc, L. de Grandpré, D.D. Kneeshaw, H. Morin, P. Drapeau & Y. Bergeron. 2008. *Aménagement écosystémique en forêt boréale*: Presses de l'Université du Québec, Québec, 568 p.

Goering, H.K, & P.J Van Soest. 1970. «Forage fibre analysis (apparatus, reagents and some applications)». In *U.S. Department of Agriculture Agricultural Handbook No. 379.*, p. 1-19: U.S. Department of Agriculture, Washington. D.C.

Gray, N. D., R. C. Hastings, S. K. Sheppard, P. Loughnane, D. Lloyd, A. J. McCarthy & I. M. Head. 2003. «Effects of soil improvement treatments on bacterial community

structure and soil processes in an upland grassland soil». *FEMS Microbiology Ecology*, vol. 46, no 1, p. 11-22.

Green III, F., & T. L. Highley. 1997. «Mechanism of brown-rot decay: Paradigm or paradox». *International Biodeterioration and Biodegradation*, vol. 39, no 2-3, p. 113-124.

Griffiths, B. S., K. Ritz, R. D. Bardgett, R. Cook, S. Christensen, F. Ekelund, S. J. Sørensen, E. Bååth, J. Bloem, P. C. De Ruiter, J. Dolfing & B. Nicolardot. 2000. «Ecosystem response of pasture soil communities to fumigation-induced microbial diversity reductions: An examination of the biodiversity-ecosystem function relationship». *Oikos*, vol. 90, no 2, p. 279-294.

Griffiths, B. S., K. Ritz, R. Wheatley, H. L. Kuan, B. Boag, S. Christensen, F. Ekelund, S. J. Sørensen, S. Muller & J. Bloem. 2001. «An examination of the biodiversity-ecosystem function relationship in arable soil microbial communities». *Soil Biology and Biochemistry*, vol. 33, no 12-13, p. 1713-1722.

Grove, S. J. 2002. «Saproxylic insect ecology and the sustainable management of forests». *Annual Review of Ecology and Systematics*, vol. 33, p. 1-23.

Haeussler, S., Y. Bergeron, S. Brais & B. D. Harvey. 2007. «Natural dynamics-based silviculture for maintaining plant biodiversity in Populus tremuloides-dominated boreal forests of eastern Canada». *Canadian Journal of Botany*, vol. 85, no 12, p. 1158-1170.

Hafner, S. D., & P. M. Groffman. 2005. «Soil nitrogen cycling under litter and coarse woody debris in a mixed forest in New York State». *Soil Biology and Biochemistry*, vol. 37, no 11, p. 2159-2162.

Hagemann, U., M. T. Moroni, J. Gleißner & F. Makeschin. 2010. «Disturbance history influences downed woody debris and soil respiration». *Forest Ecology and Management*, vol. 260, no 10, p. 1762-1772.

Hall, T. A. 1999. «BioEdit: A user-friendly biological sequence alignment editor and analysis». *Nucleic Acids Symp. Ser.*, vol. 41, p. 95-98.

Hallaksela, Anna-Maija. 1993. «Early interactions of Heterobasidion annosum and Stereum sanguinolentum with non-decay fungi and bacteria following inoculation into stems of Picea abies». *European Journal of Forest Pathology*, vol. 23, no 6-7, p. 416-430.

Hannam, K. D., S. A. Quideau & B. E. Kishchuk. 2006. «Forest floor microbial communities in relation to stand composition and timber harvesting in northern Alberta». *Soil Biology and Biochemistry*, vol. 38, no 9, p. 2565-2575.

Harmon, M. E., J. F. Franklin, F. J. Swanson, P. Sollins, S. V. Gregory, J. D. Lattin, N. H. Anderson, S. P. Cline, N. G. Aumen, J. R. Sedell, G. W. Lienkaemper, K. Cromack Jr, K. W. Cummins, A. MacFadyen & E.D Ford. 1986. «Ecology of coarse woody debris in temperate ecosystems». *Advances in Ecological Research*, vol. 15, p. 133-302.

Harmon, M. E., & Jay Sexton. 1996. *Guidelines for measurements of woody detritus in forest ecosystems*: U.S. Long-term Ecol. Res. Netw. Off. Publ. No. 20 p.

Harvey, B. 1999. «The Lake Duparquet Research and Teaching Forest: Building a foundation for ecosystem management». *Forestry Chronicle*, vol. 75, no 3, p. 389-393.

Harvey, B. D., & S. Brais. 2007. «Partial cutting as an analogue to stem exclusion and dieback in trembling aspen (Populus tremuloides) dominated boreal mixedwoods: Implications for deadwood dynamics». *Canadian Journal of Forest Research*, vol. 37, no 9, p. 1525-1533.

