



## Antagonistic effects of species on C respiration and net N mineralization in soils from mixed coniferous plantations

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### ABSTRACT

Mixtures of litter from different plant species often show non-additive effects on decomposition and net N release (i.e., observed effects in mixtures differ from predictions based on litter of the component species), with positive non-additive (i.e., synergistic effects) being most common. Although large amounts of C and N reside in soil organic matter that contribute significantly to the overall C and N cycle, only a few studies have compared species monoculture vs. mixture effects on soil C and N dynamics. We studied the interactive effects of black spruce (*Picea mariana*), tamarack (*Larix laricina*), and white pine (*Pinus strobus*) on soil C respiration and net N mineralization in a plantation in northern Minnesota, USA. The trees were planted in monoculture and in all three possible two-species combinations (mixtures). After 10 years, we measured aboveground plant biomass and soil C respiration and net N mineralization rates in long-term (266 days) and short-term (13 days) laboratory incubations, respectively. Soil C respiration and net N mineralization were significantly lower in mixtures with tamarack than would be predicted from the monocultures of the two component species. Possibly, mixing of lignin rich litter from black spruce or white pine with N rich litter from tamarack suppressed the formation of lignolytic enzymes or formed complexes highly resistant to microbial degradation. However, these antagonistic effects on soil C respiration and net N mineralization in mixtures with tamarack did not result in reduced aboveground biomass in these plots after 10 years of growth. It remains to be seen if these antagonistic effects will affect long-term forest productivity and dynamics in boreal forests.

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### 1. Introduction

Nitrogen released through decomposition of organic matter is critical for plant productivity and regeneration in many forested ecosystems (Mladenoff, 1987; Reich et al., 1997). Low nitrogen availability may be a particularly important constraint in boreal forests where decomposition and mineralization rates are slow (Swift et al., 1979; Tamm, 1991). However, plant growth in boreal forests could become P limited when aluminum and iron accumulation in the humus layer is high (Giesler et al., 2002). Litter mixtures from different plant species often decompose and release N at different rates than would be predicted from rates of all component species decomposing in isolation (i.e., non-additive effects, Wardle et al., 1997; Gartner and Cardon, 2004). These non-additive effects on litter decomposition and N dynamics often occur in mixtures containing no more than two to three species

(e.g., McTiernan et al., 1997; Wardle et al., 1997; Salamanca et al., 1998; Smith and Bradford, 2003). Because tree diversity in boreal forests is relatively low (Iwasa et al., 1993), modest changes in species composition could therefore result in large non-additive effects on litter dynamics, with potentially large implications for forest productivity and composition (Finzi and Canham, 1998; Nilsson et al., 1999).

Non-additive effects on litter decomposition and N release in litter mixtures can be synergistic (i.e., decomposition and net N mineralization are greater in litter mixtures than predicted from single-species decomposition and N release of all component species) or antagonistic (i.e., slower rates in mixtures than predicted from component single-species rates), but additive effects have also been observed (e.g., see review by Gartner and Cardon, 2004). The direction of the effect appears to be highly idiosyncratic (Chapman et al., 1988; Fyles and Fyles, 1993; Wardle et al., 1997). The non-additive effects on decomposition and net N mineralization have been related to alterations in the microbial decomposer community, interactions across trophic levels, and alterations in micro-environmental conditions (Blair et al., 1990; Wardle, 2002; Schadler and Brandl, 2005; Hättenschwiler et al., 2005). It has also been suggested that non-additive effects are

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caused by interspecific differences in litter chemistry. For instance, synergistic effects can occur when mixtures contain litter with a high initial nutrient content that stimulates decomposition of adjacent recalcitrant litter by providing a source of nutrients (Seastedt, 1984; Chapman et al., 1988), possibly via hyphal connections (McTiernan et al., 1997). Indeed, synergistic effects have been observed in mixed-litter experiments when litter of plants with a high decomposition rate (when decomposed alone) and a high N content were present (Fyles and Fyles, 1993; Wardle et al., 1997; Salamanca et al., 1998). However, when litters of the same species but with different N concentration were mixed, only antagonistic effects were observed (Smith and Bradford, 2003). Antagonistic effects could occur in mixtures of lignin-rich and high-N litter where the mineralized N suppresses the formation of enzymes that break down lignin (Carreiro et al., 2000; Saiya-Cork et al., 2002) and react with lignin residues to form complexes highly resistant to microbial degradation (Nömmik and Vahtras, 1982; Stevenson, 1994; Zech and Kögel-Knabner, 1994).

Much of the evidence for interactive effects of species on decomposition and net N mineralization comes from studies of fresh leaf litter (see review by Gartner and Cardon, 2004). However, given that non-additive effects on decomposition and N dynamics can change throughout the process of decomposition (Blair et al., 1990; Wardle et al., 1997; Scherer-Lorenzen et al., 2007), possibly due to changes in litter chemistry as decomposition proceeds, interactive effects of species on soil C respiration and net N mineralization may be very different from interactive effects on fresh leaf litter. Soil organic matter (SOM) contains large amounts of N and contributes significantly to the overall N cycle, yet experiments that are designed to study non-additive effects of species on soil C respiration and net N mineralization rates are rare (Finzi and Canham, 1998).

