Non-additive effects of invasive tree litter shift seasonal N release: a potential invasion feedback

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Many invasive plant species strongly alter ecosystem processes by producing leaf litter that decomposes faster and releases N more quickly than that of native species. However, while most studies of invasive species litter impacts have only considered the decomposition of species in monoculture, forest litter layers typically contain litter from many species. Many litter mixtures decompose in a non-additive manner, in which the mixture decomposes more quickly (synergistic effect) or more slowly (antagonistic effect) than would be expected based on decomposition of the component species’ litters in isolation. We investigated the potential for non-additive effects of invasive species’ litter by conducting a one-year litter bag experiment in which we mixed the litters of four native tree species with each of four invasive species. Litter mixtures frequently lost mass at non-additive rates, although not at every loading ratio, and the presence, sign, and strength of effects depended on species composition. Non-additive effects on N loss occurred in more litter combinations, and were almost always antagonistic at 90 days and synergistic at 365 days. Invasive species litter with lower C:N led to more strongly synergistic N loss with time. During the growing season, non-additive patterns of N loss almost always resulted in increased N release – up to six times greater than would be expected based on single-species decomposition. Consequently, we suggest that invasive species may further synchronize N release from the litter layer with plant N demand, enhancing any positive litter feedback to invasion. These results highlight the need to consider non-additive effects of litter mixing in invaded forest communities, and suggest that estimates of invasive species’ impacts on ecosystem processes would be improved by considering these effects.
Card 2004). Several mechanisms could potentially explain non-additivity in litter mixtures (Hättenschwiler et al. 2005). These include nutrient transfer theory (McTiernan et al. 1997, Gartner and Cardon 2004, Liu et al. 2007, Ball et al. 2008, 2009), which suggests that NAE can occur when nutrients from high-quality (low C:N ratio) litter transfer to low-quality (high C:N ratio) litter via leaching or microbial activity. Since litters with greater concentrations of N often decompose more quickly than those with lower concentrations of N (Melillo et al. 1982), this process accelerates decomposition by enhancing nutrient availability to microbial decomposers on low-quality litter (Hättenschwiler et al. 2005). This suggests that high-quality litter of invasive plant species can stimulate synergistic NAE, augmenting soil nutrient availability.

Soil fertility strongly influences plant community composition (Tilman 1987), which can dramatically regulate the presence, strength, and sign of NAE (Wardle et al. 2003, Bonanomi et al. 2010). However, the influence of plant community and litter layer evenness on NAE varies widely across observations (Smith and Bradford 2003, Ball et al. 2008, Dickson and Wilsey 2009, Meier and Bowman 2010, Mao and Zeng 2012). Most natural plant communities are uneven (Wilsey and Polley 2004) and, in these systems, the traits of a few dominant species strongly influence ecosystem processes (Dickson and Wilsey 2009). Thus the sign and strength of NAE are likely to change as invasive plant species become more prevalent within a community and alter the composition of the litter layer.

A positive feedback to invasion through nutrient cycling could be enhanced by synergistic NAE. If invasive plants benefit more from elevated soil fertility than native plants, as has been documented in many studies (Huenneke et al. 1990, Funk and Vitousek 2007, Drenovsky et al. 2012), increased nutrient cycling rates via NAE have the potential to favor invasive plant species. Greater relative abundances of invasive species litter could facilitate stronger NAE by increasing nutrient transfer to other litters. Therefore, mixed litter feedbacks to invasion may intensify as invasive plant species comprise a greater proportion of the litter layer.