Harvey, B. D., A. Leduc, S. Gauthier & Y. Bergeron. 2002. «Stand-landscape integration in natural disturbance-based management of the southern boreal forest». *Forest Ecology and Management*, vol. 155, no 1-3, p. 369-385.

Hättenschwiler, S., A. V. Tiunov & S. Scheu. 2005. «Biodiversity and litter decomposition in terrestrial ecosystems». *Annual Review of Ecology, Evolution, and Systematics*, vol. 36, p. 191-218.

Hautala, H., J. Jalonen, S. Laaka-Lindberg & I. Vanha-Majamaa. 2004. «Impacts of retention felling on coarse woody debris (CWD) in mature boreal spruce forests in Finland». *Biodiversity and Conservation*, vol. 13, no 8, p. 1541-1554.

Heilmann-Clausen, J., E. Aude & M. Christensen. 2005. «Cryptogam communities on decaying deciduous wood - Does tree species diversity matter?». *Biodiversity and Conservation*, vol. 14, no 9, p. 2061-2078.

Heilmann-Clausen, J., & L. Boddy. 2005. «Inhibition and stimulation effects in communities of wood decay fungi: Exudates from colonized wood influence growth by other species». *Microbial Ecology*, vol. 49, no 3, p. 399-406.

Heilmann-Clausen, J., & M. Christensen. 2003. «Fungal diversity on decaying beech logs - Implications for sustainable forestry». *Biodiversity and Conservation*, vol. 12, no 5, p. 953-973.

--------. 2004. «Does size matter? On the importance of various dead wood fractions for fungal diversity in Danish beech forests». *Forest Ecology and Management*, vol. 201, no 1, p. 105-117.

Hiscox, J., P. Baldrian, H. J. Rogers & L. Boddy. 2010. «Changes in oxidative enzyme activity during interspecific mycelial interactions involving the white-rot fungus Trametes versicolor». *Fungal Genetics and Biology*, vol. 47, no 6, p. 562-571.

Hoadley, R.B. 1990. *Identifying wood: accurate results with simple tools*: Taunton Press, Newtown, Connecticut, 223 p.

Hodge, S. J., & G.F. Peterken. 1998. «Deadwood in British forests: Priorities and a strategy». *Forestry*, vol. 71, no 2, p. 99-112.

Holm, S. 1979. «A Simple Sequentially Rejective Multiple Test Procedure». *Scandinavian Journal of Statistics*, vol. 6, no 2, p. 65-70.

Hood, I. A., P. N. Beets, M. O. Kimberley, J. F. Gardner, G. R. Oliver & S. Pearce. 2004. «Colonisation of podocarp coarse woody debris by decomposer basidiomycete fungi in an indigenous forest in the central North Island of New Zealand». *Forest Ecology and Management*, vol. 196, no 2-3, p. 311-325.

Hooper, D. U. 2002. *Species diversity, functional diversity and ecosystem functioning. Biodiversity and Ecosystem Functioning: Synthesis and Perspectives*. New York: Oxford Univ Press, 195-208 p.

Hottola, Jenni, Otso Ovaskainen & Ilkka Hanski. 2009. «A unified measure of the number, volume and diversity of dead trees and the response of fungal communities». *Journal of Ecology*, vol. 97, no 6, p. 1320-1328.

Hou, P., & C. Pan. 2001. «Coarse woody debris and its function in forest ecosystem». *Chinese Journal of Applied Ecology*, vol. 12, no 2, p. 309-314.

Humphrey, J. W. 2005. «Benefits to biodiversity from developing old-growth conditions in British upland spruce plantations: A review and recommendations». *Forestry*, vol. 78, no 1, p. 33-53.

Jabin, M., D. Mohr, H. Kappes & W. Topp. 2004. «Influence of deadwood on density of soil macro-arthropods in a managed oak-beech forest». *Forest Ecology and Management*, vol. 194, no 1-3, p. 61-69.

Jackson, J. A., & B. J. S. Jackson. 2004. «Ecological relationships between fungi and woodpecker cavity sites». *Condor*, vol. 106, no 1, p. 37-49.

Jasalavich, C. A., A. Ostrofsky & J. Jellison. 2000. «Detection and identification of decay fungi in spruce wood by restriction fragment length polymorphism analysis of amplified genes encoding rRNA». *Applied and Environmental Microbiology*, vol. 66, no 11, p. 4725-4734.

Johannesson, H., & J. Stenlid. 1999. «Molecular identification of wood-inhabiting fungi in an unmanaged Picea abies forest in Sweden». *Forest Ecology and Management*, vol. 115, no 2-3, p. 203-211.