Here we study the interactive effects of the deciduous conifer tamarack (*Larix laricina*) and the evergreen conifers black spruce (*Picea mariana*) and white pine (*Pinus strobus*) on soil C respiration and net N mineralization in a plantation in northern Minnesota. The trees were planted in monoculture and in all three possible two-species combinations. Litter produced by boreal forest species tends to have a high C:N ratio and polyphenol content (Lindahl et al., 2002), and the three species in our study vary significantly in foliar N concentration (tamarack  $\gg$  white pine  $>$  black spruce, Reich et al., 1998). We hypothesized that the mixed plots would show antagonistic effects on soil C respiration and net N mineralization, particularly in mixed plots containing tamarack, which produces N-rich litter. We assessed soil C respiration and potential N mineralization rates using laboratory incubations where all samples were kept at the same soil moisture and temperature conditions. This allowed us to isolate effects of species differences in substrate chemistry on C respiration and net N mineralization from those that might arise from species effects on moisture and temperature.

## 2. Methods and materials

A tree species mixture experiment was conducted at the Cloquet Forestry Center of the University of Minnesota in Cloquet, northern Minnesota, USA (Gerlach et al., 1997). The experiment used two sites (“East” and “West”) located approximately 5 km apart from each other. Mean annual precipitation is 761 mm with mean monthly temperatures of  $-14^{\circ}\text{C}$  in January and  $19^{\circ}\text{C}$  in July. The soil is a loamy sand of the Omega and Cloquet series. Prior to establishing this experiment, the East site was dominated by aspen (*Populus* spp.) with basal area from 18 to 23  $\text{m}^2 \text{ha}^{-1}$  while the West site was dominated by white pine (*Pinus strobus*) with paper birch (*Betula papyrifera*), aspen, and a mixed conifer understory (basal area 14–18  $\text{m}^2 \text{ha}^{-1}$ , Boyden et al., in review). Both sites

were clear-cut in the winter of 1991–1992. Slash was piled and burned.

In the spring of 1992 seedlings of black spruce (*Picea mariana* (Miller) BSP), tamarack (*Larix laricina* (Duroi) K. Koch), and white pine (*Pinus strobus* L.) were planted at both sites. Dead individuals were replanted in the spring of 1993. The three species differ in shade tolerance (black spruce  $>$  white pine  $>$  tamarack) and have different needle life spans (tamarack: 5–6 months, white pine: 20–32 months, black spruce: 50–70 months (Reich et al., 1998)). Each of the species was planted in monoculture and in all three possible two-species combinations (mixtures) at four different densities. The seedlings were planted in 12 rows of 12 trees (total of 144 trees) with 0.125, 0.25, 0.5, and 1 m spacing between rows and between trees within rows (variable plot size). In the mixed plots the two species were planted in an alternating manner. For each of the six species compositions (3 monocultures, 3 mixtures) and four densities, three replicates were established (two at the East site and one at the West site). The plots were weeded in the 2 years following planting. For our study we avoided plots with very close spacing (0.125 m) because some of these plots had high tree mortality, or distant spacing (1 m) because these plots were not completely covered by the tree canopies after 10 years of growth, and sampled a total of 36 plots (3 monocultures and 3 mixtures planted at 0.25 and 0.5 m spacing, replicated 3 times). The 0.25 and 0.5 m spaced plots had no undercover vegetation at the time of sampling.

In the summer of 2002 we measured height and stem diameter at breast height in each plot for all trees, except for trees in the outer two rows to control for edge effects. We calculated the aboveground biomass ( $\text{kg m}^{-2}$ ) using species-specific biomass equations from the literature (Ter-Mikaelian and Korzukhin, 1997). Because tree density can influence the architecture of trees (Mäkinen, 1999; Will et al., 2005), biomass equations may be tree density dependent. Unfortunately, we were unable to find density dependent biomass equations from the literature. As assessment of biomass is of secondary interest compared to our main focus on soil organic matter dynamics, any errors that would result should not materially influence the conclusions of our study. We calculated the relative aboveground biomass ( $RAB_i$ ) of black spruce, tamarack, and white pine grown in mixtures as

$$RAB_i = AB_{\text{mix},i} / AB_{\text{mono},i} \quad (1)$$

where  $AB_{\text{mix},i}$  is the aboveground biomass of species  $i$  grown in mixture and  $AB_{\text{mono},i}$  is the aboveground biomass of species  $i$  grown in monoculture. If aboveground biomass of the trees in the mixed plots is not affected by the other species,  $RAB_i$  should be 0.5 and a  $RAB_i > 0.5$  indicates better performance of species  $i$  in the mixed plot than in monoculture. The sum of  $RAB_i$  of the two species in mixture can deviate from 1, indicating absolute changes in total aboveground biomass in the mixed plots compared to the average of the two monoculture plots. Thus,  $RAB_i$  does not only reflect relative, but also absolute changes in aboveground biomass in the mixed plots compared to the monoculture plots.

