DISENTANGLING EFFECTS OF LITTER DIVERSITY: NON-RANDOM SPECIES LOSS, CROSS-SYSTEM LINKAGES, AND ECOSYSTEM FUNCTION

by

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(Under the Direction of Mark A. Bradford)

ABSTRACT

Litter decomposition is a fundamental process about which a great deal is known, but most knowledge comes from studies of single-species decay. Litter-mixing studies have tested whether monoculture data can be applied to mixed-litter systems and have mainly attempted to detect non-additive effects of litter mixing, which addresses consequences of random species loss. Under global change, non-random species loss, characterized by the loss of species more susceptible to changes in environmental factors, is more likely to occur. With this scenario, individual species effects (additivity) as well as species interactions (non-additivity) may alter decomposition processes, potentially showing consequences that differ from those of random loss. To determine the impacts of non-random species loss on decomposition, we looked for both additive and non-additive effects of litter mixing on mass loss, nutrient dynamics, and the decomposer community. To do this, a full-factorial litterbag experiment of four deciduous leaf species was conducted. Data were analyzed using a statistical method that first looks for additive effects based on the presence or absence of species, then significant species interactions occurring beyond that. We found additive species composition (identity) effects on substrate mass loss and most aspects of the decomposer community, suggesting that differences in litter

quality override mixing effects for these variables, and the consequences of non-random species loss will be predictable. Additive effects on carbon loss were more evident when the substrate was analyzed separately from microbial biomass colonizing the litter. We found non-additive effects on nutrient dynamics driven by both species richness and composition, with less overall release from multiple-species mixtures than monocultures. This led to great overestimations of ecosystem-level nutrient release when calculated from dynamics of monocultures, as is usually done by other studies, with no net immobilization as was identified by estimations based on the non-additive litter mixtures. Our results suggest a potentially large impact of non-random species loss on this system, which has not been addressed for decomposition, with large repercussions on organic matter and nutrient turnover. Together, these data demonstrate an effect of plant community composition on decomposition and related properties, confirming a link between above- and belowground communities.

INDEX WORDS: Ecosystem function, decomposition, litter mixtures, species diversity, species composition, non-random species loss, random species loss, biodiversity, litter quality

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

In forested ecosystems, litter from aboveground plant communities represents a significant addition of energy and nutrients to belowground systems (Wardle, 2002). Decomposition of this organic material is a fundamental ecological process integral to nutrient cycling, energy flow in foodwebs, and the structure and dynamics of ecosystems (Swift et al., 1979, Moore et al., 2004). This control over the availability of resources necessary for plant productivity forms a feedback from belowground systems to aboveground processes and communities (De Ruiter et al., 2005). Recently, there has been increasing interest in the reverse: how aboveground systems in turn affect belowground communities and processes (Wardle et al., 2004, De Deyn and Van der Putten, 2005, Wardle, 2006, Bardgett et al., 2005). With this interest in mind, there has been a large focus specifically on the effects of altered plant litter diversity on decomposition and its associated properties (reviewed by Hättenschwiler et al., 2005, Gartner and Cardon, 2004). This introductory chapter serves to organize what is already known about the controls of decomposition dynamics and how these might be altered through changes in leaf litter species diversity.

Influences on decomposition in terrestrial environments

The rate at which decomposition occurs is influenced by a number of biotic and abiotic factors, the most influential of which are litter chemical and structural quality, the nature of the

microbes and fauna in the soil, and climate (Aerts, 1997, Melillo et al., 1982, Seastedt, 1984, Lavelle et al., 1993, Swift et al., 1979). Though these three factors interact with each other and do not independently exert an influence on decomposition, many believe that climate has the greatest effect, followed by litter chemistry, and then soil biota (Knutson, 1997, Meentemeyer, 1978, Couteaux et al., 1995). This may be true at continental scales, but at local scales it is often litter chemistry that exerts the greatest effect, followed by microclimate and soil biota (Aerts, 1997).

The biochemical content of litter, meaning the nature of its compounds and the relative concentration of different elements and compounds, is often referred to as substrate quality. Complexity of carbon (C) compounds reflects the amount of energy that can be yielded by breaking them down (Aber and Melillo, 2001). Simple sugars, carbohydrates, and starches contain energy-rich bonds and are decomposed very quickly. Conversely, structural compounds such as cellulose and lignin and secondary plant chemicals such as polyphenols and tannins are more complex and often shown to have controls over decay rate (Hagerman and Butler, 1991, Hammel, 1994, Taylor et al., 1989). In addition to C content, nutrients in litter also determine overall decomposability. Litters with greater nutrient concentrations such as nitrogen (N) and phosphorus (P) generally decay faster than those with smaller initial content (Blair et al., 1992, Cotrufo et al., 1995, McClaugherty and Berg, 1987).

Litter horizons contain very diverse communities of biota that function as decomposers. Microbes, including bacteria and fungi, are the principal players in decomposition and can account for up to 90% of the decomposer biomass and activity (Swift et al., 1979, Chapin et al., 2002). Fungi are usually more abundant than bacteria on decomposing litter, and are the most active organisms in lignin and cellulose degradation (Chapin et al., 2002, Hammel, 1994, Atlas

and Bartha, 1998). There is also a large number and diversity of invertebrate fauna in the soil that colonize leaf litter, forming the next levels of the foodweb. Microbivorous fauna, including nematodes and microarthropods, influence decomposition through feeding activities on bacteria and fungi, regulating populations and altering nutrient turnover (Coleman et al., 2004, Seastedt, 1984). Predatory fauna, including nematodes, micro-, and macroarthropods, regulate the populations and activity of the microbial-feeding fauna, thus indirectly influencing decomposition (Hunter et al., 2003).

Mixed-species effects on decomposition

Much of our understanding about which factors control the process is derived from studies following the decay dynamics of single species, though most forested ecosystems consist of multiple tree species. To address this, many studies have attempted to determine whether decomposition dynamics differ under multi-species mixtures (reviewed by Gartner and Cardon, 2004) or are affected by litter diversity (reviewed by Hättenschwiler et al., 2005). Effects of mixing could be additive, where nutrient dynamics would result from the independent influence of individual species, with diverse litter mixes showing enhanced (or lowered) decomposition dynamics due to increased probability of including species with greater (or lesser) decomposition dynamics (Johnson et al., 2006). If decay dynamics in mixtures are the sum of their parts, nutrient dynamics of single litters can be used to predict nutrient dynamics in multi-species litter layers. Alternatively, decomposition dynamics in mixture could be dependent on other litter species, giving rise to non-additive dynamics. If this were the case, research on decomposition of mixtures would be required for us to understand nutrient dynamics in species-rich systems. Effects of litter mixing have been measured for most decomposition-related parameters,

including decay rate, nutrient dynamics, and decomposer community, and conclusions from these studies vary. Since very comprehensive literature reviews of litter-mixing and diversity effects have already been conducted in the literature, this will not be repeated here. Overall, however, there is little evidence for an effect of species richness *per se* on decomposition dynamics, but the composition of the litter mixture (i.e. the identity of species involved) often but not always generates non-additivity (see reviews by Gartner and Cardon, 2004, Hättenschwiler et al., 2005).

Non-random species loss

Non-additivity due to interactions among species has been the primary focus of previous work (Gross and Cardinale, 2005, Schläpfer et al., 2005). As a consequence, a lack of interactions, where the results of litter mixing can be predicted based on the individual species present, has been considered a null effect of mixing. Experiments for which non-additive effects are the focus generally test only for consequences of random species loss, where all species are equally likely to be lost from the system (Smith and Knapp, 2003). Under global environmental change, such as altered climate, land-use and resource availability, much of the change in biodiversity is likely to be through non-random species loss (Grime, 1998, Vitousek et al., 1997, Loreau et al., 2001, Ellison et al., 2005, Schläpfer et al., 2005, Smith and Knapp, 2003), generating different outcomes on ecosystem functioning than random species loss (Gross and Cardinale, 2005, Schläpfer et al., 2005). Thus, there is a pressing need to understand how ecosystems will function as species are lost non-randomly. While this work has begun for plant productivity, it has not been addressed explicitly for litter decomposition or its associated processes.

Current Study System

The southern Appalachian Mountains have undergone rapid land-use change due to changes in human decision-making, reflecting a response to socioeconomic and biogeophysical conditions. The area underwent massive deforestation at the turn of the 20th century, followed by agricultural uses (Wear and Bolstad, 1998). The current shift is towards agricultural abandonment and residential development, with a near tripling of the average building density, accompanied by a filling in of the road network. Additionally, land uses in the area are greatly influenced by topographic features. Slope and elevation limit intensive land uses to certain portions of the area, particularly in riparian areas. Riparian areas are relatively level, close to roads, and concentrated around important water courses (Bolstad and Swank, 1997). Additionally, the abundance and distribution of species in this area are projected to change due to invasive pests and pathogens. For example, the invasive hemlock woolly adelgid is projected to extirpate eastern hemlock from much of its range, and at our field site will likely be replaced by tulip poplar or rhododendron (Orwig and Foster, 1998, Ellison et al., 2005). Similarly, there are predicted declines in rhododendron caused by the invasive pathogen sudden oak death (Rizzo et al., 2002). Thus, the abundance and distribution of plants in riparian ecosystems in the southern Appalachians are likely to be greatly altered due to various aspects of global environmental change.