Despite the potential for NAE to strongly influence decomposition and nutrient cycling, very few studies have used litter mixtures to study invasive plant species’ impacts on ecosystem processes. Earlier studies have found that litter mixtures containing Rhamnus cathartica (Heneghan et al. 2002) and Lonicera maackii (Poulette and Arthur 2012) experience enhanced decomposition and N loss, respectively, but others suggest decomposition and N loss in native–invasive mixtures to be either slower (Hickman et al. 2013) or to not differ from expected rates (Blair and Stowasser 2009). Thus, previous findings do not provide a consistent framework for forming expectations of native–invasive litter mixtures. Consequently, we hypothesized that invasive plant species have higher quality litter that stimulates synergistic NAE when mixed with native species litter and that this effect is increasingly pronounced in mixtures containing a greater relative abundance of invasive species litter, as would be expected under nutrient transfer theory. To test these hypotheses, we conducted a litter bag experiment to measure decomposition and N loss in mixtures of leaf litter from native and invasive tree species.

**Methods**

**Litter bag experiment**

In order to examine differences in litter quality, decomposition, and N loss between native and invasive species, we selected four common native tree species and four invasive tree or shrub species naturally co-occurring in our study area (West Lafayette, IN). These species consisted of the natives Carya glabra, Cercis canadensis, Liriodendron tulipifera and Quercus palustris, and the invasives Acer ginnala, Elaeagnus umbellata, Lonicera maackii and Morus alba. Leaf litter from each species was collected in October 2010 from mixed species stands on two adjacent properties (40°26′47″N, 87°03′15″W). The two properties, The Purdue Wildlife Area and Martell Forest, contain 64 ha and 193 ha, respectively, and are 1.3 km apart at their closest points. Litters from both properties were pooled by species for use throughout the experiment. To ensure litter was freshly senesced, we exposed individuals to simulated gentle wind by lightly shaking their branches, which stimulated litter fall (Robertson 1999). This technique was used to collect litter from all species except C. glabra, which was collected from the branches of underlying shrubs following a strong wind storm, and Q. palustris, which was collected using litter traps that were checked at least once every five days. Litter was immediately dried at 40°C until constant weight was reached. Once dried, leaf tissue of each species was separated from petioles and broken into 2 cm segments to facilitate litter bag construction and to control for effects of litter fragment size, which can influence mixed litter decomposition rates (Tiunov 2009).

Subsamples of each litter type were set aside to test for initial tissue chemistry.

We constructed 10 × 10 cm litter bags from 1 mm fiberglass mesh. Each bag contained a total of 2.5 g of litter, comprised of either a single species or a mixture of one native species’ litter and one invasive species’ litter (16 species combinations). Single-species litter bags allowed us to compare decomposition rates of each species and also provided a baseline for expectations of mixed litter decomposition and N loss, which we later compared against the observed values obtained from mixed litter bags. To test effects of mixture evenness on non-additive decomposition and N loss, we filled mixed litter bags at three loading ratios: 2.25:0.25, 1.25:1.25 or 0.25:2.25 g native:invasive species litter (10, 50 and 90% invasive species litter), which correspond to relative abundances associated with progressively more invaded communities. We filled enough bags to allow for two collection dates and four replicates. In total, 448 bags were constructed (4 native species × 4 invasive species × 3 loading ratios × 2 collection dates × 4 replicates + 8 single species × 2 collection dates × 4 replicates). We deployed bags in December 2010 by placing them directly on the soil surface at least 10 cm apart in a randomized block design. The four blocks were located within a single stand of Alnus glutinosa in order to maintain a similar
canopy structure (i.e. light availability and rain interception) as would be experienced under our study species, but avoid any bias induced by decomposing litter bags in a stand of any one of those species. However, any effects of differences in soil microbial communities resulting from A. glutinosa, a N-fixing, are uncertain.