Jonsell, M., & G. Nordlander. 2002. «Insects in polypore fungi as indicator species: A comparison between forest sites differing in amounts and continuity of dead wood». *Forest Ecology and Management*, vol. 157, no 1-3, p. 101-118.

Jonsson, B. G., N. Kruys & T. Ranius. 2005. «Ecology of species living on dead wood - Lessons for dead wood management». *Silva Fennica*, vol. 39, no 2, p. 289-309.

Jönsson, M. T., M. Edman & B. G. Jonsson. 2008. «Colonization and extinction patterns of wood-decaying fungi in a boreal old-growth *Picea abies* forest». *Journal of Ecology*, vol. 96, no 5, p. 1065-1075.

Julien, M. C., P. Dion, C. Lafrenière, H. Antoun & P. Drouin. 2008. «Sources of clostridia in raw milk on farms». *Applied and Environmental Microbiology*, vol. 74, no 20, p. 6348-6357.

Junninen, K., & A. Komonen. 2011. «Conservation ecology of boreal polypores: A review». *Biological Conservation*, vol. 144, no 1, p. 11-20.

Junninen, K., M. Similä, J. Kouki & H. Kotiranta. 2006. «Assemblages of wood-inhabiting fungi along the gradients of succession and naturalness in boreal pine-dominated forests in Fennoscandia». *Ecography*, vol. 29, no 1, p. 75-83.

Juutilainen, K., P. Halme, H. Kotiranta & M. Mönkkönen. 2011. «Size matters in studies of dead wood and wood-inhabiting fungi». *Fungal Ecology*, vol. 4, no 5, p. 342-349.

Kebli, H., P. Drouin, S. Brais & G. Kernaghan. 2011. «Species Composition of Saproxylic Fungal Communities on Decaying Logs in the Boreal Forest». *Microbial Ecology*, vol. 61, no 4, p. 898-910.

Keith, H., & S. C. Wong. 2006. «Measurement of soil CO2 efflux using soda lime absorption: both quantitative and reliable». *Soil Biology and Biochemistry*, vol. 38, no 5, p. 1121-1131.

Kernaghan, G., L. Sigler & D. Khasa. 2003. «Mycorrhizal and root endophytic fungi of containerized Picea glauca seedlings assessed by rDNA sequence analysis». *Microbial Ecology*, vol. 45, no 2, p. 128-136.

Kernaghan, G., P. Widden, Y. Bergeron, S. Légaré & D. Paré. 2003. «Biotic and abiotic factors affecting ectomycorrhizal diversity in boreal mixed-woods». *Oikos*, vol. 102, no 3, p. 497-504.

Kneeshaw, D. D., A. Leduc, P. Drapeau, S. Gauthier, D. Pare, R. Carignan, R. Doucet, L. Bouthillier & C. Messier. 2000. «Development of integrated ecological standards of sustainable forest management at an operational scale». *Forestry Chronicle*, vol. 76, no 3, p. 481-493.

Komonen, A. 2003. «Distribution and abundance of insect fungivores in the fruiting bodies of Fomitopsis pinicola». *Annales Zoologici Fennici*, vol. 40, no 6, p. 495-504.

Kruys, N., & B. G. Jonsson. 1999. «Fine woody debris is important for species richness on logs in managed boreal spruce forests of northern Sweden». *Canadian Journal of Forest Research*, vol. 29, no 8, p. 1295-1299.

Kruys, N., B. G. Jonsson & G. Ståhl. 2002. «A stage-based matrix model for decay-class dynamics of woody debris». *Ecological Applications*, vol. 12, no 3, p. 773-781.

Kubartová, A., J. Moukoumi, T. Béguiristain, J. Ranger & J. Berthelin. 2007. «Microbial diversity during cellulose decomposition in different forest stands: I. Microbial communities and environmental conditions». *Microbial Ecology*, vol. 54, no 3, p. 393-405.

Kubartová, A., J. Ranger, J. Berthelin & T. Beguiristain. 2009. «Diversity and decomposing ability of saprophytic fungi from temperate forest litter». *Microbial Ecology*, vol. 58, no 1, p. 98-107.

Küffer, N., & B. Senn-Irlet. 2005. «Influence of forest management on the species richness and composition of wood-inhabiting basidiomycetes in Swiss forests». *Biodiversity and Conservation*, vol. 14, no 10, p. 2419-2435.

Kulhánkova, A., T. Béguiristain, J. Moukoumi, J. Berthelin & J. Ranger. 2006. «Spatial and temporal diversity of wood decomposer communities in different forest stands, determined by ITS rDNA targeted TGGE». *Annals of Forest Science*, vol. 63, no 5, p. 547-556.