In September 2002 we sampled the mineral soil to 20 cm soil depth in each plot. Because the forest floor was very thin ( $<0.5$  cm) we only collected mineral soil for incubations. We collected and composited three soil cores (diam. 2.5 cm) in each plot after separating the mineral soil from the overlying forest floor. We stored samples at  $4^{\circ}\text{C}$  for 24 h, then sieved (2 mm) and weighed 20 g of field-moist mineral soil into a 100 ml filter funnel unit (Pall Microfunnel, Pall Corporation, East Hills, NY) where the filter at the bottom of the unit was replaced with a layer of quartz wool. On a sub sample we measured soil moisture content (oven-drying, 48 h,  $105^{\circ}\text{C}$ ). We incubated soils at field capacity in the dark at room temperature for 266 days, during which the funnel units were

covered with a polyethylene film that allows gas exchange but prevents soils from drying out.

We modified a method described by Nadelhoffer (1990) to measure soil C respiration and net N mineralization. We measured soil C respiration by measuring respiration on days 1, 3, 7, 12, 17, 39, 70, 170, and 266 of the incubation. We removed the polyethylene film from the filter funnel units and placed the units into one-quart wide-mouth glass jars and covered the jars with a lid fitted with a septum. Immediately after closing off the glass jar, we sampled the headspace gas with a syringe. After 24 h a final air sample was drawn from the jar. Headspace samples were immediately analyzed for CO<sub>2</sub> on a gas chromatograph (Shimadzu GC14A, Shimadzu Scientific Instruments, Wood Dale, IL) using a thermal conductivity detector and a Poropak N column.

We calculated daily respiration rates by subtracting the CO<sub>2</sub> concentration in the initial sample from the CO<sub>2</sub> concentration in the final sample. We calculated the cumulative amount of C respired by multiplying the average daily soil respiration rate between two measurements by the time interval between the two measurements, and by adding the preceding soil respiration. We fitted the cumulative C respiration over time for each sample with a one-pool model (Swift et al., 1979):

$$C_t = C_0(1 - e^{-kt}) \quad (2)$$

where  $C_t$  is the cumulative amount of C respired at time  $t$ ,  $C_0$  is the amount of decomposable C in the sample at  $t = 0$ , and  $k$  is the decomposition rate constant for  $C_0$ . We used Sigmaplot (version 5.0) for curve-fitting the parameters  $C_0$  and  $k$ .

We measured potential net N mineralization by leaching the samples with ultra-filtered (Nanopure) distilled water on days 2, 4, 8, and 13 of the incubation, directly after the soil respiration measurements. With each leaching we may have removed other nutrients important for microbial activity. This periodic nutrient removal could have reduced net N mineralization rates overall, but should not have affected any relative differences between treatments. We removed the filter unit from the glass jars and added 40 ml of nanopure water to the funnel unit. After 1 h we drained the solution from the filter funnel unit using a filter dome (Kontes Ultra-ware, Kontes, Vineland, NJ) attached to a vacuum pump set at 20 kPa. Leachates were frozen until analyses for NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> on an Alpkem auto-analyzer (OI Analytical, College Station, TX). We calculated net N mineralization (mg kg<sup>-1</sup> soil) during days 2–13 as the sum of NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N extracted at days 4, 8 and 13.

We compared observed values of  $C_0$ ,  $k$ , and net N mineralization in mixed plots to expected values. Rather than calculating expected values by averaging the values observed in the monoculture plots of the component species, we weighted the expected values by aboveground biomass of each species in the mixed plots to reflect differences in dominance between species in the mixed plots. We calculated expected values ( $E$ ) of  $C_0$  and net N mineralization as

$$E = \sum O_{\text{mono},i} \times RAB_i \quad (3)$$

where  $O_{\text{mono},i}$  is the observed value of  $C_0$  or net N mineralization for species  $i$  in the monoculture plots. Expected values were thus adjusted for changes in absolute and relative aboveground biomass in the mixtures. Because the decomposition rate constant  $k$  by definition is not influenced by absolute amounts of litter inputs, but is affected by litter quality (i.e., the proportion of litter inputs from each of the two species), we calculated expected values of  $k$  ( $E_k$ ) as

$$E_k = \sum O_{\text{mono},i} \times PAB_i \quad (4)$$

where  $PAB_i$  is the proportional aboveground biomass of species  $i$  in the mixtures (i.e., the aboveground biomass of species  $i$  in the mixture divided by the total aboveground biomass in the mixture; note that the sum of  $PAB_i$  of the two species in mixture is always 1).

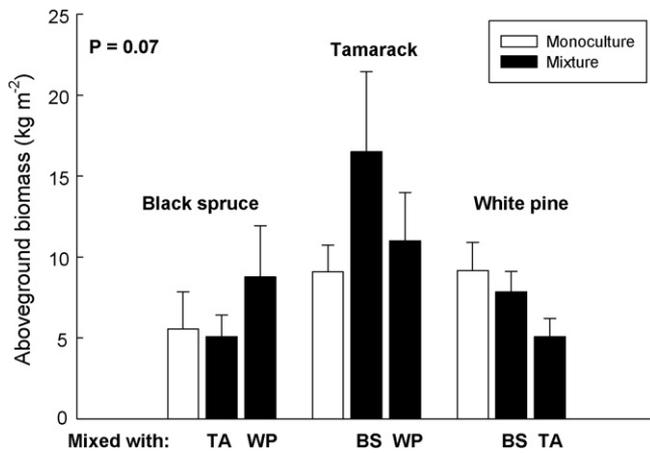
To determine litter and root quality, we collected aboveground litter and roots from each plot in the summer of 2007. We sampled aboveground litter by collecting the forest floor ( $O_i + O_e$ ) from a 10 cm × 10 cm area in the center of each plot. We sampled and composited three soil cores (diam. 2.5 cm) to 20-cm soil depth randomly from each plot. Roots were separated from the soil through sieving (2 mm) and then washed with DI water. Aboveground litter and roots were dried (65 °C), ground, and analyzed for total N (C and N Analyzer, CE Instruments, Milan, Italy) and lignin (Ankom Fiber Analyzer, Ankom Tech., Fairport, NY).