We allowed litter bags to decompose for either 90 or 365 days prior to collection. Once collected, litter was dried at 40°C for seven days and cleaned. We cleaned litter by gently brushing to remove soil, small rocks, roots and other contaminants. Heavy soil contamination of bags collected after 365 days required that all bags from the second collection date be washed. Because we did not wash litter from the first collection date, we attempted to minimize the amount of time litter was exposed to moisture to avoid unintended loss of litter nutrients. Leaching due to washing would have reduced differences in N loss between litters with greater concentrations and those with lower concentrations of N remaining, indicating that our calculations of non-additivity at 365 days should be treated as conservative estimates. To wash litter bags, we placed the contaminated litter in a 1 mm mesh bag and gently gyrated it in deionized water for 60 s. After washing, we immediately dried the litter at 75°C for 48 h, then at 40°C for seven days in accordance with the methods we used for litter collected at 90 days and cleaned to remove any remaining contaminants. Once cleaned, all litter was weighed, ground through a Wiley mill and pulverized in a ball mill. We followed the same grinding procedure for samples of undecomposed litter in order to analyze initial litter chemistry. Pulverized materials were then subsampled and analyzed for C and N concentration with an element analyzer. We were unable to fully clean some litter bags due to the presence of non-detritus insect products (e.g. webbing) that could not be separated from the litter surface. Additionally, some litter bags became buried in the soil over the course of the experiment and thus experienced a considerably different environment than undisturbed litter bags. Because these litter bags were likely to be strongly influenced by these changes in their environment, we excluded them from our statistical analyses.

Data analysis

To test whether invasive plant species produced litter of higher quality, we analyzed initial tissue chemistry using analysis of variance (ANOVA). We treated the initial C:N ratio and N concentration (%) as measures of initial tissue chemistry, and analyzed them by each species' native/invasive status. We evaluated mass loss (%) and N loss (%) of single-species litter types with custom general linear models to investigate if litter decomposition and N loss were strongly correlated with traditional measures of litter quality and species identity. N loss was calculated as the percentage of initial N (g) lost at each time point, where initial N was estimated based on the N concentration of the undecomposed litter set aside earlier. Models for mass and N loss were constructed with the date of collection (90 or 365 days), block, and either species identity, initial C:N ratio or initial N concentration as independent factors. We then compared native and invasive species' mass loss and N loss at each time point collectively using planned comparisons considering all litter bags for each species. Prior to inclusion in our statistical analyses, all mass and N loss values were adjusted using the C correction technique (Robertson 1999) in order to account for any residual soil contamination. For analyses that did not include a fixed factor term for species identity, we included species identity as a random factor.

We evaluated the presence of NAE by comparing observed mass loss and N loss to expected values. Expected mass loss values for mixed litter bags were calculated as \( P_N S_N + P_I S_I \) where \( P_N \) and \( P_I \) represent the proportion of the mixed litter initially comprised by the native and invasive species, respectively, and where \( S_N \) and \( S_I \) represent the mean mass loss of single species litter bags across blocks \((n=4)\) of the native and invasive species, respectively, at the relevant collection date. Expected N loss \((g)\) was calculated as \( S_N R_N + S_I R_I \), where \( S_N \) and \( S_I \) represent the starting amount of N \((g)\) in the mixture originating from the native and invasive species, respectively, based on N concentrations from initial tissue samples, and where \( R_N \) and \( R_I \) represent the proportion of initial N lost from single species litter bags of native and invasive species, respectively. This value was then divided by the total initial N of each litter bag to determine expected N loss \((\%)\) as a proportion of initial N. We then assessed if the mixed litter exhibited an NAE by calculating the signed non-additivity value \((\text{observed} / \text{expected}) – 1\) following Wardle et al. (1997). If the expected value was negative, we applied the opposite sign to the non-additivity equation in order to produce the appropriately signed value \((\text{e.g. N loss when N immobilization was expected resulted in a positive non-additivity value to indicate a synergistic effect})\). We then constructed 95% confidence intervals for the non-additivity value of each litter combination. If a confidence interval did not include zero, we categorized that litter mixture as exhibiting an NAE. Positive non-additivity values indicated synergistic effects, and negative non-additivity values indicated antagonistic effects. We then log-transformed unsigned non-additivity values and re-applied the appropriate sign to meet the assumptions of ANOVA, and analyzed them for effects of time, loading ratio, species identity and differences in tissue chemistry. In order to examine drivers of NAE in native-invasive tree litter mixtures, we used three statistical models for both mass loss and N loss, all of which used the Satterthwaite approximation to calculate degrees of freedom. The first model included time and loading ratio \((\text{‘mix’}; \text{percentage of the mixture that was derived from the invasive species})\) as categorical explanatory factors, as well as the identity of the native and the invasive component species. In the second model, we substituted the ratio of N concentrations between native and invasive species \((‘N ratio’; \text{native:invasive initial tissue N concentration})\) for the two species identity terms, and in the third model we substituted the difference between invasive and native C:N ratios \((‘C:N distance’; \text{native litter initial C:N ratio – invasive litter initial C:N ratio})\) for the identity terms. By using the signed difference between native and invasive C:N ratios, we could investigate the specific influence of...
invasive species’ relative litter quality; using the unsigned difference in C:N ratios would require a more generalized interpretation. For mixed litter models that did not include terms for the native and invasive species’ identities, we included the species combination (16 levels) and all of its possible interactions as random factors. This was not done for models that included species identity since the random factor would be collinear with species identity. Block was included as a random factor for all single-species and mixed litter decomposition analyses. We performed all analyses using SAS ver. 9.3.