Lee, S. D. 2004. «Population dynamics and demography of deermice (Peromyscus maniculatus) in heterogeneous habitat: Role of coarse woody debris». *Polish Journal of Ecology*, vol. 52, no 1, p. 55-62.

Legendre, P., & M. J. Andersson. 1999. «Distance-based redundancy analysis: Testing multispecies responses in multifactorial ecological experiments». *Ecological Monographs*, vol. 69, no 1, p. 1-24.

Li, C. Y., R. H. Crawford & T. T. Chang. 1997. «Frankia in decaying fallen trees devoid of actinorhizal hosts and soil». *Microbiological Research*, vol. 152, no 2, p. 167-169.

Lindahl, B., R. Finlay & S. Olsson. 2001. «Simultaneous, bidirectional translocation of ^{32}P and ^{33}P between wood blocks connected by mycelial cords of Hypholoma fasciculare». *New Phytologist*, vol. 150, no 1, p. 189-194.

Linder, P., B. Elfving & O. Zackrisson. 1997. «Stand structure and successional trends in virgin boreal forest reserves in Sweden». *Forest Ecology and Management*, vol. 98, no 1, p. 17-33.

Lindhe, A., N. Åsenblad & H. G. Toresson. 2004. «Cut logs and high stumps of spruce, birch, aspen and oak - Nine years of saproxylic fungi succession». *Biological Conservation*, vol. 119, no 4, p. 443-454.

Lindner, D. L., R. Vasaitis, A. Kubartová, J. Allmér, H. Johannesson, M. T. Banik & J. Stenlid. 2011. «Initial fungal colonizer affects mass loss and fungal community development in Picea abies logs 6yr after inoculation». *Fungal Ecology*, vol. 4, no 6, p. 449-460.

Lõhmus, A. 2011. «Silviculture as a disturbance regime: The effects of clear-cutting, planting and thinning on polypore communities in mixed forests». *Journal of Forest Research*, vol. 16, no 3, p. 194-202.

Lonsdale, D., M. Pautasso & O. Holdenrieder. 2008. «Wood-decaying fungi in the forest: Conservation needs and management options». *European Journal of Forest Research*, vol. 127, no 1, p. 1-22.

Lumley, T. C., L. D. Gignac & R. S. Currah. 2001. «Microfungus communities of white spruce and trembling aspen logs at different stages of decay in disturbed and undisturbed sites in the boreal mixedwood region of Alberta». *Canadian Journal of Botany*, vol. 79, no 1, p. 76-92.

Mantel, N. 1967. «The detection of disease clustering and a generalized regression approach». *Cancer Research*, vol. 27, no 2, p. 209-220.

Martínez, A. T., M. Speranza, F. J. Ruiz-Dueñas, P. Ferreira, S. Camarero, F. Guillén, M. J. Martínez, A. Gutiérrez & J. C. Del Río. 2005. «Biodegradation of lignocellulosics: Microbial, chemical, and enzymatic aspects of the fungal attack of lignin». *International Microbiology*, vol. 8, no 3, p. 195-204.

Mazerolle, M. J. 2006. «Improving data analysis in herpetology: Using Akaike's information criterion (AIC) to assess the strength of biological hypotheses». *Amphibia Reptilia*, vol. 27, no 2, p. 169-180.

Mazerolle, Marc J. 2009. «AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). R package version 1.01. http://CRAN.R-project.org/package=AICcmodavg».

McCay, T. S., & M. J. Komoroski. 2004. «Demographic responses of shrews to removal of coarse woody debris in a managed pine forest». *Forest Ecology and Management*, vol. 189, no 1-3, p. 387-395.

McClelland, B. R., & P. T. McClelland. 1999. «Pileated woodpecker nest and roost trees in Montana: Links with old-growth and forest 'health'». *Wildlife Society Bulletin*, vol. 27, no 3, p. 846-857.

McComb, W., & D. Lindenmayer. 1999. «Dying, dead, and down trees». In *Maintaining Biodiversity in Forest Ecosystems,* UK Cambridge, M.L. Hunter, p. 335-372: Cambridge University Press.

Meyer, P. 1999. «Dead wood research in forest reserves of Northwest-Germany: Methodology and results». *Totholzuntersuchungen in nordwestdeutschen Naturwaldern: Methodik und erste ergebnisse*, vol. 118, no 3, p. 167-180.

Meysami, P., & H. Baheri. 2003. «Pre-screening of fungi and bulking agents for contaminated soil bioremediation». *Advances in Environmental Research*, vol. 7, no 4, p. 881-887.

