Tree communities rapidly alter soil microbial resistance and resilience to drought

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Summary

1. The ability of soil microbial communities to withstand and recover from disturbance or stress is important for the functional stability of forest ecosystems. However, the relationship between the community responses of soil microbes and variation in tree mixtures vs functional composition remains poorly understood.

2. We investigated soil biochemical properties and soil microbial resistance and resilience to drought in three 4-year-old tree monocultures (Acer saccharum Marsh, Larix laricina (Duroi) K. Koch and Pinus strobus L.) and two tree species combinations (L. laricina/A. saccharum and L. laricina/P. strobus) planted in a high-density tree field experiment located in southern Quebec, Canada. The experimentally imposed drought stress consisted of maintaining soil material for 30 days at 25% of water-holding capacity (WHC). Microbial biomass was assessed immediately after the water stress (resistance) and 15 and 30 days following drought (resilience).

3. Results showed that tree communities influenced soil chemistry, soil respirometry properties and microbial resistance and resilience. We measured significant non-additive (i.e. both synergistic and antagonistic) effects of mixing tree species in some of the soil biochemical properties measured, mostly in the L. laricina/A. saccharum mixture. However, we did not find non-additive effects of tree mixtures on microbial resistance and resilience. A structural equation modelling analysis revealed that resistance and resilience were mostly modulated by direct effects of community-weighted means (CWM) of leaf litter lignin content and mineralizable N, and by indirect links from tree density and CWM of leaf litter N content via mineralizable N.

4. This study suggests that tree species identity surpassed species mixtures as a key driver of soil microbial resistance and resilience. We showed a trade-off between microbial resistance and resilience in soil food webs, which is consistent with ecological theory. Our results indicate that differences in functional traits between tree species may rapidly be reflected in divergent soil biochemical properties and that these differences can in turn drive soil microbial resistance and resilience to drought.

Key-words: ecosystem functioning, high-density tree-based field experiment, microbial stability, soil biochemical properties, tree functional traits, tree mixture, tree–soil interaction

Introduction

The soil biota is an essential component of forest ecosystems, mediating fundamental nutrient and energy flow processes that ensure primary production, regulating climate changes and sustaining biodiversity (Wardle et al. 2004; Dominati, Patterson & Mackay 2010). However, the stability of these ecosystem processes and services may be altered by different environmental and anthropogenic disturbances (Allison & Martiny 2008; Hartmann et al. 2014). Ecosystem stability encompasses two components: resistance, which is the ability of a system to withstand a
disturbance without much change in functioning or composition; and resilience, which is the extent and speed of recovery of ecosystem properties following a disturbance (Pimm 1984). In this paper, we have concentrated only upon the extent component of recovery. Resistant or resilient soil microbial communities are desirable for sustainable soil use and management, as they tend to better maintain soil functions for sustained tree productivity or other ecosystem services (Griffiths & Philippot 2013). Therefore, it is important to understand how soil microbial communities respond to disturbance or to chronic stress, and the factors that determine this response, such as forest structure and composition.

Resistance and resilience of soil biota may be controlled by multiple soil properties, including microbial biomass and community diversity, pH, nutrient availability and texture (e.g. DeAngelis et al. 1989; Orwin, Wardle & Greenfield 2006; Royer-Tardif, Bradley & Parsons 2010). Yet, many of these soil properties can be altered by tree community composition. For example, Jiang et al. (2012) showed that microbial functional diversity under broadleaf tree species was much higher than under conifers. Likewise, differences among temperate tree species in terms of their litter production and chemistry (e.g. N content, lignin content, lignin:N ratio) were sufficient to cause divergence in soil properties such as pH, C and N pools, as well as N mineralization rates (Lovett et al. 2004; Reich et al. 2005; Mueller et al. 2012). Consequently, the presence of different tree species having different above- and below-ground functional traits may result in soil microbial communities with different abilities to resist and recover after a disturbance.