All of the work reported was conducted at Coweeta Hydrologic Laboratory in the southern Appalachian Mountains near Otto, North Carolina, U.S.A. (35º00'N, 83º30'W; elevation 1300 m). The mean annual rainfall is approx. 1700 mm and the mean annual temperature 13ºC (Heneghan et al., 1999). The study was conducted in Watershed 20 on Ball Creek, which drains into Coweeta Creek, a tributary of the Little Tennessee River. To study the

impacts of altered plant communities in this area, we conducted a decomposition study over a gradient of litter species diversity using the four most abundant tree species at Coweeta: *Liriodendron tulipifera* L. (tulip poplar, L), *Acer rubrum* L. (red maple, A), *Quercus prinus* L. (chestnut oak, Q), and *Rhododendron maximum* L. (rhododendron, R). These species cover a gradient of litter quality and decay rate in monoculture (see Chapter 2, Table 1). We used all possible combinations of these species to conduct a full-factorial study of leaf litter decomposition. The fifteen possible combinations were as follows:

1	L
2	A
3	Q
4	R
5	L, A
6	L, Q
7	L, R
8	A, Q
9	A, R
10	Q, R
11	L, A, Q
12	L, A, R
13	L, Q, R
14	A, Q, R
15	L, A, Q, R

Purpose and Hypotheses

To determine the consequences of non-random species loss in aboveground plant communities for decomposition and its related processes, we conducted a long-term litterbag decomposition study manipulating leaf litter diversity. We discuss the effects of species loss, simulated through alterations in litter diversity, on several parameters often associated with decomposition: litter mass loss (Chapter 2), nutrient dynamics (Chapter 3), and decomposer community (Chapter 4). First, in Chapter 2, we outline the importance of considering the consequences of non-random species loss for mass loss and nitrogen content of decomposing

litter. Additionally, to determine if interpretations of litter-mixing effects on mass loss are obscured by the microbial biomass associated with litter, we independently analyze substrate mass loss for effects of species loss in Chapter 5. In Chapter 3, we investigate the consequences of non-random loss on nutrient dynamics from the litter layer, also demonstrating the importance of considering non-additive dynamics when estimating ecosystem-level nutrient dynamics. To explore links between aboveground and belowground communities, in Chapter 4 we describe the effects of alterations in plant community through non-random species loss on the decomposer community.

Specifically, in the following chapters I explore and address these specific hypotheses:

Chapter 2. Consequences of non-random species loss for decomposition dynamics: Experimental evidence for additive and non-additive effects.

H1: Given that our chosen litters form a gradient in litter nutrient content, loss of any one of the four species will produce an additive change in decomposition dynamics.

H2: Given the expectation that non-additive, compositional effects arise when litters of markedly differing nutrient content are present, non-additivity will only arise when a litter species is lost that is at the high or low end of the spectrum.

H3: Since the overwhelming evidence to date indicates that species composition is more important than species richness *per se* on decay of mixed-species litter, there will be no relationship between litter species richness and decomposition rate.

Chapter 3. Nitrogen and phosphorus release from mixed litter layers is lower than predicted from single species decay.

H1: Given the gradient in initial nutrient content, we will see non-additive effects on nutrient dynamics. Litter with greater nutrient content could stimulate decomposition, and

thus subsequent nutrient release, of lower quality litter, showing more net release in mixture than would be expected, or

H2: Translocation of nutrients from litter with high nutrient content to lower could lead to immobilization, rather than release, of those nutrients in mixture.

H3: We expect those non-additive effects to be due to composition, rather than richness, due to the lack of evidence for richness effects on nutrient dynamics in the literature.

Chapter 4. Additive linkages between below- and aboveground communities: decomposer responses to non-random tree species loss.

H1: Given the gradient in initial litter quality, structure, and decomposition rate, there will be compositional effects of litter mixing on the decomposer community, suggesting a feedback between aboveground plant communities and belowground communities.

H2: We expect each of the four species to exert an additive individual influence in mixture with similar species, but support a synergistically larger decomposer community when species are very different from one another.

Chapter 5. Does microbial biomass confound litter-mixing effects on mass loss?

We tested two competing hypotheses:

H1: Microbial biomass and litter substrate mass loss respond to litter-mixing in comparable manners, allowing the overall effects on litter (microbes + substrate) mass loss to reflect that of the actual substrate, or

H2: Microbial biomass and litter substrate mass loss do not respond to litter mixing in comparable manners, causing the dynamics of substrate mass loss to be obscured when only total litter mass loss is analyzed.

References

Aber, J. D. & Melillo, J. M. (2001) Chemical properties of litter and soil organic matter: the decomposition continuum. *Terrestrial Ecosystems* pp. 205-226. Academic Press, San Diego.

Aerts, R. (1997) Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: A triangular relationship. *Oikos,* **79,** 439-449.

Atlas, R. M. & Bartha, R. (1998) Soil microbial communities. *Microbial Ecology: Fundamentals and Applications*pp. 367-374. Benjamin/Cummings Publishing Co., Inc., Menlo Park, CA.

Bardgett, R. D., Bowman, W. D., Kaufmann, R. & Schmidt, S. K. (2005) A temporal approach to linking aboveground and belowground ecology. *Trends in Ecology & Evolution,* **20,** 634-641.

Blair, J. M., Crossley, D. A. & Callaham, L. C. (1992) Effects of Litter Quality and Microarthropods on N-Dynamics and Retention of Exogenous N-15 in Decomposing Litter. *Biology and Fertility of Soils,* **12,** 241-252.

Bolstad, P. V. & Swank, W. T. (1997) Cumulative impacts of landuse on water quality in a southern Appalachian watershed. *Journal of the American Water Resources Association,* **33,** 519-533.

Chapin, F. S., Matson, P. A. & Mooney, H. A. (2002) *Principles of Terrestrial Ecosystem Ecology.* Springer-Verlag New York, Inc., New York, NY.

Coleman, D. C., Crossley, D. A. & Hendrix, P. F. (2004) *Fundamentals of Soil Ecology.* Elsevier Inc., Burlington, MA.

Cotrufo, M. F., Ineson, P. & Roberts, J. D. (1995) Decomposition of Birch Leaf Litters with Varying C-to-N Ratios. *Soil Biology & Biochemistry,* **27,** 1219-1221.

Couteaux, M. M., Bottner, P. & Berg, B. (1995) Litter Decomposition, Climate and Litter Quality. *Trends in Ecology & Evolution,* **10,** 63-66.

De Deyn, G. B. & Van der Putten, W. H. (2005) Linking aboveground and belowground diversity. *Trends in Ecology & Evolution,* **20,** 625-633.

De Ruiter, P., Neutel, A. M. & Moore, J. (2005) The balance between productivity and food web structure in soil ecosystems. *Biological Diversity and Function in Soils* (eds R. D. Bardgett, M. B. Usher & D. W. Hopkins), pp. 139-153. Cambridge University Press.

Ellison, A. M., Bank, M. S., Clinton, B. D., Colburn, E. A., Elliott, K., Ford, C. R., Foster, D. R., Kloeppel, B. D., Knoepp, J. D., Lovett, G. M., Mohan, J., Orwig, D. A., Rodenhouse, N. L., Sobczak, W. V., Stinson, K. A., Stone, J. K., Swan, C. M., Thompson, J., Von Holle, B. & Webster, J. R. (2005) Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. *Frontiers in Ecology and the Environment,* **3,** 479-486.

Gartner, T. B. & Cardon, Z. G. (2004) Decomposition dynamics in mixed-species leaf litter. *Oikos,* **104,** 230-246.

Grime, J. P. (1998) Benefits of plant diversity to ecosystems: immediate, filter and founder effects. *Journal of Ecology,* **86,** 902-910.

Gross, K. & Cardinale, B. J. (2005) The functional consequences of random vs. ordered species extinctions. *Ecology Letters,* **8,** 409-418.

Hagerman, A. E. & Butler, L. G. (1991) Tannins and Lignins. *Herbivores: Their interactions with secondary plant metabolites, Vol I: The Chemical Participants* (eds G. A. Rosenthal & M. R. Berenbaum), pp. 355-388. Academic Press, San Diego, CA.

Hammel, K. E. (1994) Fungal degradation of lignin. *Driven by Nature: Plant Litter Quality and Decomposition* (eds G. Cadisch & K. E. Giller), pp. 33-45. CAB International, Oxon, UK.

Hansen, R. A. & Coleman, D. C. (1998) Litter complexity and composition are determinants of the diversity and species composition of oribatid mites (Acari : Oribatida) in litterbags. *Applied Soil Ecology,* **9,** 17-23.

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

Heneghan, L., Coleman, D. C., Crossley, D. A. & Zou, X. M. (1999) Nitrogen dynamics in decomposing chestnut oak (Quercus prinus L.) in mesic temperate and tropical forest. *Applied Soil Ecology,* **13,** 169-175.

Hunter, M. D., Adl, S., Pringle, C. M. & Coleman, D. C. (2003) Relative effects of macro invertebrates and habitat on the chemistry of litter during decomposition. *Pedobiologia,* **47,** 101-115.

Johnson, M. T. J., Lajeunesse, M. J. & Agrawal, A. A. (2006) Additive and interactive effects of plant genotypic diversity on arthropod communities and plant fitness. *Ecology Letters,* **9,** 24-34.

Knutson, R. M. (1997) An 18-year study of litterfall and litter decomposition in a Northeast Iowa deciduous forest. *American Midland Naturalist,* **138,** 77-83.

Lavelle, P., Blanchart, E., Martin, A., Martin, S., Spain, A., Toutain, F., Barois, I. & Schaefer, R. (1993) A Hierarchical Model for Decomposition in Terrestrial Ecosystems - Application to Soils of the Humid Tropics. *Biotropica,* **25,** 130-150.

Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J. P., Hector, A., Hooper, D. U., Huston, M. A., Raffaelli, D., Schmid, B., Tilman, D. & Wardle, D. A. (2001) Biodiversity and ecosystem functioning: Current knowledge and future challenges. *Science,* **294,** 804-808.

McClaugherty, C. & Berg, B. (1987) Cellulose, Lignin and Nitrogen Concentrations as Rate Regulating Factors in Late Stages of Forest Litter Decomposition. *Pedobiologia,* **30,** 101-112.