Mikola, J., & H. Setälä. 1998. «Relating species diversity to ecosystem functioning: Mechanistic backgrounds and experimental approach with a decomposer food web». *Oikos*, vol. 83, no 1, p. 180-194.

Montes, F., & I. Cañellas. 2006. «Modelling coarse woody debris dynamics in even-aged Scots pine forests». *Forest Ecology and Management*, vol. 221, no 1-3, p. 220-232.

Moore, J. C., E. L. Berlow, D. C. Coleman, P. C. De Suiter, Q. Dong, A. Hastings, N. C. Johnson, K. S. McCann, K. Melville, P. J. Morin, K. Nadelhoffer, A. D. Rosemond, D. M. Post, J. L. Sabo, K. M. Scow, M. J. Vanni & D. H. Wall. 2004. «Detritus, trophic dynamics and biodiversity». *Ecology Letters*, vol. 7, no 7, p. 584-600.

Moorhead, D. L., & R. L. Sinsabaugh. 2000. «Simulated patterns of litter decay predict patterns of extracellular enzyme activities». *Applied Soil Ecology*, vol. 14, no 1, p. 71-79.

Morin, H., D. Laprise & Y. Bergeron. 1993. «Chronology of spruce budworm outbreaks near Lake Duparquet, Abitibi region, Quebec». *Canadian Journal of Forest Research*, vol. 23, no 8, p. 1497-1506.

Müller, A. K., K. Westergaard, S. Christensen & S. J. Sørensen. 2002. «The diversity and function of soil microbial communities exposed to different disturbances». *Microbial Ecology*, vol. 44, no 1, p. 49-58.

Müller, J., H. Engel & M. Blaschke. 2007. «Assemblages of wood-inhabiting fungi related to silvicultural management intensity in beech forests in southern Germany». *European Journal of Forest Research*, vol. 126, no 4, p. 513-527.

Müller, J., T. Hothorn & H. Pretzsch. 2007. «Long-term effects of logging intensity on structures, birds, saproxylic beetles and wood-inhabiting fungi in stands of European beech Fagus sylvatica L». *Forest Ecology and Management*, vol. 242, no 2-3, p. 297-305.

Muyzer, Gerard, & Kornelia Smalla. 1998. «Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology». *Antonie van Leeuwenhoek*, vol. 73, no 1, p. 127-141.

Naeem, S., Loreau, M., Inchausti, P. 2002. «Biodiversity and ecosystem functioning: The emergence of a synthetic ecological framework ». In *Biodiversity and Ecosystem Functioning. Synthesis and Perspectives*, p. 3-11.

Niemela, T., P. Renvall & R. Penttila. 1995. «Interactions of fungi at late stages of wood decomposition». *Annales Botanici Fennici*, vol. 32, no 3, p. 141-152.

Nordén, B., F. Götmark, M. Ryberg, H. Paltto & J. Allmér. 2008. «Partial cutting reduces species richness of fungi on woody debris in oak-rich forests». *Canadian Journal of Forest Research*, vol. 38, no 7, p. 1807-1816.

Nordén, B., & H. Paltto. 2001. «Wood-decay fungi in hazel wood: Species richness correlated to stand age and dead wood features». *Biological Conservation*, vol. 101, no 1, p. 1-8.

Nordén, B., M. Ryberg, F. Götmark & B. Olausson. 2004. «Relative importance of coarse and fine woody debris for the diversity of wood-inhabiting fungi in temperate broadleaf forests». *Biological Conservation*, vol. 117, no 1, p. 1-10.

O'Connor, N. E., & T. P. Crowe. 2005. «Biodiversity loss and ecosystem functioning: Distinguishing between number and identity of species». *Ecology*, vol. 86, no 7, p. 1783-1796.

Økland, B. 1996. «Unlogged forests: Important sites for preserving the diversity of mycetophilids (Diptera: Sciaroidea)». *Biological Conservation*, vol. 76, no 3, p. 297-310.

Ostlund, L., O. Zackrisson & A. L. Axelsson. 1997. «The history and transformation of a Scandinavian boreal forest landscape since the 19th century». *Canadian Journal of Forest Research*, vol. 27, no 8, p. 1198-1206.

Ovaskainen, O., J. Nokso-Koivisto, J. Hottola, T. Rajala, T. Pennanen, H. Ali-Kovero, O. Miettinen, P. Oinonen, P. Auvinen, L. Paulin, K. H. Larsson & R. Mäkipää. 2010.