Soil properties are also likely to be affected by mixing tree species. For example, trees growing in mixed-species stands may lead to increases in net tree nutrient uptake (Richard et al. 2010) and to increased above- and below-ground tree productivity (Brassard et al. 2011; Gamfeldt et al. 2013), which may ultimately increase rhizodeposition and the amount of litter that is returned to the soil. Litter mixtures from different plant species often decompose and release nutrients at different rates than would be expected from the rates of the component species decomposing alone, when these are averaged (Gartner & Cardon 2004). These non-additive effects on nutrient cycling often can occur in mixtures containing as few as two species (McTieran, Ineson & Coward 1997; Dijkstra et al. 2009). Because tree diversity is relatively low in the northern temperate biome, both in natural forests and in human-made tree ecosystems (e.g. plantation forests, agroforestry systems), modest changes in species richness could result in large non-additive effects on litter dynamics and tree production (Finzi & Canham 1998; Paquette & Messier 2011), with potentially broad implications for soil biochemical properties and soil stability. Because soil microbial communities will be affected by soil physical and biochemical properties, we might expect that variation in tree species mixtures would affect the stability of these microbial communities as well. Yet, in an experiment using three common pasture plant species, Orwin & Wardle (2005) found no effect of plant species mixtures on microbial resistance and resilience to drought; rather, this microbial response was strongly affected by plant species composition. In contrast, Pfisterer & Schmid (2002) showed that an inverse relationship existed between grassland plant species richness and microbial resistance and resilience to drought. Surprisingly, little is known about the specific effects of tree species composition and mixtures on soil microbial resistance and resilience to drought in tree communities, which would have obviously different above- and below-ground functional traits compared to those of grassland communities.

The relationship between the community responses of soil microbes and variation in tree species mixtures vs. functional composition is therefore unclear. Here, we investigated soil chemical and microbial community properties, and soil microbial resistance and resilience to drought in different 4-year-old tree monocultures and two-species combinations that were planted in a high-density tree experiment that was located in southern Quebec, Canada (Tobner et al. 2014). Tree monocultures included the deciduous broadleaf sugar maple (Acer saccharum Marsh), the deciduous conifer eastern larch or tamarack (Larix laricina (Du Roi) K. Koch) and the evergreen conifer white pine (Pinus strobus L.), while the mixtures included sugar maple with tamarack, and white pine with tamarack. These three North American temperate tree species have contrasting ecological strategies for successful establishment. They differ in shade tolerance (sugar maple > white pine > tamarack), litter lignin content (tamarack > white pine > sugar maple) and leaf life span (sugar maple and tamarack, 5–6 months; white pine, 20–32 months), among others. We hypothesized (i) that differences in functional traits between tree species would be reflected in divergent soil biochemical properties and that these differences, in turn, would drive soil microbial resistance and resilience to a drying stress. We next posited (ii) that growing species in mixtures would result in non-additive effects on soil biochemical properties and on microbial resistance and resilience. Given that fungi-based soil food webs are expected to be more resistant and less resilient than those composed of fast-growing organisms such as bacteria (de Vries et al. 2012), we also posited (iii) that soils in plots that included conifers only, which produce lignin-rich litters that can increase the fungi/bacterial ratio (Neely et al. 1991), would be more resistant but less resilient than those plots that included sugar maple. Given the previous hypothesis (iii), we expected (iv) that there would be a trade-off between microbial resistance and resilience (Orwin, Wardle & Greenfield 2006; de Vries et al. 2012).

Materials and methods

SITE DESCRIPTION AND SOIL SAMPLING

The experimental site is located on the Macdonald College Campus of McGill University (Sainte-Anne-de-Bellevue, Quebec,
Canada, 45°28’N, 73°45’W; 36 m asl). The region is characterized by an annual temperature of 6-1 °C and 967 mm of precipitation (Dorval climate station; Environment Canada, 2014). The soil is a Humic Gleysol (Typic Endoaquent) with a pHwater of 6-3 in the 0- to 20-cm sandy loam layer (containing, on average, 78% sand, 6% silt and 16% clay). The experiment, which is part of the International Diversification Experiment Network with Trees (IDENT) that is linked to TreeDivNet (Tobner et al. 2014), was established in spring 2009 on a former agricultural field. Among the species that were present on this experiment, three were selected for our present study. One-year-old seedlings of sugar maple and two-year-old seedlings of tamarack and white pine were planted to create five different tree communities, each of which was replicated in four blocks. Each species was planted in monoculture and in two-species combinations, viz. sugar maple with tamarack and tamarack with white pine. Tree community plots, which measured 4 × 4 m, consisted of 64 individual trees that had been planted every 50 cm in eight rows. Rows were separated by 25-m-wide corridors to reduce interactions between communities and to allow for the movement of personnel and equipment without disturbing the plots. These corridors were trenched down to a 30 cm depth during the summers of 2011 and 2012 to prevent roots from neighbouring tree communities from interacting. Growth on the site was rapid and, together with the high density at which they were planted and negligible mortality, the trees already had formed a closed canopy by the fourth year after planting. Component species of each mixed-species community were planted in equal densities. Individual community locations within a block and tree locations within a community were randomized with restrictions on the latter to prevent clumping of species and to maintain the same diversity both outside the perimeter (i.e. a buffer) and inside the 6 × 6 grid. Communities were periodically weeded to maintain desired composition and diversity.