Results

Single species litter

For the eight species we selected, native and invasive tree litters did not differ categorically in either N concentration (F\textsubscript{1,7} = 0.58, p = 0.47) or C:N ratio (F\textsubscript{1,7} = 0.06, p = 0.82; Supplementary material Appendix 1 Fig. A1). While the two highest quality litters (lowest C:N) were produced by invasive species, *M. alba* (20.81 ± 0.32 g C g N\textsuperscript{-1}) and *E. umbellata* (21.80 ± 0.26 g C g N\textsuperscript{-1}), *A. ginnala* litter had a relatively low N concentration (6.4 mg g\textsuperscript{-1}, SE = 0.01) and exhibited the highest C:N ratio (71.81 ± 1.61 g C g N\textsuperscript{-1}) out of all eight species. Consequently, litter from invasive species (39.92 ± 12.04 g C g N\textsuperscript{-1}) had a much broader range of elemental ratios than native species (43.04 ± 4.93 g C g N\textsuperscript{-1}, Supplementary material Appendix 1 Fig. A1–A2).

The eight species we considered varied greatly in total mass and N loss (Fig. 1). Mass loss differed among species, but initial tissue chemistry was not a consistent predictor of single-species decomposition (Supplementary material Appendix 1 Table A1). Surprisingly, *C. glabra* litter had gained 3.4 ± 2.9% mass by our first collection, which we expect is the result of fungal growth within the litter itself. While mass loss was not related to initial C:N ratio or N concentration (GLM main factors; C:N ratio: F\textsubscript{1,48} = 0.21, p = 0.65, N concentration: F\textsubscript{1,48} = 0.07, p = 0.80), litters with lower C:N ratios had lost a greater percentage of their N after 365 days (GLM; C:N × time: F\textsubscript{1,48} = 6.92, p = 0.01). This trend was largely driven by *L. maackii* and *M. alba*, since *E. umbellata* continued to retain N after 365 days.

After 365 days of decomposition, native species litter types had lost about half of their original mass, but continued to exhibit net N immobilization (Fig. 2). Of the four native species we considered, *C. canadensis* litter lost the greatest proportion of mass and was the only native species litter to lose N at that time point (Fig. 1). Conversely, *Q. palustris* lost the least mass and showed the greatest net N immobilization.

Invasive species litter decomposed faster than native species litter (Fig. 2). Invasive species litter, considered together, lost almost three-quarters of its original mass and released N over the experimental period. *M. alba* lost the greatest percentage of mass and N, whereas *E. umbellata* lost the lowest percentage of mass and immobilized the most N of the four invasive species we considered (Fig. 1).