Meentemeyer, V. (1978) Macroclimate and Lignin Control of Litter Decomposition Rates. *Ecology,* **59,** 465-472.

Melillo, J. M., Aber, J. D. & Muratore, J. F. (1982) Nitrogen and Lignin Control of Hardwood Leaf Litter Decomposition Dynamics. *Ecology,* **63,** 621-626.

Moore, J. C., Berlow, E. L., Coleman, D. C., de Ruiter, P. C., Dong, Q., Hastings, A., Johnson, N. C., McCann, K. S., Melville, K., Morin, P. J., Nadelhoffer, K., Rosemond, A. D., Post, D. M., Sabo, J. L., Scow, K. M., Vanni, M. J. & Wall, D. H. (2004) Detritus, trophic dynamics and biodiversity. *Ecology Letters,* **7,** 584-600.

Orwig, D. A. & Foster, D. R. (1998) Forest response to the introduced hemlock woolly adelgid in southern New England, USA. *Journal of the Torrey Botanical Society,* **125,** 60-73.

Rizzo, D. M., Garbelotto, M., Davidson, J. M., Slaughter, G. W. & Koike, S. T. (2002) Phytophthora ramorum as the cause of extensive mortality of Quercus spp. and Lithocarpus densiflorus in California. *Plant Disease,* **86,** 205-214.

Schläpfer, F., Pfisterer, A. B. & Schmid, B. (2005) Non-random species extinction and plant production: implications for ecosystem functioning. *Journal of Applied Ecology,* **42,** 13-24.

Seastedt, T. R. (1984) The Role of Microarthropods in Decomposition and Mineralization Processes. *Annual Review of Entomology,* **29,** 25-46.

Smith, M. D. & Knapp, A. K. (2003) Dominant species maintain ecosystem function with non-random species loss. *Ecology Letters,* **6,** 509-517.

Swift, M. J., Heal, O. W. & Anderson, J. M. (1979) *Decomposition in Terrestrial Ecosystems.* University of California Press, Los Angeles, CA.

Taylor, B. R., Parkinson, D. & Parsons, W. F. J. (1989) Nitrogen and Lignin Content as Predictors of Litter Decay-Rates - a Microcosm Test. *Ecology,* **70,** 97-104.

Vitousek, P. M., Mooney, H. A., Lubchenco, J. & Melillo, J. M. (1997) Human domination of Earth's ecosystems. *Science,* **277,** 494-499.

Wardle, D. A. (2002) *Communities and Ecosystems: Linking the Aboveground and belowground components.* Princeton University Press, Princeton, NJ, USA.

Wardle, D. A. (2006) The influence of biotic interactions on soil biodiversity. *Ecology Letters,* **9,** 870-886.

Wardle, D. A., Bardgett, R. D., Klironomos, J. N., Setälä, H., van der Putten, W. H. & Wall, D. H. (2004) Ecological Linkages Between Aboveground and Belowground Biota. *Science,* **304,** 1629-1633.

Wardle, D. A., Yeates, G. W., Barker, G. M. & Bonner, K. I. (2006) The influence of plant litter diversity on decomposer abundance and diversity. *Soil Biology and Biochemistry,* **38,** 1052-1062.

Wear, D. N. & Bolstad, P. (1998) Land-use changes in Southern Appalachian landscapes: Spatial analysis and forecast evaluation. *Ecosystems,* **1,** 575-594.

CHAPTER 2

CONSEQUENCES OF NON-RANDOM SPECIES LOSS FOR DECOMPOSITION DYNAMICS: EXPERIMENTAL EVIDENCE FOR ADDITIVE AND NON-ADDITIVE EFFECTS[1]

[1] Ball, B.A., M.D. Hunter, J.S. Kominoski, C.M. Swan, and M.A. Bradford. To be submitted to *Journal of Ecology*.

Abstract

Litter decomposition is a fundamental process about which a great deal is known, but most knowledge comes from studies of single-species decay. Litter-mixing studies have tested whether monoculture data can be applied to mixed-litter systems and have mainly attempted to detect non-additive effects of litter mixing, which addresses potential consequences of random species loss. Under global change, species loss is more likely to be non-random, characterized by the loss of species more susceptible to changes in environmental factors. With non-random species loss, individual species effects (additivity) as well as species interactions (non-additivity) may alter decomposition rates, potentially showing consequences that differ from those of random loss. To determine the impacts of non-random species loss, we looked for both additive and non-additive effects of litter mixing on mass loss and litter nutrients. To do this, a full-factorial litterbag experiment of four deciduous leaf species was conducted, from which mass loss and nitrogen content were measured. Data were analysed using a statistical method that first looks for additive effects based on the presence or absence of species and then any significant species interactions occurring beyond that and partitions non-additive effects into those caused by richness and/or composition. This approach deviates from the typical methods used to analyse litter mixtures, but addresses questions key to understanding the potential effects of global change. If additive effects dominate, the consequences for decomposition dynamics will be predictable based on our knowledge of individual species, but not predictable if non-additive effects dominate. We found additive effects on mass loss and non-additive composition effects on litter nitrogen. We were able to identify the species responsible for these composition effects that would otherwise have been considered idiosyncratic or absent when analysed by the methods of previous work. Our results suggest a potentially large impact of non-random species

loss on this system, which has not been addressed for decomposition. We were able to identify the species driving additive and non-additive interactions, aiding predictions of the consequences of the loss of these dominant species on organic matter and nutrient turnover.

Key Words: Ecosystem function, decomposition, litter mixtures, species diversity, species composition, non-random species loss, random species loss, biodiversity, litter quality

Introduction

Decomposition of plant litter is a fundamental ecological process, integral to nutrient cycling, energy flow in foodwebs, and the structure and dynamics of ecosystems (Swift et al., 1979, Stevenson, 1994, Aber and Melillo, 2001, Schnitzer and Khan, 1978, Moore et al., 2004). Much of our understanding about which factors control the process is derived from studies following the decay dynamics of single species. Whether this understanding can be used to predict how litters decompose within litter mixtures was the focus of a number of studies in the 1980's and 90's (Fyles and Fyles, 1993, Chapman et al., 1988, Blair et al., 1990, Salamanca et al., 1998, Rustad, 1994). These early litter-mixing studies followed from the suggestion that differences in substrate nutrient content between litters might generate non-additive decay dynamics (Seastedt, 1984), and they tested our understanding of nutrient cycling in multi-species plant communities (Rustad, 1994). If decay dynamics in mixtures were the sum of their parts (i.e. additive), decay dynamics of single litters could be used to predict decay dynamics in the multi-species litter layers of most ecosystems. Alternatively, if decay dynamics of mixtures were non-additive, research on decay rates of mixtures would be required for us to understand nutrient dynamics in mutli-species systems.

Following from the study by Wardle et al. (1997), litter-mix studies have proliferated in the context of the biodiversity and ecosystem function debate. In contrast to earlier studies, the central focus of this body of work (reviewed by Gartner and Cardon, 2004, Hättenschwiler et al., 2005) has been whether biodiversity (primarily species richness and composition) is related to ecosystem function. The main conclusions from this work are: (1) there is little evidence that litter species richness generates non-additive decay dynamics; and (2) the composition of the litter mixture (i.e. the identity of species involved) often but not always generates non-additivity (see reviews by Gartner and Cardon, 2004, Hättenschwiler et al., 2005). In other words, non-additivity due to interactions among species has been the primary focus of previous work. As a consequence, a lack of interactions, where the results of litter mixing can be predicted based on the individual species present, has been considered a null effect of mixing. However, when litter mixtures behave as predicted (with no non-additive interactions), there may still be effects of composition through the identity of the species involved (i.e. additivity). This individual influence of species is sometimes referred to as the sampling effect and considered an artifact of experimental design (Huston, 1997). Thus, in the context of litter-mixing and diversity studies, additivity *per se* has been used to imply that biodiversity does not beget ecosystem function. Nonetheless, there may remain valid compositional effects based on the individual properties of the species present. If non-additive effects are the focus, the interest is in the consequence of loss of any species, regardless of its individual properties. Such a focus considers the consequences of random species loss (see Gross and Cardinale, 2005), where all species are equally likely to be lost, and non-additive effects that describe interactions among species are most important.

Under global environmental change, such as altered climate, land-use and resource availability, much of the change in biodiversity is likely to be through non-random species loss

(Grime, 1998, Vitousek et al., 1997, Loreau et al., 2001, Ellison et al., 2005, Schläpfer et al., 2005, Smith and Knapp, 2003), generating different outcomes on ecosystem functioning than random species loss (Gross and Cardinale, 2005, Schläpfer et al., 2005). Thus it is clear there is a pressing need to understand how ecosystems will function as species are lost non-randomly. To achieve this understanding, experimental designs that remove vulnerable species (Schläpfer et al., 2005, Smith and Knapp, 2003) or statistical models that can identify additive and non-additive effects of component species are required. While this work has begun for plant productivity, it has not been explicitly addressed for litter decomposition.

In the context of non-random species loss, either additive or non-additive effects of a component species imply that ecosystem functioning will be altered because of a shift in community composition. Additive effects of species loss are predictable from an understanding of the decay dynamics of that litter in isolation (i.e. monoculture), whereas non-additive effects are inherently unpredictable because the dynamics of a mixture differ from those of the component species in monoculture. As described above, these differential effects reflect either an independent influence of species on ecosystem functioning (additivity) versus emergent dynamics that arise because species effects are dependent on the presence of other species (non-additivity) (Johnson et al., 2006). Non-additive effects of litter species richness on decay dynamics have been detected (Hättenschwiler and Gasser, 2005, McTiernan et al., 1997, Leroy and Marks, 2006, Swan and Palmer, 2004, Wardle et al., 1997), and the mechanistic explanations for non-additivity in litter mixtures generally revolve around differences in litter quality among component species (where species of differing litter quality will drive additive effects through their individual characteristics, but might also drive non-additive interactions by stimulation of mass loss rates of lower-nutrient litters by adjacent higher-nutrient litter (Seastedt, 1984,

Hättenschwiler et al., 2005)). Thus litter species composition, specifically the presence of litters differing markedly in quality, is likely to generate non-additivity, and this is borne out by experimental data.