SOIL SAMPLING AND ANALYSES OF BIOCHEMICAL PROPERTIES

In each tree community plot, four soil cores (7 cm diameter, 0-20 cm depth) were randomly collected on 5 June 2012 (preceded by four days without precipitation), passed through a 4 mm mesh screen and bulked into two composite samples. Soil samples were transported on ice to the laboratory and stored at 4 °C prior to analyses. Soil moisture was determined gravimetrically from oven-dried subsamples (c. 10 g, 105 °C for 48 h). Water-holding capacity (WHC) of each soil sample was determined as the mass of water that was retained in a soil following saturation and 18 h after drainage. Bulk pH was measured in slurries containing 5 g of oven-dried (65 °C) soil and 25 mL of deionized water. Total C and N were determined by high-temperature combustion (150 mg oven-dried, ground subsamples) followed by thermo-conductimetric detection, on a CN Analyser (Vario Macro, Elementar GmbH, Hanau, Germany). Bray-extractable P was measured by ICP (Optima 4300 DV, PerkinElmer, Waltham, MA, USA).

Nutrient availability was measured by routine soil extractions. Mineralizable N was measured as the net increase in inorganic N (NH₄⁺ + NO₃⁻) over 30-day aerobic, dark incubations in the laboratory (at 22 °C). Fresh subsamples (c. 300 g dry mass equiv.) were transferred to 500-mL glass jars. Sample moisture was gravimetrically adjusted and maintained at 60% WHC by periodically adding deionized water. Before and after incubation, soils were extracted in 1 m KCl and analysed for NH₄⁺ and NO₃⁻. Fresh soil subsamples (c. 20 g) were extracted in 100 mL of 1 m KCl solution, shaken for 1 h on a reciprocal shaker and gravity-filtered through cellulose papers (Whatman No. 5). Filtrates were analysed for NH₄⁺ and NO₃⁻ by flow injection (QuikChem FIA 4000, Lachat Instruments, Loveland, CO, USA).

Microbial dynamics were assessed by respirometry and by measurements of metabolic efficiency (μCO₂). Basal respiration was determined by weighing fresh subsamples (c. 32 g dry mass equiv.) into 126-mL gas sampling jars, allowing 1 week of conditioning at room temperature (c. 22 °C), flushing the headspace with ambient air for 5 min, sealing jars with air-tight lids equipped with rubber septa and sampling aliquots of the headspace with a needle and syringe after 4 h. CO₂ concentrations were analysed with a gas chromatograph (341-GC, Varian Canada; Mississauga, ON, Canada) that was equipped with a TCD and which used high-purity helium as the carrier gas. Room temperature and ambient CO₂ concentrations were noted during each measurement. Ambient CO₂ was subtracted from sample CO₂ concentrations, and the differences were adjusted according to ideal gas laws and centred at 22 °C using Q₁₀ = 2. Microbial biomass C was determined from substrate-induced respiration (SIR) measurements (Anderson & Domsch 1978) following the procedure described in Rivest et al. (2013). Values of metabolic quotient (qCO₂) were calculated as the ratio of basal respiration to microbial biomass C.

SOIL MICROBIAL RESISTANCE AND RESILIENCE

To determine the effects of tree species composition and richness on soil microbial resistance and resilience, we performed an assay involving a drying stress. Soil subsamples (c. 300 g dry mass equiv.) were incubated in 500-mL glass jars for 30 days using two levels of soil WHC (25% and 65%, respectively referred to as stressed and control). After this stress period of 30 days, soil samples were adjusted and maintained at 65% of WHC for an additional 30 days (adapted from Hueso, Hernández & García 2011; and de Vries et al. 2012). Moisture levels were maintained gravimetrically twice a week. For each site, the glass jars were randomly assigned to four closed chambers (one per block) under controlled conditions of temperature (22 °C) and humidity (60%). Microbial biomass was measured using the aforementioned procedure at days 30 (i.e. t₀, immediately after the disturbance), 45 (i.e. t₁₅, 15 d after disturbance) and 60 (i.e. t₃₀, 30 d after disturbance). Microbial biomass was simultaneously measured in control subsamples that had not been stressed. Microbial resistance and resilience were determined as follows (Banning & Murphy 2008):