We used ANOVA to test for main effects of species number (two levels: 1 or 2 species) and tree spacing (two levels: 0.25 or 0.5 m), and their interaction on aboveground biomass, decomposable C,  $k$ , and net N mineralization. For species-specific biomass responses (expressed per unit ground area), we used ANOVA to test for main effects of species combination (nine levels: black spruce in monoculture and grown with tamarack and white pine, tamarack in monoculture and grown with black spruce and white pine, white pine in monoculture and grown with black spruce and tamarack) and tree spacing. We further used ANOVA to test for main effects of species composition (six levels: black spruce, tamarack, white pine, black spruce with tamarack, black spruce with white pine, and tamarack with white pine) and tree spacing on decomposable C,  $k$ , and net N mineralization. For each of the models, we also tested for the random effect of the location of the two sites (East or West). The effect of location was never significant and was subsequently not included in the ANOVAs. Whenever the ANOVA was significant ( $P < 0.05$ ) we compared the means using a post hoc Tukey test. In some cases, data were log-transformed to meet assumptions of ANOVA. We tested if the relative aboveground biomass of each species grown in mixture deviated from 0.5 (two-tailed  $t$ -test). We tested if observed values of  $C_0$ ,  $k$ , and net N mineralization differed from expected values (matched-pairs  $t$ -test). All statistics were done with JMP (version 4.0.4; SAS Institute, Cary, NC, USA).

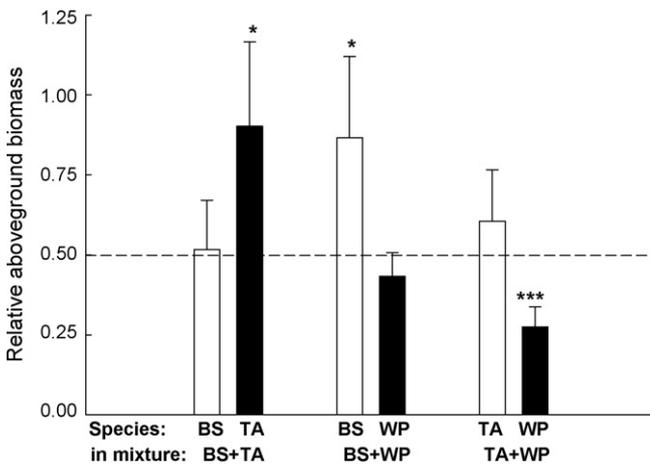
### 3. Results

Species-specific biomass responses were expressed per unit ground area. Because in the mixed plots each species only occupies half the area, we multiplied aboveground biomass (in kg m<sup>-2</sup>) of the species grown in mixture by two to compare with aboveground biomass in monoculture plots. There was no significant species number effect on aboveground biomass ( $P = 0.56$ ). However, we observed a marginally significant species combination effect on aboveground biomass ( $P = 0.07$ , Fig. 1). Black spruce had 58% more aboveground biomass when grown with white pine than grown in monoculture. Tamarack had greater aboveground biomass when grown in mixture than in monoculture, particularly when grown with black spruce (82% increase compared to monoculture). White pine was less productive in terms of aboveground biomass when grown in mixture, particularly with tamarack (45% decrease compared to monoculture). Not surprisingly, plots with 0.25-m tree spacing had significantly greater aboveground biomass ( $P = 0.02$ ,  $10.7 \pm 1.6$  kg m<sup>-2</sup>) than plots with 0.5-m spacing ( $6.6 \pm 0.7$  kg m<sup>-2</sup>), but there were no significant interactions with species composition.

We calculated relative aboveground biomass ( $RAB_i$ ) with equation (1). Here, aboveground biomass was not adjusted for unit ground area. Thus, if aboveground biomass of the trees in the mixed plots is not affected by the other species,  $RAB_i$  should be 0.5. The  $RAB_i$  was significantly smaller than 0.5 for white pine when grown with tamarack ( $P < 0.01$ , Fig. 2). The  $RAB_i$  of tamarack in the



**Fig. 1.** Average aboveground biomass per unit ground area planted to black spruce, tamarack, and white pine, grown as monoculture and in mixture with one of the other two species (averaged across tree spacing). Error bars represent  $\pm$  S.E. Aboveground biomass is calculated using measured DBH for each species in biomass equations (black spruce: Grigal and Kernik, 1984a, tamarack: Carpenter, 1983, white pine: Perala and Alban, 1994, all cited by Ter-Mikaelian and Korzukhin, 1997), divided by the ground area planted. For mixtures, this is half of the total plot area for each species, given that species were planted in alternate rows.