To investigate the potential consequences of non-random species loss for litter decomposition dynamics, we utilised a three-year, full-factorial, litter mixture study in a southeastern U.S. temperate forest. We used litters from the four dominant tree species within our study system, which differed in their chemical composition and decomposition rate in monoculture. We used a statistical model that sequentially tests for additive effects of the loss of each component species, then whether any of the remaining variance is explained by interactions between the main factors (i.e. whether a species is present or absent). Significant interactions indicate non-additivity, and this behaviour was explored using *post hoc* analyses to determine whether the non-additivity was explained by species richness and/or composition (Drake, 2003, Mikola et al., 2002). The strength of the approach is that we could first ask whether loss of a particular species is likely to affect ecosystem functioning. If it does, then second we could ask whether the effects of its loss are likely to be predictable (i.e. additive) or whether the consequence of its loss will be dependent on the presence of some or all of the other species in the community (i.e. non-additive).

We hypothesised that (1) given that our chosen litters form a gradient in litter nutrient content, loss of any one of the four species will produce an additive change in decomposition dynamics; (2) given the expectation that non-additive, compositional effects arise when litters of markedly differing nutrient content are present, non-additivity will only arise when a litter species is lost that is at the high or low end of the quality spectrum; and (3) since the overwhelming evidence to date indicates species composition is more important than species

richness *per se* on decay of mixed-species litter, there will be no relationship between litter species richness and decomposition rate. Note that richness effects are by definition non-additive, whereas compositional effects may be additive (Hypothesis 1) or non-additive (Hypothesis 2).

Our approach is to determine whether there are neutral, additive or non-additive consequences of the loss of a particular species for ecosystem functioning. The focus then is on non-random species loss. To place our analyses in the context of previous work on the consequences of random species loss for decomposition dynamics, we evaluated a number of the analytical approaches commonly used in prior litter-mix studies. Specifically, we were interested in whether these approaches can identify compositional effects and whether these effects could be classified as additive or non-additive and linked to the identities of particular species.

Materials and Methods

Study site

The experiment was conducted at Coweeta Hydrologic Laboratory in the southern Appalachian Mountains near Otto, North Carolina, U.S.A. (35º00'N, 83º30'W; elevation 1300 m). The mean annual rainfall is approx. 1700 mm and the mean annual temperature 13ºC (Heneghan et al., 1999). The study was conducted in Watershed 20 on Ball Creek, which drains into Coweeta Creek, a tributary of the Little Tennessee River.

Experimental design

The litters used were collected from the four most abundant tree species at Coweeta: *Liriodendron tulipifera* L. (tulip poplar, L), *Acer rubrum* L. (red maple, A), *Quercus prinus* L.

(chestnut oak, Q), and *Rhododendron maximum* L. (rhododendron, R). The litters from these species cover a range of chemical composition and decay rate in monoculture (Table 2.1). Senesced leaves of each species were collected in October 2003 and air-dried at room temperature in paper bags in the lab for one week. Leaves were put into litterbags in each of the possible 15 combinations of the four species. Litterbags (15 cm × 15 cm) were constructed from 1 mm nylon mesh and heat-sealed at the edges. Each litterbag contained 5 g of leaves, and all species in any one combination were equally represented in mass. On November 17, 2003, one set of all 15 combinations was placed in each of four replicate blocks for each of 9 collection dates across three years: 0, 92, 181, 273, 365, 546, 730, 911, and 1065 days. At each collection date, one set from each replicate plot was randomly chosen for processing, and litterbags were transported back to the laboratory on ice. Litter was dried, ground using a Spex CertiPrep 8000-D Mixer Mill (Spex CertiPrep, Metuchen, USA), and the ash free dry mass (AFDM) remaining for each litterbag was determined by incineration at 550°C for one hour. Nitrogen content was determined by combustion in a Carlo Erba Elemental Analyser (Carlo Erba, Milan, Italy) and reported as percentage nitrogen (%N) of litter dry mass.

Data Analyses

Mass loss data were expressed as proportion AFDM remaining and subjected to each of four commonly used analytical methods to detect effects of diversity on decomposition dynamics. Since a linear fit did not fit our decomposition data, decay rate (k) was not calculated and instead mass loss data were analysed using time (days) as a discrete, rather than continuous, factor. This also avoids the problems caused by attempting to take averages of log-transformed data (see Ostrofsky, 2007). In this manner we could test whether any effects of species loss were

consistent across time. All statistical calculations were conducted in S-Plus 7.0 for Windows using 0.05 as the critical level of alpha.

Testing for additivity and non-additivity

Following the approach of Kominoski et al. (2007), an Analysis of Variance (ANOVA), using Type I Sums of Squares (SS), was performed to test for additivity and non-additivity of species effects. Block, time, and the presence/absence of each of the four species were added sequentially as terms to the model. Block had four levels and Time eight levels (the Day 0 data were not included). The term representing each species had two levels: present or absent. To test for non-additivity, this was followed by a species interaction term (SpInt). This term had 15 levels, each representing one of the specific litterbag combinations. Lastly, interactions between time and block, the species, and SpInt terms were included.

A significant SpInt term (and/or its interaction with time) indicates a significant non-additive interaction among species, due to richness or composition, which is not explained by simple presence or absence of individual species. To explore potential richness effects we replaced the SpInt term with a Richness term, composed of four levels (1 to 4 species). In the absence of a significant effect of Richness or its interaction with time, a significant SpInt term must arise through non-additive composition effects. If a Richness term is significant, a Composition term, with 15 possible levels and thereby equivalent to the SpInt term, can be added to the model, while retaining Richness, to evaluate if both non-additive richness and composition effects manifest. Non-additive composition effects could be further explored to determine which of the species were interacting.

If SpInt was not significant, the model was re-run with each of the four species' presence/absence terms added first. This was done to determine which of the species had significant additive effects on decay dynamics. Since Type I SS was used, the F-values of the species terms were sensitive to the order in which they were added.

Alternative analytical methods

To examine how the analytical methods used in previous litter mixing and diversity studies might have influenced our conclusions, we analysed our data using several alternative models that are typically applied to such data. These include models for which the focus is on the effects of random species loss on litter decay dynamics.

Observed versus Expected models

First, following Wardle et al. (1997), expected values for a variable (such as mass remaining) were calculated for each mixture as an average of the monoculture values for each species involved using the following equation:

$$R_e = \sum_{i=1}^{S} \frac{M_i}{S}$$

where M_i is the monoculture value for species i, and S is the total number of species in the mixture. This was then compared to the observed value that was found experimentally for the mixture treatment as:

100*[(observed – expected)/expected]

which was plotted against species richness. This was done for each sample, and the average was taken for each treatment. 95% Confidence Intervals (CI) were also calculated for each treatment, and if the CI for each point did not cross y = 0, the effect is considered to be non-additive. This was done separately for each sampling period.

In addition, following Hättenschwiler and Gasser (2005), expected values were calculated for each mixture as above, and the relationship between it and the observed was assessed through simple linear regression where deviations from the 1:1 line indicate non-additivity. Deviations were considered significant if the CI, both on the x- and y-axis, did not cross the line. A single-factor ANOVA across treatments was used to test for significant differences between observed and expected values. A Calculation term was used to describe the values for each treatment that had two values: observed or expected. The ANOVA determined if there were significant differences between the two. Since we had multiple sampling dates, an additional two-way ANOVA was run including time as a main and interacting factor, as well as a paired t-test that has also been used in some literature (e.g. Johnson et al., 2006, Schweitzer et al., 2005).

Nested model

As per Smith and Bradford (2003), composition was nested within richness in an ANOVA to test for effects of richness beyond those explained by composition. Block, time, and both richness and composition were terms added to the model. These had the same number of levels as with the initial model. Lastly, the interactions of time with Block, Richness, and Composition were added to the model. The resulting ANOVA table was recalculated for the Richness term (and its interaction with time) so that its F value was calculated against the Mean Sum of Squares (MS) of composition (or its interaction with time), rather than the residual (Crawley, 2002). Significant richness terms would then indicate significant non-additive effects between at least two richness levels, whereas a significant composition effect may arise through additive or non-additive effects.

Results

Testing for additivity and non-additivity

Mass Loss

Litter mixing did not generate any non-additive effects on mass loss, given that the SpInt term and its interaction with time were not significant ($P > 0.05$), but there were significant additive effects of composition (Table 2.2). Specifically, the presence/absence of each of the four species had a significant effect on mass loss, and those of *A. rubrum* and *L. tulipifera* were consistent over time. Their main effects could therefore be pooled across time, which in turn revealed that their presence in mixture accelerated mass loss (Fig. 2.1a). The additive effects of *R. maximum* and *Q. prinus* were, however, time dependent. In general, the presence of these two species decreased rates of mass loss, but at days 273, 546, and 730, mass loss appears to be equivalent in both their presence and absence (Fig. 2.1b).

A hurricane prior to the 365-day sampling period deposited organic sediment in the litterbags, causing an increase in mass remaining that could not be corrected by measuring AFDM.

Nitrogen

In contrast to mass loss, there were significant non-additive effects of litter mixing on N content of litter (Table 2.2). Replacing the SpInt term with Richness did not identify richness to be driving that non-additivity ($F_{2,442} = 0.54$, $P > 0.50$), so it was caused by compositional interactions among the species present. Since the composition effect did not interact with time, results could be pooled across time.