Resistance = \(-100 \left( \frac{Co - So}{Co} \right) \) at \(t₀\)

Resilience =

\(-100 \left( \frac{Co - So}{Co} \right) - 100 \left( \frac{Cx - Sx}{Cx} \right) \) at \(t₁₅ \) or \(t₃₀\)

where \(Co\) is the control sample microbial biomass and \(So\) is the stressed soil microbial biomass at time 0, and \(Cx\) and \(Sx\) are soil microbial biomass at time 15 or 30 days after the drought period. Thus, resistance was measured as the per cent change in microbial biomass under stressed condition relative to the control immediately after the stress was removed. A microbial resistance value of zero indicates no difference between the stressed and control soils (i.e. maximal resistance). Increasingly negative values indicate lowering resistance up to a value of −100, which occurs when the response variable in the treated soil is zero (i.e. minimal resistance). The index is unbounded for positive values, which occur when the response variable in the test soil is higher than in the control. The resilience index expresses the % difference from the control at each point in time, relative to the size of the initial change. A value of zero indicates no recovery, negative index values indicate decline rather than recovery, and positive values indicate recovery relative to the controls for a given point in time.

DATA ANALYSES

Effects of tree communities on soil biochemical properties and microbial resistance were analysed using a mixed-effects model ANOVA that included two factors: block (random) and tree community (fixed). Mixed-effects models investigating tree community effects on microbial resilience also included time as a second fixed factor. Data deviating from normality or homoscedasticity assumptions were log-transformed. When the ANOVA was significant ($P < 0.05$), we compared the means using least significant difference (LSD) post hoc tests. Analyses were conducted in SAS (version 9.2, SAS Institute, Inc., Cary, NC, USA).

We calculated non-additive tree mixture effects on soil properties according to the formula $(O-E)/E$, where $O$ is the observed soil response for each replicate within a tree community and $E$ is the expected response (Wardle, Bonner & Nicholson 1997; Meier & Bowman 2010). Expected values ($E$) were calculated for each tree community according to the following equation:

$$E = \sum_{i=1}^{S} R_i / S$$

where $R_i$ is the soil response when tree species $i$ was added alone, and $S$ is the total number of species in the mixture (i.e. two). To assess whether $(O-E)/E$ responses were significantly different from zero (i.e. the response was non-additive as opposed to additive), we used a $t$-test.

We used structural equation modelling (SEM) to study the direct and indirect effects of a series of factors on soil microbial resistance and resilience. SEM provides a means of representing complex hypotheses about causal networks, accounting for factors that are both causes and effects, and testing for model data consistency (Shipley 2000; Grace 2006). We developed an initial conceptual model as the starting point of a SEM investigation (Fig. 1). This initial model was based on our incomplete knowledge of individual relationships among tree traits, soil chemical and microbial community properties, and soil stability (see Introduction for predicted relationships and respective references). We first created six conceptual groups of measured variables, which were measured either in the present study or at the same experimental site during 2012 in companion studies (Tobner, Paquette & Messier 2013; Archambault 2014; Jewell 2013; Tobner 2014). These conceptual groups represented (i) environmental variables that varied spatially but not temporally, (ii) community-weighted trait means (CWM) that reflected the functional identity of the tree communities, (iii) two measures of tree density, (iv) five variables that were related to soil chemical responses, (v) four variables that were related to soil microbial response, and (vi) our two measures of soil stability. Conceptually, CWM are based on the ‘mass ratio hypothesis’, which states that the effect of each species on an ecosystem process is proportional to its relative abundance in the community (Grime 1998). Thus, the effects of the different trait values of each species on an ecosystem process were proportional to the mean trait value of each species, weighted by its relative abundance (Diaz et al. 2004; Garnier et al. 2004). CWM were weighted by species abundances in 2012, which were based on stem volumes (i.e. multiplying total height by the square of diameter measured at 5 cm above the ground level), and calculated following the methods of Lavorel et al. (2008), using the FD package (Laliberté & Shipley 2011) in R (R Development Core Team 2008) for each functional trait (see Table 1, Fig. 1 and Appendix S1, Supporting information). Because our previous knowledge did not allow us to formulate precise multivariate causal hypotheses linking all of these variables, we used SEM in an exploratory mode to obtain hypothesized causal models that were both consistent with our data and which made biological sense. Thus, our resulting SEM should be viewed as a hypothesis rather than a test of a pre-existing hypothesis. Our relatively small sample size (40) and large number of potential variables did not allow for the computation and comparison of all possible path models with high statistical power. Instead, we used a stepwise procedure to obtain the most parsimonious set of predictors, beginning with all plausible interaction paths among the factors that were contained in our a priori model, based on the Akaike Information Criterion (AIC). Chi-square tests were used to assess model fit (other tests are also reported). SEM analyses were conducted in R using the lavaan package (Rosseel 2012).