Our planned comparisons between native and invasive species revealed that mass loss from invasive species litter was 12% greater than mass loss from native species litter after 90 days (F\textsubscript{1,21} = 32.67, p < 0.01) and 31% greater after 365 days (F\textsubscript{1,18} = 61.68, p < 0.01; Fig. 2a). Invasive species litter released an average of 13% more N after 90 days (F\textsubscript{1,21} = 28.11, p < 0.01) compared to native species litter, and released 38% of initial N after 365 days, whereas native species litter gained an additional 16% of initial N (F\textsubscript{1,18} = 36.23, p < 0.01; Fig. 2b). Although invasive species litter had, on average, lost N after 365 days, litters of two species (*M. alba* and *L. maackii*) drove this pattern.

Mixed litter decomposition and N loss

We detected non-additive mass loss in almost all species pairings, although not at every loading ratio, and the presence, sign, and strength of observed NAE varied over time (Fig. 3, see Supplementary material Appendix 1, Fig. A3 and A4 for mean mass and N loss of each type of litter bag). In general, NAE became more synergistic and less antagonistic with time (time or its interaction with another factor was significant in all models; Table 1). Greater relative differences in litter chemistry between native and invasive species also resulted in more synergistic NAE over time. Both C:N distance and N ratio interacted with time to promote antagonistic and synergistic NAE with higher quality invasive litter at 90 and 365 days, respectively.

Native and invasive species did not act uniformly to promote NAE in either mass loss or N loss, and not all species acted consistently across both time points, either (Table 1). However, while species differed relative to each other, no species was found to have an effect that significantly differed from zero (Tukey HSD), indicating that the inclusion of any single species in mixture did not predispose that mixture to NAE.

We detected NAE more frequently for N loss than for mass loss. Thirty-six percent of all combinations across both times were non-additive for N loss, whereas 31% of combinations were non-additive for mass loss. NAE for N loss were almost always antagonistic after 90 days and synergistic after 365 days of decomposition (Fig. 3). However, transitions from antagonistic effects to synergistic effects for a single litter mixture were only observed in mixtures containing *M. alba*. All other transitions after 365 days were the result of the absence of antagonistic NAE detected earlier or the presence of synergistic NAE that were previously absent within the same species pairing.

Litter mixtures consistently experienced more synergistic and less antagonistic NAE on N loss after 365 days than after 90 days (Table 1). This trend was strongly correlated with relative differences in initial litter quality. Invasive species that had higher quality were equally likely as not to promote NAE after 90 days, but made non-additivity values more positive after 365 days of decomposition. We also found significant three-way interactions between C:N distance or N ratio, time, and loading ratio ('Mix'; Table 1). Simple linear regressions for each collection date and loading ratio revealed that relatively higher quality invasive species litter enhanced synergistic effects to a greater extent in mixtures containing a greater
proportion of invasive species litter after 365 days (Supplementary material Appendix 1 Table A2). Although statistically significant, relative litter quality did not explain the majority of variation in non-additivity ($r^2 \leq 0.20$, Supplementary material Appendix 1 Table A2). However, if C:N distance was considered as an absolute value in these regressions, thus representing total dissimilarity between litters regardless of native status, all significant effects of C:N distance were lost (data not shown), indicating that native and invasive litters were not equally able to promote NAE via differences in C:N ratio.