«Identifying wood-inhabiting fungi with 454 sequencing - what is the probability that BLAST gives the correct species?». *Fungal Ecology*, vol. 3, no 4, p. 274-283.

Palviainen, M., L. Finér, R. Laiho, E. Shorohova, E. Kapitsa & I. Vanha-Majamaa. 2010. «Carbon and nitrogen release from decomposing Scots pine, Norway spruce and silver birch stumps». *Forest Ecology and Management*, vol. 259, no 3, p. 390-398.

Parks, C. G., & D. C. Shaw. 1996. «Death and decay: A vital part of living canopies». *Northwest Science*, vol. 70, no SPEC. ISS., p. 46-53.

Parsons, S., K. J. Lewis & J. M. Psyllakis. 2003. «Relationships between roosting habitat of bats and decay of aspen in the sub-boreal forests of British Columbia». *Forest Ecology and Management*, vol. 177, no 1-3, p. 559-570.

Paul, L. R., B. K. Chapman & C. P. Chanway. 2006. «*Suillus tomentosus* tuberculate ectomycorrhizal abundance and distribution in Pinus contorta woody debris». *Canadian Journal of Forest Research*, vol. 36, no 2, p. 460-466.

Penttilä, R., M. Lindgren, O. Miettinen, H. Rita & I. Hanski. 2006. «Consequences of forest fragmentation for polyporous fungi at two spatial scales». *Oikos*, vol. 114, no 2, p. 225-240.

Penttilä, R., J. Siitonen & M. Kuusinen. 2004. «Polypore diversity in managed and old-growth boreal *Picea abies* forests in southern Finland». *Biological Conservation*, vol. 117, no 3, p. 271-283.

Persiani, A. M., P. Audisio, D. Lunghini, O. Maggi, V. M. Granito, A. B. Biscaccianti, U. Chiavetta & M. Marchetti. 2010. «Linking taxonomical and functional biodiversity of saproxylic fungi and beetles in broad-leaved forests in southern Italy with varying management histories». *Plant Biosystems*, vol. 144, no 1, p. 250-261.

Porter, A. D., C. C. St. Clair & A. De Vries. 2005. «Fine-scale selection by marten during winter in a young deciduous forest». *Canadian Journal of Forest Research*, vol. 35, no 4, p. 901-909.

Preston, C. M., & J. A. Trofymow. 2000. «Variability in litter quality and its relationship to litter decay in Canadian forests». *Canadian Journal of Botany*, vol. 78, no 10, p. 1269-1287.

Progar, R. A., T. D. Schowalter, C. M. Freitag & J. J. Morrell. 2000. «Respiration from coarse woody debris as affected by moisture and saprotroph functional diversity in Western Oregon». *Oecologia*, vol. 124, no 3, p. 426-431.

Pyle, C., & M. M. Brown. 1999. «Heterogeneity of wood decay classes within hardwood logs». *Forest Ecology and Management*, vol. 114, no 2-3, p. 253-259.

R Development Core Team. 2010. *R Development Core Team (2010). R: A language and environment for statistical computing, reference index version 2.10.1. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0,* http://www.R-project.org/. p.

Råberg, U., M. L. Edlund, N. Terziev & C. J. Land. 2005. «Testing and evaluation of natural durability of wood in above ground conditions in Europe - An overview». *Journal of Wood Science*, vol. 51, no 5, p. 429-440.

Rajala, T., M. Peltoniemi, T. Pennanen & R. Mäkipää. 2010. «Relationship between wood-inhabiting fungi determined by molecular analysis (denaturing gradient gel electrophoresis) and quality of decaying logs». *Canadian Journal of Forest Research*, vol. 40, no 12, p. 2384-2397.

Rajala, Tiina, Mikko Peltoniemi, Jarkko Hantula, Raisa Mäkipää & Taina Pennanen. 2011. «RNA reveals a succession of active fungi during the decay of Norway spruce logs». *Fungal Ecology*, vol. 4, no 6, p. 437-448.

Ramette, A. 2007. «Multivariate analyses in microbial ecology». *FEMS Microbiology Ecology*, vol. 62, no 2, p. 142-160.

Ranius, T., O. Kindvall, N. Kruys & B. G. Jonsson. 2003. «Modelling dead wood in Norway spruce stands subject to different management regimes». *Forest Ecology and Management*, vol. 182, no 1-3, p. 13-29.

Rayner, A. D. M., & L. Boddy. 1988. *Fungal Decomposition of Wood: Its Biology and Ecology*. New-York: Wiley.

Rothman, J. M., P. J. Van Soest & A. N. Pell. 2006. «Decaying wood is a sodium source for mountain gorillas». *Biology Letters*, vol. 2, no 3, p. 321-324.