Results

Tree communities differed in their effects on soil biochemical properties (Table 2). Soil moisture was highest in the sugar maple monoculture plots and lowest in the tamarack monoculture and tamarack/white pine mixture plots.
Table 1. Traits of three species included in the present study and microbial community structure under these species planted in monocultures at the IDENT Montreal site (Québec, Canada)

<table>
<thead>
<tr>
<th>Trait</th>
<th>Units</th>
<th>Sugar maple</th>
<th>Tamarack</th>
<th>White pine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broad group</td>
<td></td>
<td>Deciduous broadleaf</td>
<td>Deciduous conifer</td>
<td>Evergreen conifer</td>
</tr>
<tr>
<td>Shade tolerance†</td>
<td>4.8</td>
<td>1</td>
<td>1.36</td>
<td>1.42</td>
</tr>
<tr>
<td>Leaf traits§</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrogen concentration</td>
<td>%</td>
<td>1.83</td>
<td>1.36</td>
<td>1.42</td>
</tr>
<tr>
<td>Phosphorus concentration</td>
<td>%</td>
<td>0.72</td>
<td>0.08</td>
<td>0.1</td>
</tr>
<tr>
<td>Leaf dry matter*</td>
<td>g m⁻¹</td>
<td>412</td>
<td>295</td>
<td>344</td>
</tr>
<tr>
<td>Specific leaf area*</td>
<td>m² g⁻¹</td>
<td>11.27</td>
<td>8.26</td>
<td>6.54</td>
</tr>
<tr>
<td>Leaf litter traits§</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lignin concentration</td>
<td>%</td>
<td>16.6</td>
<td>33.3</td>
<td>22.3</td>
</tr>
<tr>
<td>Nitrogen concentration*</td>
<td>%</td>
<td>43.5</td>
<td>51.2</td>
<td>52.5</td>
</tr>
<tr>
<td>Carbon concentration*</td>
<td>%</td>
<td>63.0</td>
<td>74.0</td>
<td>70.7</td>
</tr>
<tr>
<td>C:N*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lignin:N</td>
<td></td>
<td>2.4</td>
<td>4.82</td>
<td>3.00</td>
</tr>
<tr>
<td>Decomposition rate*</td>
<td>g g⁻¹ d⁻¹</td>
<td>0.00276</td>
<td>0.00197</td>
<td>0.00145</td>
</tr>
<tr>
<td>Fine root traits¶</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diameter*</td>
<td>mm</td>
<td>0.33</td>
<td>0.38</td>
<td>0.56</td>
</tr>
<tr>
<td>Specific root length*</td>
<td>m g⁻¹</td>
<td>57.8</td>
<td>41.3</td>
<td>16.1</td>
</tr>
<tr>
<td>Branching intensity*</td>
<td>tips cm⁻¹</td>
<td>2.7</td>
<td>2.8</td>
<td>3.2</td>
</tr>
<tr>
<td>Tree above-ground traits</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height *</td>
<td></td>
<td></td>
<td>cm</td>
<td>242</td>
</tr>
<tr>
<td>Relative growth rate*¶**</td>
<td></td>
<td>0.67</td>
<td>0.88</td>
<td>0.70</td>
</tr>
<tr>
<td>Microbial community structure††</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fungal biomass*</td>
<td>ng g⁻¹</td>
<td>125</td>
<td>256</td>
<td>146</td>
</tr>
<tr>
<td>Bacterial biomass*</td>
<td>ng g⁻¹</td>
<td>1081</td>
<td>1343</td>
<td>877</td>
</tr>
<tr>
<td>Fungal:bacterial ratio*</td>
<td></td>
<td>0.11</td>
<td>0.19</td>
<td>0.16</td>
</tr>
</tbody>
</table>