To detect which species were involved in non-additive interactions, we compared the observed value for all mixtures involving each species against those that would be expected

based on the average of that species in monoculture and the treatment that contained the other species involved. For example, to explore possible non-additivity of *L. tulipifera*, we compared the observed and expected values for LA, LQ, LR, LAQ, LAR, LQR, and LAQR (where each of these is the mixture treatment consisting of the species each letter represents; see Methods). The expected values were the average between the observed values for treatments L and A, Q, R, AQ, AR, QR, and AQR, respectively. Observed minus expected values were plotted, and CI's that did not cross the x-axis were considered to be non-additive (Fig. 2.2). By doing this, we found that each species was involved in a non-additive interaction at some level, especially at the higher richness levels. *L. tulipifera* and *Q. prinus* tended to decrease %N, while *A. rubrum* and *R. maximum* tended to increase it.

Testing Alternative Models

Mass Loss

The observed/expected model showed that there were idiosyncratic, sometimes non-additive, effects on mass remaining (Fig. 2.3). Mixing effects were strongly non-additive for some compositions at some time points, but in most cases the difference between observed and expected did not appear to differ significantly from zero, therefore showing only additive effects. There was also the potential for the relationship to vary with time, with stronger interactions occurring later in time. However, error also increased (data not shown), and it was difficult to identify a significant relationship with certainty. The regression method showed no significant difference between observed and expected values when averaged over time ($F_{1,652} = 0.21$, $P = 0.65$, Figure 4a), so there was no overall mixing effect. Again, stronger effects tended to occur later in time, but when time was added to the model, there was still no significant Calculation

effect ($F_{1,650}$ = 0.80, P = 0.37) or its interaction with time ($F_{1,650}$ = 1.38, P = 0.24). The nested model identified significant composition effects (Table 2.3), but we could not determine the drivers, whether it was additive or non-additive, or direction of that effect. In agreement with the previous models, there was no interaction of either composition or richness with time, so effects were consistent throughout the experiment. Neither of the methods that test for an effect of species richness identified a significant impact on mass loss.

Nitrogen

As with mass loss, the observed/expected model showed idiosyncratic effects on N content, with both additive and non-additive effects present (Fig. 2.5). Again, strength varied with time, but a trend is difficult to determine. The regression method showed that actual %N was lower than expected, but not significantly so ($F_{1,652}$ = 0.05, P = 0.83, Fig. 2.4b). Again, the strength of this appeared to vary with time, but an interaction with time was not identified as significant if added to the ANOVA model ($F_{1,650}$ = 1.88, P = 0.17). It is important to note that the overall average showed that observed and expected %N were virtually the same, but the majority of samples were above the 1:1 line, showing positive effects, for all but two time points. The nested model shows that there was an effect of composition on %N, but does not identify if it is due to additive or non-additive mechanisms (Table 2.3). As with mass loss, no effects of richness were identified by any of the methods.

Discussion

We sought to determine if there were additive or non-additive effects of litter diversity, through richness or composition, on leaf litter mass loss and N content in a southern Appalachian riparian zone. We were primarily interested in the relative importance of additive and non-

additive effects in order to assess potential consequences of non-random species loss. Given the variation in litter quality represented by our four species (Table 2.1), we expected additive effects on decomposition based on species identity. This was confirmed for mass loss, where there were significant effects of the presence/absence of each of the four species. Given previous work (Wardle et al., 1997, Hättenschwiler and Gasser, 2005), we also expected non-additive effects due to the large difference in litter quality between some of the species. Indeed, non-additive effects on litter N content were detected and determined to be due to species composition rather than species richness. Overall, our data suggest that effects of litter diversity on the decomposition process are mediated by species composition rather than species richness.

Given the presence of both additive and non-additive effects, we suggest that non-random species loss from our system would influence significantly the dynamics of decomposition. Because additive effects alone drive mass loss, the consequences of species loss on this variable should be predictable from studies of individual species, or their chemical properties, in isolation. Given the plethora of work investigating the decay rate of single plant species, we may already have abundant information to predict the consequence of species loss and/or gain on litter decay rates. This is valuable given changing distribution and abundances of species. For example, the invasive hemlock woolly adelgid is projected to extirpate eastern hemlock from much of its range and at our field site will likely be replaced by tulip poplar or rhododendron (Orwig and Foster, 1998, Ellison et al., 2005). Replacement by tulip poplar would likely increase rates of litter loss (Fig. 2.1a) whereas replacement with rhododendron would likely decrease rates of litter loss (Fig. 2.1b). Similarly, potential declines in rhododendron caused by the invasive pathogen sudden oak death (Rizzo et al., 2002) would likely increase rates of litter

decay, although the strength of rhododendron's influence on mass loss appears to vary over time (Fig. 2.1b).

Changes in nutrient dynamics caused by species loss may be less predictable, given that we observed pervasive non-additive effects based on interactions among species (Fig. 2.2). Each of the four species are involved in non-additive interactions, but this was not only true for mixtures containing species of very different initial chemical qualities, in contrast to the general theory behind litter-mixing studies (Seastedt, 1984, Blair et al., 1990). Normally, these species interactions would be investigated with a full interaction model, where interactions between each of the species terms would be investigated (e.g. Kominoski et al., 2007). However, this method is flawed in diversity studies where the main effects (i.e. species presence/absence) are endogenous, rather than exogenous, to the aggregated property under investigation (e.g. as would have been the case if we simulated additions of species not already in the litter layer). This issue arises because there is no true control (i.e. litterbags without any of the species, as there would be no decomposition). Given its relevance to scenarios of non-random species loss, we explored non-additive interactions using an altered observed/expected method that tests for non-additive interactions driven by a species in each of its possible combinations. Certainly this is not the only possible method, and more work on the best way to explore non-additive composition effects is necessary. These data do suggest, however, that each of the four species participate in non-additive interactions, especially in more species-rich compositions, and nutrient dynamics may be altered by the loss of any of them.

Using a full-factorial design and a model that allows us to look for additive and non-additive effects of species composition allows us to explore the effects of both random and non-random species loss on ecosystem processes, an issue that has been brought forth for diversity

studies of productivity (Gross and Cardinale, 2005, Schläpfer et al., 2005, Smith and Knapp, 2003), but not yet decomposition. The advantages of this method are that it (a) places an equal emphasis on additive effects, which is important if species are lost non-randomly as anticipated under global change, by looking for effects of the presence of each species; (b) permits us to ask whether there are overall effects of particular species that would otherwise appear idiosyncratic; and (c) identifies whether species loss will be predictable or not. Conversely, most litter mixing studies do not address additive effects and focus on the effects of random species loss. In these studies, if additivity is detected (i.e. by default because non-additivity is not found), the statistical approaches used do not enable one to determine which, if any of the species, might alter decomposition dynamics through additive effects. If non-random species loss is also likely in other systems, it is important to realise that the consequences of species loss may differ from those represented in the literature. Schläpfer et al. (2005) point out that the assumption of random species loss can cause results to be either over- or underestimated, depending on the correlation between species persistence and performance. To determine how information yielded from previous studies compares with our data, and what previous methods tell us about additivity and non-additivity, we used several common methods for analysing litter mixture decomposition data.

The various observed/expected models tend to treat additive effects as a null effect (see Introduction), thereby not addressing the potential for a lack of diversity effects. A lack of non-additive effects may be due to additivity, where observed values equal expected due to a dominance of species identity over interactions among species, or a dominance of exogenous driving factors, such as climate, that overshadow any species effects. In this case, observed would still equal expected, though it is not due to additive effects, but non-detrital abiotic factors.

It may not be an unreasonable assumption that diversity effects exist, given that most studies see some sort of effect, though they do not always differentiate between additive or non-additive, and cannot always identify the species driving those effects (Hättenschwiler et al., 2005, Gartner and Cardon, 2004).

In our comparison of analytical methods, we found that all methods converged on one result: that there was no effect of species richness on either litter mass loss and N content. In contrast, the methods of analysis provided different interpretations of the effects of species composition on litter decay. This is due to the differences in how additive and non-additive effects are treated by each model. Overall, all of the methods can detect additive and non-additive composition effects, but only our Type I SS model and the nested model treat additive effects as a legitimate compositional effect, though the nested model cannot differentiate them specifically from non-additivity. While non-additive effects (that can be identified by observed/expected models) drive litter N content, mass loss is driven by additive composition effects, which are identified as idiosyncratic or nonexistent by some models. Though additivity is not explicitly addressed, the information still exists via the lack of non-additive effects, but these effects tend not to be explained or investigated further. They offer no specific identification of the strength of a certain species' effect, which is an important factor in the case of non-random species loss. For models that can detect additivity but not identify it, such as the nested method, we are able to predict that there are consequences of non-random species loss, but are not able to identify which species are likely to generate consequences or how predictable those consequences will be. Therefore, we may be missing out on important information by using only methods that do not or cannot specifically identify additive effects.

To be able to detect both additive and non-additive effects of species on ecosystem processes, a full-factorial design is necessary, which generally limits species mixtures to low richness levels given the number of combinations necessary. However, patches of leaf litter in temperate forest soils are generally occupied by only a few species, so this is not unreasonable. If simpler questions pertaining to only non-additivity, and therefore random species loss, are being asked, then it is appropriate to use the methods already frequent in litter-mixing literature. However, our method allows us to look for potential effects of non-random species loss without having to identify *a priori* the most susceptible species and eliminate them. While it is often pointed out that it may be more appropriate to study the decomposition of mixed litter through identification of the litter remaining in bags (Hättenschwiler and Gasser, 2005, Gartner and Cardon, 2006), it is not always possible or practical to do this. Over long-term studies such as ours, litter species become indistinguishable later in decomposition. Our method allows us to look for the species driving compositional effects without having to identify individually their leaves in the litter layer and measure their effects as contribution of mass remaining.