To understand the relative influence NAE might have on seasonal N release from litter mixtures, we estimated mean N release under additive and non-additive scenarios across all 16 species pairings (Fig. 4). Considering the mean values for each even (50% invasive) mixture of native and invasive species litter, the mean expected N loss across species pairs was 8.61% at 90 days, and 11.21% at 365 days; translating to an effective rate of N release of $9.47 \times 10^{-3}$ %N day$^{-1}$ between those points. The slow expected rate of N release during this period resulted from the high immobilization of native species litters compensating for the high mineralization of invasive species litters. Thus, under additive N loss, very little N would have been released. In contrast, the mean observed N loss for even mixtures was 3.85% at 90 days, and 20.31% at 365 days. Following the general temporal trend of antagonistic and then synergistic N loss in litters, N loss at 90 days was much lower than expected, but was almost double the expected value at 365 days. This translates to N loss of
Mixed litter decomposition and N release

We expected mixtures of native and invasive species’ litters to exhibit synergistic NAE since differences in litter quality are generally cited as enhancing mass loss and N loss in litter mixtures (Gartner and Cardon 2004, Liu et al. 2007), and invasive species often have higher litter quality than co-occurring native species (Liao et al. 2008). Following this logic, we also expected differences in litter chemistry to be indicative of the sign and strength of NAE, and for NAE to be strongest in mixtures containing a majority of invasive species litter. These hypotheses received mixed support.

In contradiction to our hypothesis, litter mixture decomposition was largely idiosyncratic, although NAE were frequently observed and were more often synergistic than antagonistic. The lack of a consistent pattern across mixtures supports previous findings that NAE on mass loss are often highly variable, with the presence, sign, and strength of NAE changing over time and depending largely on the identity of the species contained within the mixture (Wardle et al. 1997, Jonsson and Wardle 2008, Marco et al. 2011). Although NAE were highly variable, they became more synergistic over time. That some synergistic NAE became more pronounced throughout the decomposition process is not surprising, since the processes leading to divergence in decomposition of single substrate litters and litter mixtures are likely to accumulate over time and only permit convergence once the litter has fully decomposed. However, the transition from an antagonistic effect to a synergistic effect requires a different explanation since the mechanisms driving the two types of NAE are likely to differ (Hättenschwiler et al. 2005). Others have suggested that litter mixtures can become increasingly synergistic as litter secondary compounds are degraded or leach out of the mixture (Wardle et al. 1997, Liu et al. 2007). While we cannot confirm that these mechanisms were responsible for the change in sign we observed between time points, they offer a potential explanation to the high variability of NAE on mass loss we observed.

NAE on N loss were common and were almost always antagonistic at 90 days, but synergistic at 365 days. That antagonistic effects are so prominent at 90 days and that synergistic effects mostly occur only at 365 days leads us to reject our hypothesis of mixed litter exhibiting only synergistic effects. However, the consistency of NAE at each time point is remarkable. Most studies on NAE find them to be largely idiosyncratic (Gartner and Cardon 2004, Ball et al. 2008); thus our observation of relatively uniform antagonistic then synergistic effects on N loss stands out amongst previous findings.

The clear pattern of NAE on N loss suggests a mechanism of altered N release in mixtures of native and invasive species litter. When antagonistic effects on N loss have been observed previously (Schimel and Hättenschwiler 2007, Ball et al. 2009), they have been largely attributed to the rapid uptake of N by the microbial community associated with the lower quality litter (Hättenschwiler and Gasser 2005). We speculate that the same mechanism is responsible for the antagonistic effects we observed, where N released from the higher quality litter is retained by the
Table 1. Summary of mixed model ANOVAs testing for the effects of time, loading ratio (mix), and differences in initial N concentration between species (N ratio; model 1), differences in initial C:N ratio between species (C:N distance; model 2), or native and invasive species identity (model 3) on the signed non-additivity value of all mixed litter bags for mass loss and N loss. Significant factors (p ≤ 0.05) are presented in bold.

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<td></td>
<td>T×N×M</td>
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<td></td>
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<td>0.86</td>
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Our collection dates (March and December) effectively border the growing season in the study region (April–October), allowing us to infer how litter mixtures might influence seasonal N fluxes. Litter bags collected at 90 days inform nutrient dynamics prior to the growing season, while the period between those and the litter bags collected at 365 days suggest dynamics during the growing season. Consequently, the enhanced N loss we observed at 365 days must have occurred over the growing season, which would have resulted in improved temporal synchrony between litter N release and plant N demand. By better aligning plant demand for N and its release from the litter layer, mixed litter can enhance system fertility and plant productivity (Briones and Ineson 1996, McTiernan et al. 1997). N availability is particularly important for invasive species, since the spread of many invasive species can be facilitated by enhanced nutrient availability (Ehrenfeld 2010). Thus, some invasive species are likely to disproportionately benefit from enhanced synchrony of N release.