*Traits measured locally.  
†Niinemets & Valladares (2006).  
‡Glopnet (Wright et al. 2004) for [N] and [P] and Jewell (2013) for leaf dry matter and specific leaf area.  
||Mean height of trees measured in monocultures in autumn 2012 (Tobner et al. 2014).  
**Relative growth rate (RGR) was calculated based on volume (vol = (trunk diameter at 5 cm)² × total tree height); RGR = (log vol autumn 2011 - log vol spring 2009)/3 growth periods (i.e. vegetation periods of 2009 through 2011).  
††Fungal and bacterial biomass was determined by analysis of group-specific phospholipid fatty acids (PLFAs) from composite soil samples (0–5 cm depth, collected on 7 June 2012, soil cores from each plot were bulked into one sample per tree species) according to the protocol described in Hamel et al. (2006).

Bray-extractable P concentrations in soils of the tamarack monocultures were significantly higher than in all other tree communities. Mineralizable N in the monocultures and mixed plots that contained white pine was significantly higher than in the sugar maple monoculture plots. Soil pH was significantly higher in the tamarack/sugar maple mixture than in tamarack and sugar maple monocultures. Of all tree communities, soils from the sugar maple monoculture plots exhibited the lowest means of microbial biomass, basal respiration and qCO₂. The fungi/bacterial ratio was highest in the tamarack monoculture plots and lowest in the sugar maple monoculture plots (Table 1).

Resistance of microbial biomass in the tamarack monoculture plots was significantly ($P = 0.0109$) higher than in the sugar maple and white pine monoculture plots (Fig. 2). Conversely, soil from the sugar maple and white pine monoculture plots had significantly higher resilience 30 days after water stress compared to soils from the tamarack monoculture plots, but not after 15 days (time x tree community interaction, $P = 0.0484$).

Some significant non-additive tree mixture effects on soil biochemical properties were observed (Fig. 3). The tamarack/sugar maple ($P < 0.0001$) and tamarack/white pine ($P = 0.0299$) mixtures had significantly less Bray-extractable P than expected, when compared to their respective component monocultures. Observed values of total C were significantly lower in the tamarack/sugar maple mixture than expected, compared to the component species in monoculture ($P = 0.0191$). In contrast, the tamarack/sugar maple mixture exhibited significantly higher values of mineralizable N ($P = 0.05$), pH ($P = 0.0134$) and qCO₂ ($P = 0.009$) than would be expected in the component monocultures.

Our final parsimonious SEM model linked soil microbial resistance and resilience (30 days after drying stress) to litter nitrogen and lignin contents (as CWM), stem volume (proxy for tree density) and mineralizable N. This model could not be rejected as a causal explanation since the $\chi^2$ test ($P = 0.304$) and other indices all indicated good fit (Fig. 4). Environmental factors (elevation and soil texture),
Table 2. Soil biochemical properties in different tree communities at the IDENT Montreal site (Québec, Canada)

<table>
<thead>
<tr>
<th>Soil property</th>
<th>S. Maple</th>
<th>Tamarack + S. Maple</th>
<th>Tamarack</th>
<th>Tamarack + W. Pine</th>
<th>W. Pine</th>
<th>P-value (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil moisture (%)</td>
<td>0.21 (0.01) a</td>
<td>0.19 (0.005) b</td>
<td>0.17 (0.01) c</td>
<td>0.17 (0.004) c</td>
<td>0.19 (0.01) b</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total C (mg g⁻¹)</td>
<td>22.93 (0.94)</td>
<td>2.16 (0.66)</td>
<td>24.57 (0.87)</td>
<td>22.40 (1.11)</td>
<td>23.60 (0.78)</td>
<td>0.1330</td>
</tr>
<tr>
<td>Total N (mg g⁻¹)</td>
<td>2.26 (0.07)</td>
<td>2.14 (0.06)</td>
<td>2.28 (0.10)</td>
<td>2.21 (0.08)</td>
<td>2.30 (0.08)</td>
<td>0.6167</td>
</tr>
<tr>
<td>Bray-extractable P (µg g⁻¹)</td>
<td>358.7 (17.7)</td>
<td>358.7 (17.7)</td>
<td>413.9 (15.6) a</td>
<td>357.7 (12.8) b</td>
<td>367.1 (6.6) b</td>
<td>0.0003</td>
</tr>
<tr>
<td>Mineralizable N (mg kg⁻¹ 30 d⁻¹)</td>
<td>32.33 (8.80) b</td>
<td>49.84 (8.28) ab</td>
<td>47.17 (3.95) ab</td>
<td>60.79 (6.31) a</td>
<td>64.16 (6.78) a</td>
<td>0.0010</td>
</tr>
<tr>
<td>pH (in water)</td>
<td>6.23 (0.08) b</td>
<td>6.48 (0.07) ab</td>
<td>6.26 (0.06) b</td>
<td>6.36 (0.05) ab</td>
<td>6.24 (0.06) ab</td>
<td>0.0333</td>
</tr>
<tr>
<td>Microbial biomass (mg C g⁻¹)</td>
<td>0.22 (0.01) c</td>
<td>0.25 (0.01) c</td>
<td>0.30 (0.01) a</td>
<td>0.29 (0.01) ab</td>
<td>0.26 (0.01) b</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Basal respiration (mg CO₂-C g⁻¹ h⁻¹)</td>
<td>0.49 (0.02) b</td>
<td>0.71 (0.04) a</td>
<td>0.74 (0.08) a</td>
<td>0.73 (0.04) a</td>
<td>0.75 (0.05) a</td>
<td>0.0039</td>
</tr>
<tr>
<td>Metabolic qCO₂ (mg CO₂-C g Cmic⁻¹ h⁻¹)</td>
<td>2.19 (0.07) b</td>
<td>2.84 (0.14) a</td>
<td>2.39 (0.19) b</td>
<td>2.57 (0.17) ab</td>
<td>2.91 (0.25) a</td>
<td>0.0271</td>
</tr>
</tbody>
</table>