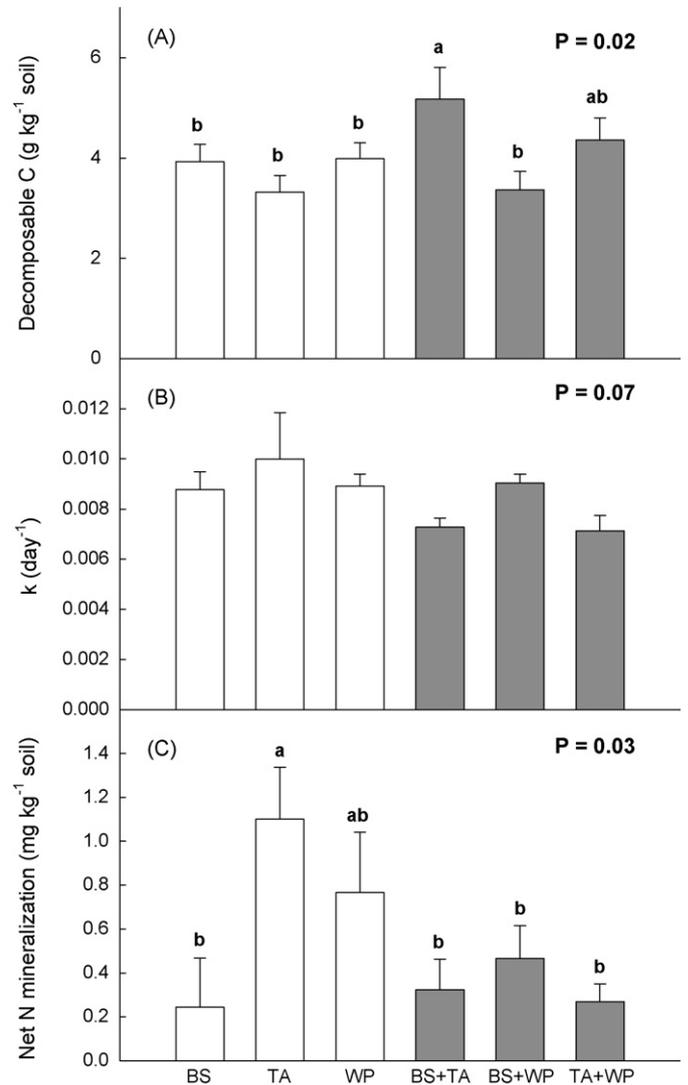


**Fig. 2.** Relative aboveground biomass ( $RAB_i$ ) of black spruce, tamarack and white pine grown in mixture with one of the other two species. If aboveground biomass of the trees in the mixed plots is not affected by the other species,  $RAB_i$  should be 0.5. Error bars represent  $\pm$  S.E. Asterisks indicate how significantly different the mean is from 0.5 (\* $P < 0.1$ , \*\*\* $P < 0.01$ ).

black spruce/tamarack mixture and of black spruce in the black spruce/white pine mixture was marginally greater than 0.5 ( $P < 0.1$ ).

We observed significant species composition effects on  $C_0$ ,  $k$ , and net N mineralization in the mineral soil (Fig. 3). Spacing caused marginally significant effects on the decomposition rate constant  $k$  ( $P = 0.06$ ) where  $k$  was greater in the 0.5 m spaced plots ( $0.0090 \pm 0.0005 \text{ d}^{-1}$ ) than in the 0.25 m spaced plots ( $0.0080 \pm 0.0005 \text{ d}^{-1}$ ). Interactions between species composition and spacing were never significant.

We observed no significant species number effect on the decomposable C pool in the soil ( $C_0$ ,  $P = 0.16$ ), but did observe significant species composition effects ( $P = 0.02$ ). Of all monoculture and mixture plots the black spruce/tamarack mixture showed the greatest average decomposable C pool in the soil. This pool was significantly greater than in the monocultures of both black spruce (by 32%) and tamarack (by 56%, Fig. 3A). The decomposable C pool in the soil of the tamarack/white pine mixture was on average also



**Fig. 3.** (A) Average decomposable C, (B) its decomposition rate constant  $k$ , and (C) net N mineralization in the mineral soil (0–20 cm) for the different monocultures (white bars) and mixtures (gray bars, averaged across tree spacing). Decomposable C and  $k$  were estimated from a 266-day lab incubation and net N mineralization from an 11-day lab incubation (see Section 2). Error bars represent  $\pm$  S.E. Different letters above bars indicate significant differences ( $P < 0.05$ , post hoc Tukey test).

greater than in the monocultures of the component species (31 and 9% greater than in tamarack and white pine monocultures, respectively), although in this case not significantly so.

The decomposition rate constant  $k$  was significantly smaller in the mixed plots ( $0.0078 \pm 0.0003 \text{ d}^{-1}$ ) than in the monoculture plots ( $0.0091 \pm 0.0006 \text{ d}^{-1}$ ,  $P = 0.05$ ). While differences among species composition in  $k$  were only marginally significant (Fig. 3B), patterns mirrored the decomposable C pool data. The mixed plots with tamarack as one of the component species had smaller  $k$  values than each of the component monoculture species. Smaller  $k$  values in the mixed plots when tamarack is present indicate that soil C respiration slowed down in these plots compared to soil C respiration in the component monoculture plots.