Conclusion

We have shown significant additive effects of litter mixing on mass loss and non-additive effects of species composition on nutrient content in decomposing litter. This suggests that there will be potential consequences of both random and non-random species loss for this system, with predictable results for mass loss, but less so for nutrient dynamics. Given that non-random species loss is more likely to occur under global change, more attention needs to be paid to its effects on decomposition in litter mixing studies. We have shown here that the additive effects of species identity have a large impact that is usually not addressed in litter-mixing studies. Since

the dominant tree species used in this study are likely to change in relative abundance due to invasive pathogens and pests, this research also indicates potential significant changes in organic matter processing and nutrient dynamics that might result. Finally, we stress that, even in studies where diversity-ecosystem function relationships have not been identified (using designs that test for random species loss), species loss may still markedly alter ecosystem function through unexplained additive effects.

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References

Aber, J. D. & Melillo, J. M. (2001) Chemical properties of litter and soil organic matter: the decomposition continuum. *Terrestrial Ecosystems* pp. 205-226. Academic Press, San Diego.

Blair, J. M., Parmelee, R. W. & Beare, M. H. (1990) Decay-Rates, Nitrogen Fluxes, and Decomposer Communities of Single-Species and Mixed-Species Foliar Litter. *Ecology,* **71,** 1976-1985.

Chapman, K., Whittaker, J. B. & Heal, O. W. (1988) Metabolic and Faunal Activity in Litters of Tree Mixtures Compared with Pure Stands. *Agriculture Ecosystems & Environment,* **24,** 33-40.

Crawley, M. J. (2002) Nested designs and variance components analysis. *Statistical Computing: An Introduction to Data Analysis using S-Plus*pp. 361-375. John Wiley & Sons, Ltd., West Sussex.

Drake, J. M. (2003) Why does grassland productivity increase with species richness? Disentangling species richness and composition with tests for overyielding and superyielding in biodiversity experiments. *Proceedings of the Royal Society of London Series B-Biological Sciences,* **270,** 1713-1719.

Ellison, A. M., Bank, M. S., Clinton, B. D., Colburn, E. A., Elliott, K., Ford, C. R., Foster, D. R., Kloeppel, B. D., Knoepp, J. D., Lovett, G. M., Mohan, J., Orwig, D. A., Rodenhouse, N. L., Sobczak, W. V., Stinson, K. A., Stone, J. K., Swan, C. M., Thompson, J., Von Holle, B. & Webster, J. R. (2005) Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. *Frontiers in Ecology and the Environment,* **3,** 479-486.

Fyles, J. W. & Fyles, I. H. (1993) Interaction of douglas-fir with red alder and salal foliage litter during decomposition. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere,* **23,** 358-361.

Gartner, T. B. & Cardon, Z. G. (2004) Decomposition dynamics in mixed-species leaf litter. *Oikos,* **104,** 230-246.

Gartner, T. B. & Cardon, Z. G. (2006) Site of leaf origin affects how mixed litter decomposes. *Soil Biology and Biochemistry,* **38,** 2307-2317.

Grime, J. P. (1998) Benefits of plant diversity to ecosystems: immediate, filter and founder effects. *Journal of Ecology,* **86,** 902-910.

Gross, K. & Cardinale, B. J. (2005) The functional consequences of random vs. ordered species extinctions. *Ecology Letters,* **8,** 409-418.

Hättenschwiler, S. & Gasser, P. (2005) Soil animals alter plant litter diversity effects on decomposition. *Proceedings of the National Academy of Sciences of the United States of America,* **102,** 1519-1524.

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

Heneghan, L., Coleman, D. C., Crossley, D. A. & Zou, X. M. (1999) Nitrogen dynamics in decomposing chestnut oak (Quercus prinus L.) in mesic temperate and tropical forest. *Applied Soil Ecology,* **13,** 169-175.

Huston, M. A. (1997) Hidden treatments in ecological experiments: Re-evaluating the ecosystem function of biodiversity. *Oecologia,* **110,** 449-460.

Johnson, M. T. J., Lajeunesse, M. J. & Agrawal, A. A. (2006) Additive and interactive effects of plant genotypic diversity on arthropod communities and plant fitness. *Ecology Letters,* **9,** 24-34.

Kominoski, J. S., Pringle, C. M., Ball, B. A., Bradford, M. A., Coleman, D. C., Hall, D. B. & Hunter, M. D. (2007) Nonadditive effects of leaf litter species diversity on breakdown dynamics in a detritus-based stream. *Ecology,* **88,** 1167-1176.

Leroy, C. J. & Marks, J. C. (2006) Litter quality, stream characteristics and litter diversity influence decomposition rates and macroinvertebrates. *Freshwater Biology,* **51,** 605-617.

Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J. P., Hector, A., Hooper, D. U., Huston, M. A., Raffaelli, D., Schmid, B., Tilman, D. & Wardle, D. A. (2001) Biodiversity and ecosystem functioning: Current knowledge and future challenges. *Science,* **294,** 804-808.

McTiernan, K. B., Ineson, P. & Coward, P. A. (1997) Respiration and nutrient release from tree leaf litter mixtures. *Oikos,* **78,** 527-538.

Mikola, J., Salonen, V. & Setälä, H. (2002) Studying the effects of plant species richness on ecosystem functioning: does the choice of experimental design matter? *Oecologia,* **133,** 594-598.

Moore, J. C., Berlow, E. L., Coleman, D. C., de Ruiter, P. C., Dong, Q., Hastings, A., Johnson, N. C., McCann, K. S., Melville, K., Morin, P. J., Nadelhoffer, K., Rosemond, A. D., Post, D. M., Sabo, J. L., Scow, K. M., Vanni, M. J. & Wall, D. H. (2004) Detritus, trophic dynamics and biodiversity. *Ecology Letters,* **7,** 584-600.

Orwig, D. A. & Foster, D. R. (1998) Forest response to the introduced hemlock woolly adelgid in southern New England, USA. *Journal of the Torrey Botanical Society,* **125,** 60-73.

Ostrofsky, M. L. (2007) A comment on the use of exponential decay models to test nonadditive processing hypotheses in multispecies mixtures of litter. *Journal of the North American Benthological Society,* **26,** 23-27.

Rizzo, D. M., Garbelotto, M., Davidson, J. M., Slaughter, G. W. & Koike, S. T. (2002) Phytophthora ramorum as the cause of extensive mortality of Quercus spp. and Lithocarpus densiflorus in California. *Plant Disease,* **86,** 205-214.

Rustad, L. E. (1994) Element dynamics along a decay continuum in a red spruce ecosystem in Maine, USA. *Ecology,* **75,** 867-879.

Salamanca, E. F., Kaneko, N. & Katagiri, S. (1998) Effects of leaf litter mixtures on the decomposition of Quercus serrata and Pinus densiflora using field and laboratory microcosm methods. *Ecological Engineering,* **10,** 53-73.

Schläpfer, F., Pfisterer, A. B. & Schmid, B. (2005) Non-random species extinction and plant production: implications for ecosystem functioning. *Journal of Applied Ecology,* **42,** 13-24.

Schnitzer, M. & Khan, S. U. (1978) *Soil Organic Matter.* Elsevier Scientific Publishing Company, New York, NY.

Schweitzer, J. A., Bailey, J. K., Hart, S. C. & Whitham, T. G. (2005) Nonadditive effects of mixing cottonwood genotypes on litter decomposition and nutrient dynamics. *Ecology,* **86,** 2834-2840.

Seastedt, T. R. (1984) The Role of Microarthropods in Decomposition and Mineralization Processes. *Annual Review of Entomology,* **29,** 25-46.

Smith, M. D. & Knapp, A. K. (2003) Dominant species maintain ecosystem function with non-random species loss. *Ecology Letters,* **6,** 509-517.

Smith, V. C. & Bradford, M. A. (2003) Do non-additive effects on decomposition in litter-mix experiments result from differences in resource quality between litters? *Oikos,* **102,** 235-242.

Stevenson, F. J. (1994) *Humus Chemistry: Genesis, Composition, Reactions.* John Wiley & Sons, Inc., New York, NY.

Swan, C. M. & Palmer, M. A. (2004) Leaf diversity alters litter breakdown in a Piedmont stream. *Journal of the North American Benthological Society,* **23,** 15-28.

Swift, M. J., Heal, O. W. & Anderson, J. M. (1979) *Decomposition in Terrestrial Ecosystems.* University of California Press, Los Angeles, CA.

Vitousek, P. M., Mooney, H. A., Lubchenco, J. & Melillo, J. M. (1997) Human domination of Earth's ecosystems. *Science,* **277,** 494-499.

Wardle, D. A., Bonner, K. I. & Nicholson, K. S. (1997) Biodiversity and plant litter: Experimental evidence which does not support the view that enhanced species richness improves ecosystem function. *Oikos,* **79,** 247-258.

Table 2.1. Summary of initial litter chemistries and the 3-year decay rate (*k*) in monoculture for each of the four tree species used. Numbers represent Means ± 1 Standard Error (SE); n = 4.