Standard errors are given in parentheses. Means with a row not sharing the same letter are significantly different at $P < 0.05$ (least significant difference tests).

as well as soil chemical response variables (humidity, pH, or nutrient content), did not contribute any significant additional explanations and were excluded. This model explained 31% and 41% of the variation in soil resistance and resilience, respectively. There was a strong negative total correlation between resistance and resilience ($r = -0.92$). Although ~60% of this negative correlation was not related to the measured variables in this model (free correlation between the residuals = −0.6), the ~40% that was attributable to the measured variables was due to the opposite effects of litter nitrogen and lignin contents. Litter nitrogen content indirectly increased resilience (0.24 SD) via its effect on soil N mineralization and indirectly decreased resistance (−0.18 SD) via its effect on soil N mineralization. Contrary to litter nitrogen, litter lignin content directly decreased resilience (−0.6 SD) and directly increased resistance (0.5 SD) as well as indirectly decreasing resilience (−0.12 SD) via its direct effect on stem volume and then indirectly on soil N mineralization. This weak negative indirect effect was generated by a positive link between CWM litter lignin and tree stem volume that ran contrary to expectation (viz. species with high lignin content normally not being the most productive), due to tamarack, which was among both the most productive of our species and also exhibiting the highest leaf litter lignin content (Table 1).

**Discussion**

Our study hypothesized that different young monocultures and two-species combinations in a high-density plantation would alter soil biochemical properties and that these changes would in turn affect soil microbial resistance and resilience to an experimental drought stress. As expected, tree species in monoculture plots caused divergence in chemical and soil microbial community properties. These differences in soil biochemical properties were likely related to variation in tree traits that are associated with different growth strategies (Orwin et al. 2010), which may control the production and quality of resources that each tree species added or removed from the soil (Finzi & Canham 1998; Porazinska et al. 2003; Lovett et al. 2004). For instance, we found lower soil microbial biomass C and basal respiration in monoculture plots of sugar maple.
compared to tamarack (Table 2). These differences were likely related to greater growth rates and inputs of relatively fresh, easily decomposable plant material (tree roots and above-ground litter) that originated from tamarack (Table 1, Appendix S1), resulting in greater availability of metabolically accessible compounds in the monoculture plots containing tamarack. Our SEM model (Fig. 4) further revealed that mineralizable N was directly affected by the CWM leaf litter N content, which suggests yet again specific tree traits that are related to quality and quantity of organic matter inputs are sufficient to cause divergence in soil properties when species are grown on the same initial soil.