Recently, litter mixtures of the invasive vine *Mikania micrantha* and seven species native to southern China have been shown to exhibit NAE on N loss in a pattern consistent with our findings (Chen et al. 2013). These mixtures slowed N release early in decomposition, but enhanced N release after 128 and 160 days, with stronger NAE observed in mixtures containing more *M. micrantha* litter. Although not discussed by the authors, these mixtures would have released a greater proportion of their N during the growing season than would be possible at the additive rate of N loss. The clear similarities between our results and those of Chen et al. (2013) suggest that NAE-derived N synchrony is likely not confined to our study species or system, and suggest this mechanism may commonly occur in native–invasive litter mixtures.
We suggest that improved synchrony of N release through NAE may represent a previously unknown positive litter feedback to invasion. The litter of one of our study species, L. maackii, has been suggested to decompose at a rate that coincides with plant nutrient requirements (Trammell et al. 2012), and other studies have suggested positive litter feedbacks to invasion through nutrient release and alterations to soil light conditions (Eppinga et al. 2011, Tharayil et al. 2013). However, the ability of L. maackii or other invasive plants to promote N synchrony through NAE has not previously been addressed. Our findings indicate that NAE promote improved N synchrony in mixtures of native and invasive species litters by enhancing N loss rates during the growing season, and subsequently elevating the impacts of invasive plant species on litter N loss to levels consistent with a more heavily invaded system. We suggest that this enhances the influence of invasive trees on ecosystem processes at low and intermediate densities and promotes positive litter feedbacks to invasion beyond what would be expected based solely on single-species litter traits. The differences we calculated between observed and expected rates of N release from 90 to 365 days support this notion. Deviations from the expected N release of mixed litters containing 10% or 90% invasive litter are consistent with litter mixtures promoting increased synchrony of N release and demand, but are not as large as those observed in even litter mixtures. Consequently, our results suggest the influence of invasive species litter on N release is largest when invasive species constitute an even proportion of the litter mixture. Thence, the apparent benefit of NAE is noticeable in the later stages of decomposition. However, the apparent benefit of NAE is reduced as mixtures become more dominated by invasive species litter, but is compensated for by the high N release rates of invasive species litter.

We cannot exclude the possibility that native–native mixtures would have behaved similarly to our native–invasive mixtures. However, to the best of our knowledge, no study considering native–native mixtures has found a similar pattern. If we had decomposed native–native mixtures and they had exhibited the same pattern of antagonism then synergism, this would likely have still resulted in net N immobilization since all but one native species continued to immobilize N after 365 days (Fig. 1). In contrast, native-invasive mixtures consistently lost N (Fig. 4), and are thus able to contribute to any potential N feedbacks to invasion. Here, we have shown strong empirical evidence suggesting that litter from invasive plant species can enhance N release from the litter layer during the time period of greatest plant demand.

It is also important to acknowledge some of the biases introduced by our methods. Our first collection date roughly corresponded with the end of the winter season at our site. During this time, low air temperatures (mean: $-3.2 \pm 0.7^\circ C$) would have limited microbial activity, and decomposition would have been largely a physical process, which may have been accelerated as a result of our breaking litter into 2 cm fragments (Tiuov 2009). However, fragmentation of litter was unavoidable and our protocol allowed us control over fragment size and facilitated mixing of litter types. As with all litter bag experiments, the mesh size of our litter bags excluded some decomposers (mainly macrofauna) that would have otherwise acted to physically degrade our litter (Robertson 1999).