Salles, J. F., J. A. Van Veen & J. D. Van Elsas. 2004. «Multivariate analyses of *Burkholderia* species in soil: Effect of crop and land use history». *Applied and Environmental Microbiology*, vol. 70, no 7, p. 4012-4020.

Seedre, M., B. M. Shrestha, H. Y. H. Chen, S. Colombo & K. Jõgiste. 2011. «Carbon dynamics of North American boreal forest after stand replacing wildfire and clearcut logging». *Journal of Forest Research*, vol. 16, no 3, p. 168-183.

Seifert, Keith A. 1983. «Decay of Wood by the Dacrymycetales». *Mycologia*, vol. 75, no 6, p. 1011-1018.

Setäla, H., & M. A. McLean. 2004. «Decomposition rate of organic substrates in relation to the species diversity of soil saprophytic fungi». *Oecologia*, vol. 139, no 1, p. 98-107.

Siitonen, J., & P. Martikainen. 1994. «Occurrence of rare and threatened insects living on decaying Populus tremula: A comparison between Finnish and Russian Karelia». *Scandinavian Journal of Forest Research*, vol. 9, no 2, p. 185-191.

Siitonen, J., P. Martikainen, P. Punttila & J. Rauh. 2000. «Coarse woody debris and stand characteristics in mature managed and old-growth boreal mesic forests in southern Finland». *Forest Ecology and Management*, vol. 128, no 3, p. 211-225.

Siitonen, Juha. 2001. «Forest management, coarse woody debris and saproxylic organisms: Fennoscandian boreal forests as an example». *Ecological Bulletins*, vol. 49, p. 11-41.

Similä, M., J. Kouki & P. Martikainen. 2003. «Saproxylic beetles in managed and seminatural Scots pine forests: Quality of dead wood matters». *Forest Ecology and Management*, vol. 174, no 1-3, p. 365-381.

Sippola, A. L., M. Mönkkönen & P. Renvall. 2005. «Polypore diversity in the herb-rich woodland key habitats of Koli National Park in eastern Finland». *Biological Conservation*, vol. 126, no 2, p. 260-269.

Sippola, A. L., & P. Renvall. 1999. «Wood-decomposing fungi and seed-tree cutting: A 40-year perspective». *Forest Ecology and Management*, vol. 115, no 2-3, p. 183-201.

Sippola, A. L., M. Simila, M. Mönkkönen & J. Jokimäki. 2004. «Diversity of polyporous fungi (Polyporaceae) in northern boreal forests: Effects of forest site type and logging intensity». *Scandinavian Journal of Forest Research*, vol. 19, no 2, p. 152-163.

Sippola, Anna-Liisa, Timo Lehesvirta & Pertti Renvall. 2001. «Effects of Selective Logging on Coarse Woody Debris and Diversity of Wood-Decaying Polypores in Eastern Finland». *Ecological Bulletins*, no 49, p. 243-254.

Spano, S. D., M. F. Jurgensen, M. J. Larsen & A. E. Harvey. 1982. «Nitrogen-fixing bacteria in Douglas-fir residue decayed by Fomitopsis pinicola». *Plant and Soil*, vol. 68, no 1, p. 117-123.

Speight, M. C. D. 1989. *Saproxylic invertebrates and their conservation*. Strasbourg.

Steeger, C., & C. L. Hitchcock. 1998. «Influence of forest structure and diseases on nestsite selection by red-breasted nuthatches». *Journal of Wildlife Management*, vol. 62, no 4, p. 1349-1358.

Stenlid, J., R. Penttilä & A. Dahlberg. 2008. «Chapter 13 Wood-decay basidiomycetes in boreal forests: Distribution and community development». p. 239-262: British Mycological Society Symposia Series.

Stokland, J., & H. Kauserud. 2004. «*Phellinus nigrolimitatus* - A wood-decomposing fungus highly influenced by forestry». *Forest Ecology and Management*, vol. 187, no 2-3, p. 333-343.

Sverdrup-Thygeson, A., & R. A. Ims. 2002. «The effect of forest clearcutting in Norway on the community of saproxylic beetles on aspen». *Biological Conservation*, vol. 106, no 3, p. 347-357.

Takahashi, K. H., & T. Kagaya. 2005. «Guild structure of wood-rotting fungi based on volume and decay stage of coarse woody debris». *Ecological Research*, vol. 20, no 2, p. 215-222.

Tedersoo, L., T. Suvi, T. Jairus & U. Kõljalg. 2008. «Forest microsite effects on community composition of ectomycorrhizal fungi on seedlings of *Picea abies* and *Betula pendula*». *Environmental Microbiology*, vol. 10, no 5, p. 1189-1201.