As expected, we found significant non-additive effects of mixing tree species on some soil biochemical properties that we measured (Fig. 3). However, these non-additive effects appeared to be restricted to the tamarack/sugar maple mixture. This behaviour is likely the result of greater diversity of traits (i.e. functional diversity) in the tamarack/sugar maple mixture than in the tamarack/white pine mixture plots (Tobner et al. 2014). For example, Meier & Bowman (2010) found that non-additive net N mineralization responses were positively correlated with the chemical diversity of plant litter mixtures. Future research should attempt to determine whether increasing tree functional diversity affects the magnitude and direction of non-additive tree mixture effects on soil biochemical properties. Our results suggested that the direction and strength of non-additive effects on soil properties depended strongly upon which soil response variable was being measured. The considerable synergistic effects of the tamarack/sugar maple mixture on both mineralizable N and $qCO_2$ may reflect a greater supply of fresh organic residues (Anderson & Domsch 1989) as a result of overyielding (Tobner 2014). The antagonistic effect of the tamarack/sugar maple mixture on total soil C could be due to further increases in the decomposition rate of that pool. However, tree mixtures did not induce significant non-additive effects on soil microbial resistance and resilience to drought, indicating that tree species identity surpassed
species mixture as a key driver of resistance and resilience to drought. These results are consistent with another study that found no effect of common pasture plant species mixtures but a strong effect of plant species composition on the microbial resistance and resilience to a drying stress (Orwin & Wardle 2005). The authors argued that different plant species that are grown in the same soil may create soils with microbial communities which differ in their responses to a drying stress. Indeed, studies involving soil (Griffiths & Philippot 2013) or plant (MacGillivray & Grime 1995) systems have suggested that community composition is an important driver of resistance and resilience.

In our study, soil microbial resistance was highest in tamarack monoculture plots and lowest in sugar maple monoculture plots (Fig. 3). These differences were reflected in litter with much greater lignin content, lower decomposition rate and higher fungal/bacterial ratio in the tamarack than sugar maple monoculture plots (Table 1). In accordance with these findings, de Vries et al. (2012) have provided evidence that fungal-based soil food webs are more resistant to drought than those based on bacteria. Resilience is implicitly related to growth rates, and because most bacteria have higher potential growth rates than fungi (Cooke & Whipp 1993), resilience should decrease when the fungi/bacteria ratio increases or when carbon and nutrient availability limits the growth rate of resistant microbial cells (Royer-Tardif, Bradley & Parsons 2010). This conjecture was supported by our SEM, which revealed that resilience was positively affected by mineralizable N.

In accordance with our last hypothesis, microbial resistance and resilience were negatively correlated (Fig. 4). This result indicates a trade-off between resistance and resilience in soil food webs, which is consistent with ecological theory related to resistance and resilience (Pimm 1984; Hedlund et al. 2004), as well as other observational studies in natural forests (Orwin, Wardle & Greenfield 2006) or agroecosystems (de Vries et al. 2012). Orwin, Wardle & Greenfield (2006) suggested that there may be subsets of the soil microbial community that have different life-history strategies and features related to resistance and resilience that are trade-off in a similar manner to that found in plant systems. This trade-off may be related to the active and dormant fractions of the soil microbial biomass. The active fraction may represent the fast-growing and r-selected portion of the soil microbial biomass that is less resistant to drought, but more resilient because of its fast growth rate. The dormant fraction may correspond to the slow-growing and K-selected portion of the soil microbial biomass that is resistant to drought, but recovers slowly. Our SEM further suggested that the trade-off in microbial resistance and resilience was mostly modulated by direct effects of CWM leaf litter lignin content and N mineralization. CWM leaf litter lignin content imposed a positive effect on resistance and a negative effect on resilience. Conversely, mineralizable N incurred a negative effect on resistance and a positive effect on resilience. This phenomenon could be due to the fact that increasing litter lignin content and decreasing N mineralization rates are expected to increase drought-resistant, slow-growing microbes and to decrease drought-resistant, fast-growing microbes (Henriksen & Breland 1999). We recommend that future research be guided towards assessing whether the soil food webs resulting from contrasting tree communities (e.g. along a gradient of functional diversity) have significant implications for their resistance and resilience to drought, and whether these differences can have consequences for the delivery of soil ecosystem services such as C and N sequestration.

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Data accessibility
All data are included in the manuscript and Supporting Information.

References
Gamfeldt, L., Snäll, T., Bagchi, R., Jonsson, M., Gustafsson, L., Kjellander, P. et al. (2013) Higher levels of multiple ecosystem services are found in forests with more tree species. Nature Communications, 4, 1340.


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**Supporting Information**

Additional Supporting information may be found in the online version of this article:

**Appendix S1.** Explanatory variables used for SEM analyses (means across blocks; n = 40) in different tree communities at the IDENT Montreal site (Québec, Canada).