Species composition significantly affected net N mineralization, which showed a similar but more divergent pattern as the  $k$  data. Net N mineralization was significantly larger in the monoculture plots ( $0.76 \pm 0.16 \text{ mg kg}^{-1}$ ) than in the mixed plots ( $0.35 \pm 0.07 \text{ mg kg}^{-1}$ ,  $P = 0.03$ ). Net N mineralization was highest in the tamarack monoculture plots, which had on average 3.4–4.1 times higher rates than did the mixed plots with tamarack (Fig. 3C).

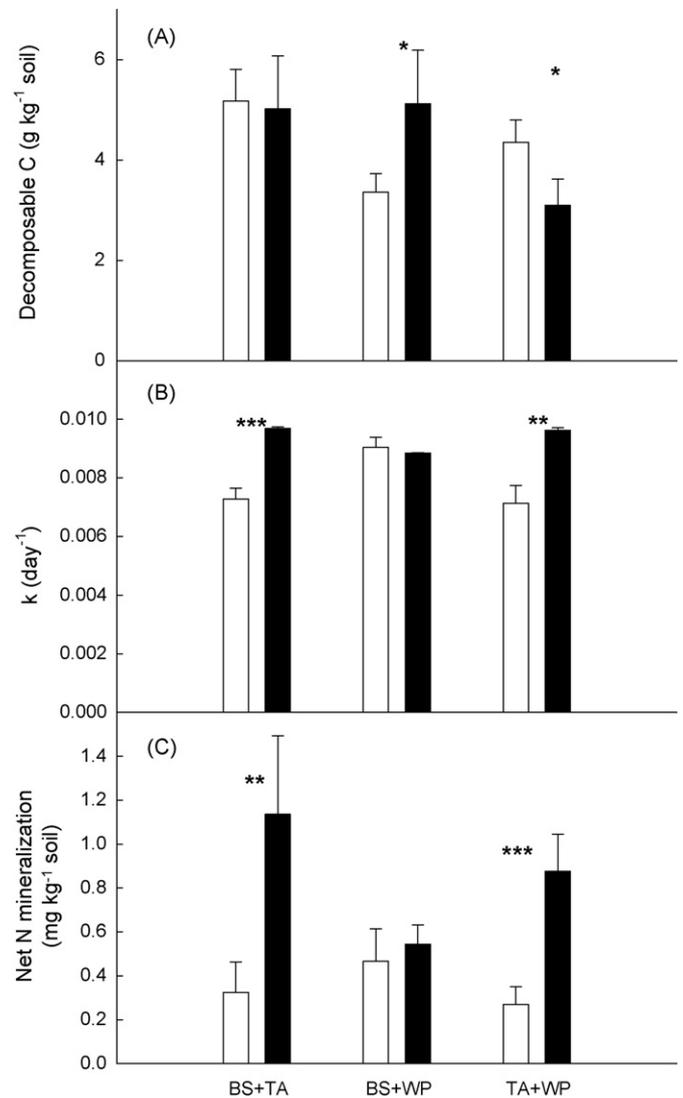
We compared observed values of decomposable C,  $k$ , and net N mineralization in the mixed plots (as shown in Fig. 3) with expected values adjusted for aboveground biomass to test if species mixtures caused non-additive effects. When observed values are not significantly different from the expected values, there are only additive effects, but when observed values are significantly different from the expected values, there are non-additive effects. Observed decomposable C pools in the soil were significantly smaller in the black spruce/white pine mixture (by 34%) and significantly larger in the tamarack/white pine mixture (40%) than predicted from component species in monoculture adjusted for  $RAB_i$  (Fig. 4A). Observed values of the decomposition rate constant  $k$  and net N mineralization were significantly smaller in the mixtures containing tamarack than expected values adjusted for proportional aboveground biomass ( $PAB_i$ ) and  $RAB_i$ , respectively (Fig. 4B and C). Observed values of net N mineralization were 71 and 69% smaller than expected values in the black spruce/tamarack and tamarack/white pine mixture, respectively.

When grown in monoculture, aboveground litter of tamarack had the highest N concentration, followed by black spruce, and white pine (Table 1). Lignin concentration in aboveground litter was significantly lower for white pine than for black spruce and tamarack. There were no significant differences in lignin:N ratio in aboveground litter among species. There were no significant species composition effects on N concentration in roots. However species composition effects on lignin concentration and lignin:N ratio in roots were marginally significant ( $P = 0.07$ ), with lowest values for tamarack and highest for black spruce monoculture and black spruce/tamarack mixed plots. We observed no significant spacing effects or species composition  $\times$  spacing interactions.

#### 4. Discussion

As we predicted, the mixed plots with tamarack showed strong and significant antagonistic effects on soil C respiration and net N mineralization rates. Observed  $k$  values and net N mineralization rates in the mixed plots with tamarack were significantly smaller than expected from their component species in monoculture adjusted for aboveground biomass.