	%N	**%C**	**%P**	**%Lignin**	**% Total Phenolics**	***k* day^{-1}**
L. tulipifera	0.95±0.04	47.87±0.60	0.43±0.002	8.58±0.36	74.46±15.17	0.00099
A. rubrum	0.70±0.06	49.75±0.95	0.33±0.009	9.14±0.42	58.56± 6.96	0.00097
Q. prinus	1.25±0.09	50.06±1.15	0.52±0.004	13.55±0.37	20.5 ±1.92	0.00092
R. maximum	0.55±0.08	48.88±1.08	0.19±0.004	12.54±1.15	9.9 ±4.54	0.00086

Table 2.2. Summary of the ANOVA's testing for additive and non-additive effects of litter mixing on mass loss (%AFDM remaining) and nitrogen content (%N) of litter. The significance of the species terms is sensitive to the order in which they were added to the models. Hence, in the absence of a significant SpInt term, the models were re-run with each species being run first in the species order (see Results).

	df	SS	MS	F	*P*
%AFDM Remaining:					
Block	3	588	196	2.97	0.032
Day	7	98592	14084	213	<0.001
L. tulipifera	1	1884	1884	29.8	<0.001
A. rubrum	1	2114	2114	32.0	<0.001
Q. prinus	1	686	686	10.8	0.001
R. maximum	1	1415	1415	22.4	<0.001
SpInt	10	868	86	1.32	0.221
Block*Day	21	9775	465	7.05	<0.001
Day**L. tulipifera*	7	379	54	0.82	0.571
Day**A. rubrum*	7	550	78	1.19	0.308
Day**Q. prinus*	7	882	126	1.91	0.067
Day**R. maximum*	7	1051	150	2.27	0.028
Day*SpInt	70	3289	46	0.71	0.957
Residuals	328	21672	66		
Total	471	143454	21158		
% Nitrogen:					
Block	3	3.15	1.05	24.0	<0.001
Day	8	45.5	5.68	130	<0.001
L. tulipifera	1	3.02	3.02	69.0	<0.001
A. rubrum	1	0.11	0.11	2.58	0.109
Q. prinus	1	2.88	2.88	65.9	<0.001
R. maximum	1	6.52	6.52	149.3	<0.001
SpInt	10	3.61	0.36	8.27	<0.001
Block*Day	24	10.8	0.45	10.2	<0.001
Day**L. tulipifera*	8	0.49	0.06	1.39	0.200
Day**A. rubrum*	8	0.47	0.06	1.35	0.220
Day**Q. prinus*	8	0.31	0.04	0.89	0.521
Day**R. maximum*	8	0.53	0.07	1.51	0.153
Day*SpInt	80	3.59	0.04	1.03	0.423
Residuals	370	16.2	0.04		
Total	531	97.1	20.4		

Table 2.3. Summary of the nested ANOVA testing for composition and richness effects of litter-mixing on mass loss (%AFDM remaining) and nitrogen content (%N) of litter.

	df	SS	MS	F	*P*
%AFDM Remaining:					
Block	3	588	196	2.18	0.090
Time	7	98593	14085	157	<0.001
Richness	3	463	154	0.27	0.840
Composition	11	6210	565	6.29	<0.001
Time:Richness	21	947	45.1	0.65	0.866
Time:Composition	77	5332	69.3	0.77	0.916
Residuals	349	31322	89.8		
Total	471	143454	305		
%Nitrogen:					
Block	3	3.15	1.05	15.48	<0.001
Time	8	45.54	5.69	83.97	<0.001
Richness	3	0.07	0.02	0.02	0.997
Composition	11	16.08	1.46	21.57	<0.001
Time:Richness	24	0.77	0.03	0.58	0.933
Time:Composition	88	4.87	0.06	0.82	0.876
Residuals	394	26.71	0.07		
Total	531	97.18	0.18		

Note: Quality richness terms (indented) are tested against the quality composition terms, while other terms are tested against the model residual.

Figure 2.1. Investigation of the direction of significant additive effects identified for %AFDM remaining, both for (a) cumulative effects for species that did not interact with time and (b) effects over time for those that did. Letters refer to the genus of each of the four tree species: *L. tulipifera* (L), *A. rubrum* (A), *Q. prinus* (Q), and *R. maximum* (R). Solid bars or symbols represent all treatments that contained that species, and open ones include all treatments that did not. Values are means ± 1 SE; $n = 4$. The spike at 365 d is due to organic sedimentation caused by a hurricane that flooded the riparian zone. While inorganic sedimentation can be corrected in the analyses, organic sedimentation could not, so we considered it to be part of the natural dynamics.

Figure 2.2. Investigation into potential non-additive interactions driven by each of the four species used. Observed values were compared to expected values calculated as the average between the observed monoculture of each species and all of its possible interaction treatments. Error bars represent 95% CI.

Figure 2.3. Litter %AFDM remaining in the mixture litterbags in relation to the expected values calculated from the corresponding monoculture litterbags. Values are plotted against the number of species involved in the mixtures. Closed circles represent points for which the 95% CI did not cross y = 0, suggesting significant non-additivity. Open circles represent points for which they did, suggesting additive effects. For clarity, CI's are not shown.

Figure 2.4. (a) Observed %AFDM remaining and (b) observed %N in litter in relation to the expected values calculated from the corresponding monoculture litterbags. The line indicates the

1:1 relationship along which observed and expected values are equal. Data points represent averages across treatments over time, where solid symbols are significantly different from 1:1 (based on the 95% CI). For clarity, CI's are not shown. The inset shows the average observed (solid) and expected (open) values across all treatments.

Figure 2.5. Litter %N content in the mixture litterbags in relation to the expected values calculated from the corresponding monoculture litterbags. Values are plotted against the number of species involved in the mixtures. Closed circles represent points for which the 95% CI did not cross zero, suggesting significant non-additivity. Open circles represent points for which they did, suggesting additive effects. For clarity, CI's are not shown.

Figure 2.1

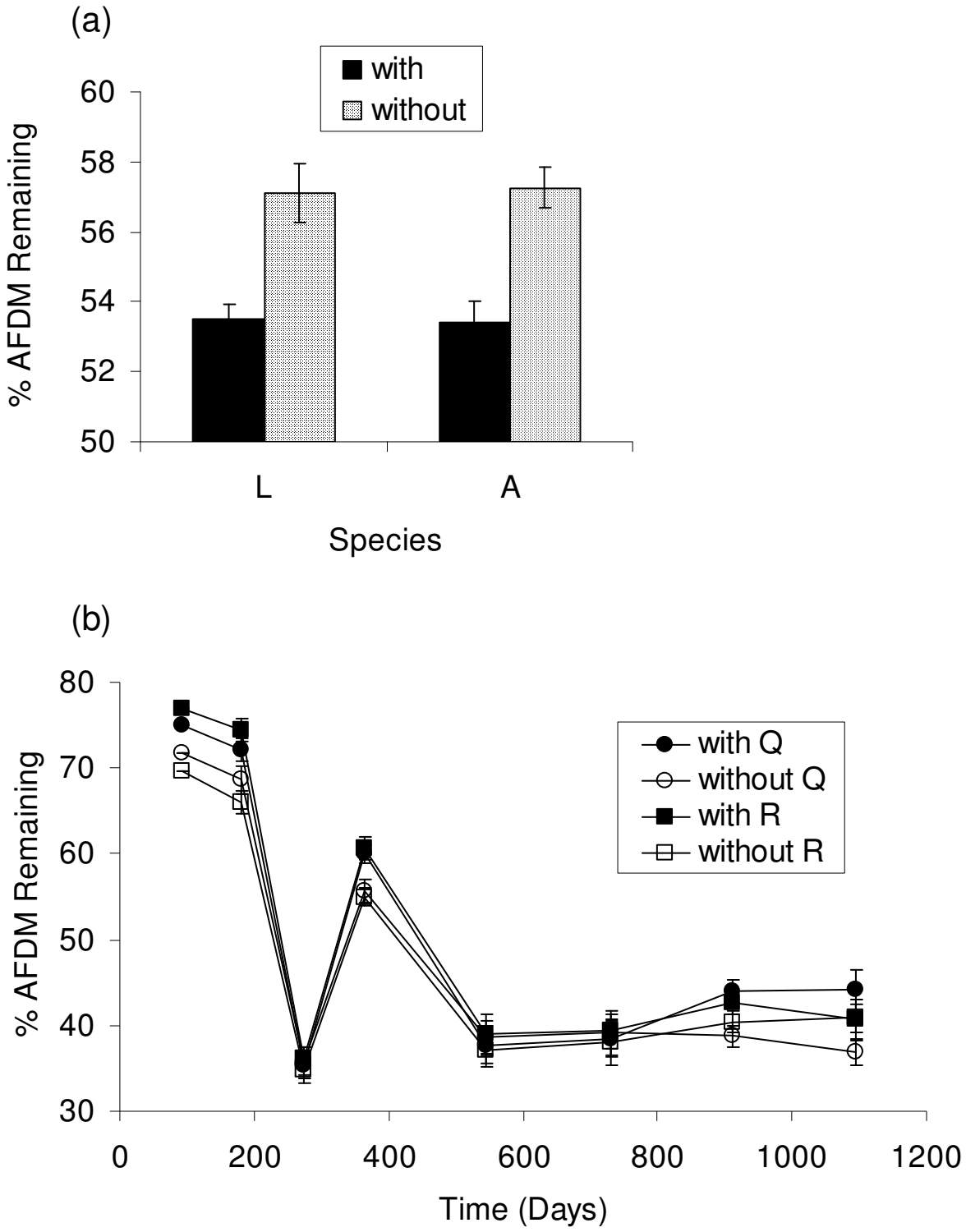
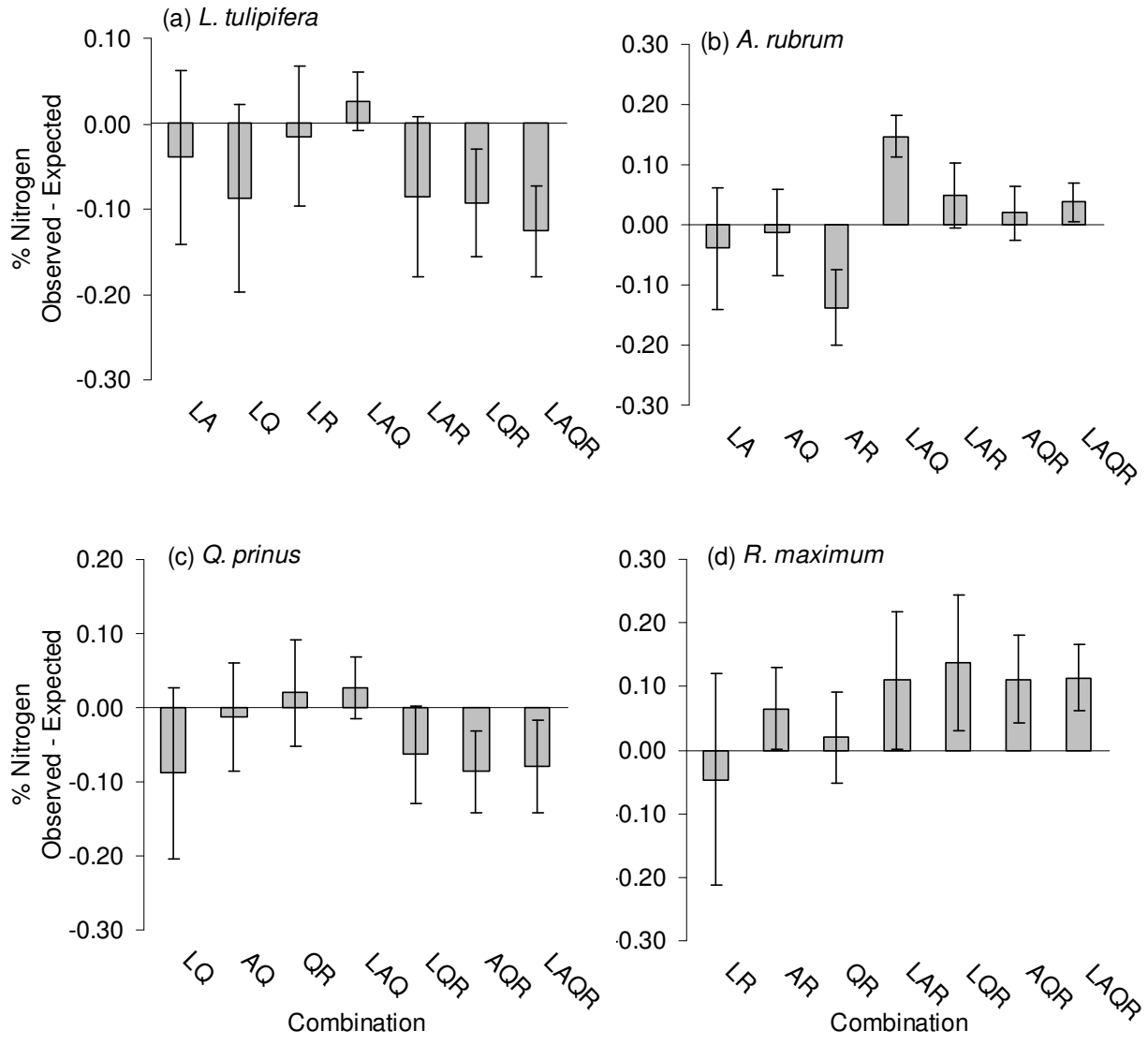