Ter Braak, Cajo J. F., & I. Colin Prentice. 1988. «A Theory of Gradient Analysis». *Advances in Ecological Research*, vol. 18, p. 271-317.

Tiunov, A. V., & S. Scheu. 2005. «Facilitative interactions rather than resource partitioning drive diversity-functioning relationships in laboratory fungal communities». *Ecology Letters*, vol. 8, no 6, p. 618-625.

Ucitel, D., D. P. Christian & J. M. Graham. 2003. «Vole use of coarse woody debris and implications for habitat and fuel management». *Journal of Wildlife Management*, vol. 67, no 1, p. 65-72.

Urcelay, C., & G. Robledo. 2004. «Community structure of polypores (Basidiomycota) in Andean alder wood in Argentina: Functional groups among wood-decay fungi?». *Austral Ecology*, vol. 29, no 4, p. 471-476.

Van Der Heijden, M. G. A., R. D. Bardgett & N. M. Van Straalen. 2008. «The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems». *Ecology Letters*, vol. 11, no 3, p. 296-310.

Van Wagner, C. E. (1982). Practical aspects of the line intersect method. Chalk River, Petawawa Natl. For. Inst.: 18 p.

Vasiliauskas, R., V. Lygis, K. H. Larsson & J. Stenlid. 2005. «Airborne fungal colonisation of coarse woody debris in North Temperate *Picea abies* forest: Impact of season and local spatial scale». *Mycological Research*, vol. 109, no 4, p. 487-496.

Vasiliauskas, R., A. Menkis, R. D. Finlay & J. Stenlid. 2007. «Wood-decay fungi in fine living roots of conifer seedlings». *New Phytologist*, vol. 174, no 2, p. 441-446.

Vasiliauskas, R., A. Vasiliauskas, J. Stenlid & A. Matelis. 2004. «Dead trees and protected polypores in unmanaged north-temperate forest stands of Lithuania». *Forest Ecology and Management*, vol. 193, no 3, p. 355-370.

Větrovský, T., J. Voříšková, J. Šnajdr, J. Gabriel & P. Baldrian. 2011. «Ecology of coarse wood decomposition by the saprotrophic fungus *Fomes fomentarius*». *Biodegradation*, vol. 22, no 4, p. 709-718.

Wardle, D. A., K. I. Bonner, G. M. Barker, G. W. Yeates, K. S. Nicholson, R. D. Bardgett, R. N. Watson & A. Ghani. 1999. «Plant removals in perennial grassland: Vegetation dynamics, decomposers, soil biodiversity, and ecosystem properties». *Ecological Monographs*, vol. 69, no 4, p. 535-568.

Wells, J. M., & L. Boddy. 2002. «Interspecific carbon exchange and cost of interactions between basidiomycete mycelia in soil and wood». *Functional Ecology*, vol. 16, no 2, p. 153-161.

Wertz, S., V. Degrange, J. I. Prosser, F. Poly, C. Commeaux, T. Freitag, N. Guillaumaud & X. L. Roux. 2006. «Maintenance of soil functioning following erosion of microbial diversity». *Environmental Microbiology*, vol. 8, no 12, p. 2162-2169.

White, T. J., T.D. Bruns, S.B. Lee & J.W. Taylor. 1990. «Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics». In *PCR - Protocols and Applications - A Laboratory Manual,* N. Innis, p. 315-322. New York, NY: Academic Press.

Wohl, D. L., S. Arora & J. R. Gladstone. 2004. «Functional redundancy supports biodiversity and ecosystem function in a closed and constant environment». *Ecology*, vol. 85, no 6, p. 1534-1540.

Woods, C. M., S. Woodward & D. B. Redfern. 2005. «In vitro interactions in artificial and wood-based media between fungi colonizing stumps of Sitka spruce». *Forest Pathology*, vol. 35, no 3, p. 213-229.

Woodward, S., & L. Boddy. 2008. «Chapter 7 Interactions between saprotrophic fungi». p. 125-141: British Mycological Society Symposia Series.

Wormington, K. R., D. Lamb, H. I. McCallum & D. J. Moloney. 2003. «The characteristics of six species of living hollow-bearing trees and their importance for arboreal marsupials in the dry sclerophyll forests of southeast Queensland, Australia». *Forest Ecology and Management*, vol. 182, no 1-3, p. 75-92.

Yamashita, S., T. Hattori & H. Abe. 2010. «Host preference and species richness of wood-inhabiting aphyllophoraceous fungi in a cool temperate area of Japan». *Mycologia*, vol. 102, no 1, p. 11-19.