Antagonistic effects on soil C respiration and net N mineralization could occur because of mixing of litter with different chemistries. For instance, N-rich litter could slow down decomposition of lignin-rich litter by suppressing the formation of enzymes responsible for breaking down lignin (Carreiro et al., 2000; Saiya-Cork et al., 2002) or by reacting with lignin residues to form complexes highly resistant to microbial degradation (Nömmik and Vahtras, 1982; Stevenson, 1994; Zech and Kögel-Knabner, 1994). Indeed, the N concentration in aboveground tamarack litter was significantly higher than for the other two species, and release of N from this litter could potentially have reduced overall soil C respiration in mixtures with tamarack. In other studies synergistic



**Fig. 4.** (A) Observed (white bars) decomposable C pool, (B) its decomposition rate constant  $k$ , and (C) net N mineralization in the mineral soil (0–20 cm) in the mixed plots, compared to expected values (black bars). Expected values are based on the means of the two species in monoculture, weighted by the amounts and proportions of biomass of each species in the mixtures (see Section 2). Error bars represent  $\pm$  S.E. Asterisks indicate significant differences between observed and expected (\* $P < 0.1$ , \*\* $P < 0.05$ , \*\*\* $P < 0.01$ ).

effects have been observed in mixed-litter experiments when litter of plants with a high decomposition rate (when decomposed alone) and a high N content were present (Fyles and Fyles, 1993; Wardle et al., 1997; Salamanca et al., 1998). However, in all of these

**Table 1**  
Average N concentration, lignin concentration, and lignin:N ratio ( $\pm$ S.E.) in aboveground litter and root biomass for each of the monoculture and mixed plots. Different letters in each column denote significant differences ( $P < 0.05$ , post hoc Tukey's test).

Species composition	Aboveground litter			Roots		
	N (%)	Lignin (%)	Lignin:N	N (%)	Lignin (%)	Lignin:N
BS	1.74 $\pm$ 0.12 bc	33.2 $\pm$ 1.4 c	19.4 $\pm$ 1.2	1.30 $\pm$ 0.09	32.8 $\pm$ 0.9	25.9 $\pm$ 2.3
TA	2.14 $\pm$ 0.07 d	33.3 $\pm$ 1.6 c	15.6 $\pm$ 0.8	1.38 $\pm$ 0.04	28.3 $\pm$ 0.7	20.6 $\pm$ 0.9
WP	1.15 $\pm$ 0.12 a	22.3 $\pm$ 1.0 a	20.8 $\pm$ 2.7	1.38 $\pm$ 0.05	29.0 $\pm$ 1.2	21.2 $\pm$ 1.5
BS + TA	1.95 $\pm$ 0.06 cd	33.2 $\pm$ 1.6 c	17.1 $\pm$ 1.1	1.33 $\pm$ 0.05	33.1 $\pm$ 1.4	25.1 $\pm$ 1.1
BS + WP	1.41 $\pm$ 0.15 ab	27.7 $\pm$ 1.2 b	20.3 $\pm$ 1.6	1.36 $\pm$ 0.05	31.2 $\pm$ 1.6	23.0 $\pm$ 1.4
TA + WP	1.55 $\pm$ 0.19 b	27.9 $\pm$ 1.2 b	19.0 $\pm$ 1.6	1.33 $\pm$ 0.07	30.0 $\pm$ 1.0	23.0 $\pm$ 1.5
ANOVA $P$ values*						
Sp. comp.	<0.0001	<0.0001	0.22	0.76	0.07	0.07

\* Spacing and Sp. comp.  $\times$  spacing were always non-significant ( $P > 0.1$ ).

studies decomposition of fresh litter was studied rather than of SOM, which mostly consists of transformed products with little resemblance to the physical and chemical characteristics of fresh litter (e.g., Stevenson, 1994; Zech and Kögel-Knabner, 1994; Kramer et al., 2003). It should be noted that differences in root chemistry among species were much less strong than for aboveground litter. We sampled aboveground litter and roots 5 years after the aboveground biomass and soil sampling. Aboveground litter and root chemistry may have changed during that time, limiting our ability to explain our measured soil C respiration and net N mineralization to aboveground litter and root chemistry.

Other chemical differences that we did not measure might also have been involved. Madritch and Cardinale (2007) suggested that secondary metabolites and phenolics leached from recalcitrant litter may have caused lower than expected decomposition rates of mixed litters when N-rich litters of American basswood (*Tilia Americana*) and sugar maple (*Acer saccharum*) were present in northern temperate forests of Wisconsin. It is well known that secondary metabolites such as tannins can affect microbial activity (Fyles and Fyles, 1993; Schimel et al., 1996; Fierer et al., 2001). It is plausible that antagonistic effects on  $k$  and net N mineralization in our study may have been caused by inhibitory effects of secondary metabolites and phenolics as well.

Observed decomposable C pools in the black spruce/white pine mixtures were smaller and in tamarack/white pine mixtures were greater than expected from component species in monoculture. This increase in decomposable C in the soil could be due to a reduced decomposition rate of that pool. This should then also be reflected in a smaller observed  $k$  value than expected. However, in the black spruce/white pine mixture the observed  $k$  value was not significantly different from the expected value. An increase in observed decomposable C in the soil compared to expected pools could also be due to greater litter production in the mixed plots compared to what can be expected from the component monoculture plots.