Although these biases are important to note, litter mixing effects are primarily driven by small scale processes (Hättenschwiler et al. 2005, Lummer et al. 2012, Makkonen et al. 2012), and thus we feel these methods are acceptably representative of reality and also provided the best opportunity for us to detect possible non-additive effects.

Although we did not empirically test any litter feedbacks to invasion, our observation of increased N release during the growing season could be the first indicator of such a mechanism. If such a feedback exists, it could indicate a higher threshold for invasive plant removal necessary to restore ecosystem function to levels more consistent with intact native communities since moderating effects of native plants on N cycling would be reduced through NAE. Thus, our findings point to research needs in the realms of forest ecology, invasion biology, and invasive plant management. Future work should seek to further examine the impact of NAE on the timing of soil N availability and plant community responses to these changes, as well as compare native–invasive mixtures to co-occurring native–native mixtures using the same species under identical environmental conditions.

Drivers of NAE

We examined multiple predictors and potential drivers of the sign and strength of NAE on mass loss and N loss, including species identity, differences in tissue chemistry, and the evenness of the litter mixture. Of these potential drivers, we determined that higher invasive species litter quality was the most consistently important determinant of NAE, but that the effects of tissue chemistry were only noticeable in the later stages of decomposition. However, the influence of relative invasive species litter quality was strongest in mixtures containing a majority of invasive species litter, suggesting that synchrony of litter N release and plant N demand will be greatest in communities dominated by invasive species.

We hypothesized that greater differences in litter quality between native and invasive species would result in more synergistic NAE in both mass loss and N loss. Contrary to this hypothesis, differences in litter quality were not significant predictors of decomposition or N loss after 90 days. However, we found that higher quality invasive species litter relative to native species litter resulted in more synergistic effects in both mass and N loss after 365 days. While statistically significant, relative litter quality did not explain the majority of variation in NAE in our regression analyses (Supplementary material Appendix 1 Table A2). Consequently, our results suggest that differences in litter quality were an important factor influencing NAE during the later stages of decomposition, but other factors largely determined the sign and strength of NAE in mixtures of native and invasive tree species. This supports previous findings that suggest that chemical differences in litters are not the primary driver of NAE (Smith and Bradford 2003, Hoorens et al. 2003, Schimel and Hättenschwiler 2007), and indicates that there might be important litter characteristics that we did not examine. Lummer et al.
(2012) suggest that NAE may be strongly influenced by absolute nutrient content of litter, as opposed to relative differences in litter quality, since nutrient content can regulate the composition of fungi and bacteria, which differ in their vulnerability to nutrient transfer mechanisms. Additionally, water saturation capacity of litters has recently been shown to strongly influence NAE (Makkonen et al. 2012) by providing more favorable microclimates for microbial communities (Hättenschwiler et al. 2005). Thus, future investigations into NAE would likely benefit from examining potential differences in microbial community composition and water saturation capacity between native and invasive species’ litters as well as differences in litter chemistry.

Conclusions

We found that mixtures of native and invasive species’ leaf litter frequently exhibited non-additive decomposition and N loss. While effects on mass loss were highly variable, we observed a strong trend of greater N retention during the early stages of decomposition, followed later by enhanced N release, especially for mixtures containing a majority of high-quality invasive species litter. We are the first to provide consistent evidence of non-additive effects on N loss in multiple litter mixtures of native and invasive tree species and suggest a novel mechanism that would enhance invasive plant species’ impacts on ecosystem processes. Non-additive effects on N loss markedly accelerated N release during the growing season relative to what would be expected based on single-species litter decomposition. Consequently, our findings strongly imply that estimates of invasive species’ impacts on ecosystem processes based only on single species’ litter traits are inaccurate. Based on the timing of our observations, we suggest that some invasive plant species improve the temporal synchrony of litter N release and speculate that this mechanism could potentially facilitate invasive plant species’ success.

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References