We have no direct measurements of above- and belowground litter production, but weighted expected values for the C respiration and N mineralization parameters by aboveground biomass of each species to reflect differences in dominance between species in the mixed plots. Aboveground biomass was calculated from species-specific biomass equations using measurements of stem diameter at breast height. For each of these species, similar equations were developed for foliage biomass and coarse root biomass (Ter-Mikaelian and Korzukhin, 1997; Bond-Lamberty et al., 2002) suggesting that litter production is also related to aboveground biomass for these species. When we calculated expected values of decomposable C,  $k$ , and net N mineralization adjusted for aboveground litter production (based on the foliage biomass equations and assuming that black spruce, tamarack, and white pine drop their needles once every 5, 1, and 2 years, respectively) and coarse root biomass, expected values were very similar to values adjusted for aboveground biomass (data not shown). We should caution again that our estimates for aboveground litter production may have some error because foliage biomass equations may be tree density dependent that we did not account for. Adjustments for differences in dominance between species provide a better estimate of expected values than do unadjusted estimates based on average values observed in the monoculture plots of the component species, although the latter gave very similar results when comparing these expected values with observed values (data not shown). Further, while in many mixed litter bag studies equal amounts of fresh litter of the component species have been used (e.g., McTiernan et al., 1997; Wardle et al., 1997; Madritch and Cardinale, 2007), we took soil samples from the field that reflected species-specific differences in litter production and that incorporated species-specific differences

in timing of litterfall in the natural setting. Non-additive effects on decomposition can change when different masses of each litter type are used (Fyles and Fyles, 1993; Salamanca et al., 1998; Scherer-Lorenzen et al., 2007), and could potentially change when mixing of litter of different age occurs. Thus, this study compliments litter bag studies that use equal amounts of fresh litter of the component species and allowed the mineral soil response to be observed after 10 years of tree growth and litter production.

Our results from incubations under constant soil moisture and temperature may, or may not, be similar to what one would find under field conditions with varying moisture and temperature. The laboratory N mineralization rankings among species found here (spruce much less than pine, with the more N-rich tamarack greatest) are consistent with field incubations in northern Minnesota, where spruce had much lower N mineralization than pine, with the more N-rich aspen stands even greater N mineralization (Reich et al., 2001). Another important species difference is leaf habit, with tamarack being a deciduous conifer and the other two evergreen conifers. As a result, the three species cause different shading effects on the soil and therefore could alter soil moisture and temperature differently. Soils in the tamarack monoculture plots may experience, at times when tamarack had no needles, drier and hotter conditions than in the mixed plots with tamarack where black spruce or white pine provided more shading, thus potentially affecting the soil C and N dynamics. However, the antagonistic effects on C respiration and net N mineralization observed here from mixed plots with tamarack were large and are likely to persist under field conditions. Indeed, at the global scale plant species traits such as litter quality had a greater effect on litter decomposition than climate effects (Cornwell et al., 2008).

The antagonistic effects on net N mineralization rates in the mixed plots with tamarack did not result in reduced aboveground biomass in these plots after 10 years of growth. The relative aboveground biomass total (RABT, the sum of the relative aboveground biomass of the two species in mixture, similar to the relative yield total concept, De Wit, 1960) did not significantly deviate from 1 for all three mixtures (two-tailed  $t$ -test). A RABT smaller than 1 would suggest that antagonistic effects on net N mineralization reduced plant N availability and thus plant biomass accumulation in the mixed plots. The RABT in the black spruce/tamarack (1.42) and black spruce/white pine (1.30) mixtures was actually larger than 1 (but not significantly so) suggesting no effect or a small synergistic effect on plant biomass accumulation, seemingly contradicting the antagonistic effects on net N mineralization in mixed plots.

Possibly, up until now, the trees in the mixed plots have exploited the available resources more completely than in monoculture (e.g., Loreau and Hector, 2001; van Ruijven and Berendse, 2003). It is likely that SOM dynamics changed only gradually over time as the forests developed in these plots, resulting in delayed antagonistic effects of net N mineralization on plant productivity that have yet not been detected in total biomass estimates. In essence, at this time scale, SOM dynamics may be much more a result of species C and N influences, than an influence on them. There are several other possible complications all of which could be complementary (rather than mutually exclusive). It is possible is that the tree species used in our study depend on organic N uptake for much of their N requirement and changes in net N mineralization rates do not affect the productivity of these species. There is evidence that plants in boreal ecosystems can take up organic forms of N, often through ectomycorrhizae (Nasholm et al., 1998; Read and Perez-Moreno, 2003). However, plants and ectomycorrhizae have been shown to take up organic N in the form of amino acids or small peptides which become available only after the initial stages of organic matter

decomposition. Thus, the antagonistic effects on SOM decomposition ( $k$ ) that we observed should also result in antagonistic effects on plant availability of organic N. It is also possible that plant growth at our site was not N limited, but may have been P limited. Giesler et al., 2002 suggested that high accumulation of aluminum and iron in the humus layer may have caused P limitation for plant growth in a boreal forest in northern Sweden.

## 5. Conclusion

Boreal forests are characterized by low tree diversity (Iwasa et al., 1993) and slow decomposition and mineralization rates (Swift et al., 1979; Tamm, 1991). Our results demonstrate antagonistic effects on soil C respiration and net N mineralization in mixtures of two species, particularly in mixtures with tamarack. Future work will determine if a link to productivity can be established, but the large negative effects on soil C respiration observed here suggests an important role of species composition on the cycling of N and C in these boreal systems.

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