(a)
with
without
% AFDM Remaining
60
58
56
54
52
50
L
A
Species
(b)
with Q
without Q
with R
without R
% AFDM Remaining
80
70
60
50
40
30
0
200
400
600
800
1000
1200
Time (Days)

Figure 2.2


(a) L. tulipifera
(b) A. rubrum
(c) Q. prinus
(d) R. maximum
% Nitrogen
Observed - Expected
0.10
0.00
-0.10
-0.20
-0.30
0.30
0.20
LA
LQ
LR
LAQ
LAR
LQR
LAQR
AQ
AR
AQR
QR
Combination

Abstract

Aboveground plant communities can influence belowground decomposer biota through litter input. Litter-mixing studies have tested whether the diversity and heterogeneity of litter input affects various aspects of the decomposer community in a manner that can be predicted from monocultures. However they have mainly attempted to detect non-additive effects of litter mixing, which addresses potential consequences of random species loss. Under projected scenarios of global change, species loss is more likely to be non-random, characterized by the loss of species more susceptible to changes in environmental factors. With non-random species loss, individual species effects (additivity) as well as species interactions (non-additivity) may alter decomposition rates, potentially showing consequences that differ from those of random loss. To determine the impacts of non-random species loss on this aboveground-belowground linkage, we looked for both additive and non-additive effects of litter mixing on the decomposer community. To do this, a full-factorial litterbag experiment of four deciduous leaf species was conducted, from which bacteria, fungi, nematodes, and all taxa of arthropods were assessed. Data were analyzed using a statistical method that first looked for additive effects based on the presence or absence of species and then any significant species interactions occurring beyond that. We found almost exclusively additive effects of all four species on decomposer biota, with each species exerting effects on different aspects of the community that often changed through time. This suggests that differences in litter quality override mixing effects and the consequences of non-random species loss will be predictable. The two species at opposite ends of the quality spectrum exerted the most effects, with high-quality *L. tulipifera* supporting a more diverse arthropod community and driving bottom-up effects on the decomposer foodweb. Low-quality *R. maximum* decreased most aspects of the biota. Litter of mid-quality species exerted fewer

additive effects, save for an increase in arthropod abundance in the presence of *Q. prinus*. The lower plant-feeding nematodes are the only group for which there were non-additive effects, and this was due to richness. Together, these data demonstrate an effect of plant community composition on decomposer biomass, abundance, and diversity, confirming a link between above- and belowground communities.

Key Words: Ecosystem function, decomposition, decomposer community, soil biota, litter mixtures, species diversity, species composition, non-random species loss, biodiversity

Introduction

Decomposer biota, including microbes and invertebrate fauna, play a pivotal role in litter decomposition, and through their feeding activity drive the amount and timing of organic matter turnover and mineral nutrient availability (Seastedt, 1984, Hunter et al., 2003, Beare et al., 1992). This control over the availability of resources for plant productivity forms a feedback from belowground systems to aboveground processes and communities (De Ruiter et al., 2005). Recently, there has been increasing interest in the reverse: how aboveground systems in turn affect belowground communities and processes (Wardle et al., 2004, De Deyn and Van der Putten, 2005, Wardle, 2006, Bardgett et al., 2005). With this interest in mind, much research has been conducted to determine how plant communities might affect soil processes and communities. Since a major input of plants to the soil system is through litter, there has been a large focus specifically on the effects of altered plant litter on decomposer communities, through litter quality (Gonzalez and Seastedt, 2001, Carrillo et al., unpublished manuscript, Saetre and Baath, 2000), species and functional diversity (Wardle et al., 2006, Milcu et al., 2006, Bardgett

and Shine, 1999), and resource heterogeneity (St John et al., 2006, Blair et al., 1990, Hansen, 1999).

From this interest, many litter-mixing studies have been conducted to determine whether decomposer communities differ under multi-species mixtures as compared to monoculture, thus altering decay dynamics (reviewed by Gartner and Cardon, 2004). Additive effects on biota would result from the independent influence of individual species on mass loss, where diverse litter mixes have more abundant or diverse communities due to increased probability of including species that supports a greater community (Johnson et al., 2006). If decay dynamics in mixtures are the sum of their parts, biota of single litters can used to predict biota colonizing multi-species litter layers. Alternatively, if species' decomposer communities in mixture were dependent on other litter species (giving rise to non-additive dynamics), research on biota of mixtures would be required for us to understand decomposer communities in multi-species systems. With this in mind, studies have attempted to identify non-additive effects of litter-mixing on a variety of decomposition parameters, both in terrestrial systems (reviewed by Gartner and Cardon, 2004, Hättenschwiler et al., 2005) and aquatic (Swan and Palmer, 2006, Lecerf et al., 2005, Leroy and Marks, 2006), and responses vary (see Gartner and Cardon, 2004). This could largely be due to the variety of parameters measured by these studies. Biotic assessments vary from measurements of abundance, density, biomass, or activity and describe various different groups of decomposers, rather than the entire community. Additionally, studies have also been conducted under a variety of plant richness levels and cover different time spans. Thus more comprehensive work addressing litter-mixing effects on decomposer communities is necessary.

Under scenarios of global environmental change, many systems are at risk of losing dominant species (Ellison et al., 2005, Vitousek et al., 1997, Loreau et al., 2001, Grime, 1997). While previous litter-mixing studies have tested for consequences of species loss, they generally tested for non-additivity, where the interactions among species are the focus, rather than the individual effects of the identity of individual species, which may also have a major impact on ecosystem processes (Gross and Cardinale, 2005, Schläpfer et al., 2005). Experiments such as these test the consequences of random species loss, where all species are equally likely to be lost from the system (Smith and Knapp, 2003). However, non-random species loss is more likely to occur, characterized by the loss of particular species that are more susceptible to extinction (such as dominant species). In this case, both additive and non-additive effects of species loss may affect ecosystem functioning, reflecting either an independent influence of species on ecosystem functioning (additivity) versus emergent dynamics that arise due to species interactions (non-additivity). Since additive effects are also considered, non-random species loss may generate different influences on ecosystem functioning than random species loss (Gross and Cardinale, 2005, Schläpfer et al., 2005, Smith and Knapp, 2003), causing important information about species loss to be overlooked.

To determine the consequences of species loss on belowground decomposer communities, we conducted a three-year, full-factorial litter-mix study in a southeastern U.S. temperate forest. We used leaf litter from the four dominant tree species within our study system, which differed in initial chemical quality. To obtain a large picture of the decomposer community, we measured all commonly studied groups of decomposers over the course of two years: microbes, nematodes, microarthropods, and small macroarthropods. To analyze these data, we used a statistical model that sequentially tests first for additive effects of the loss of each

component species, then whether any of the remaining variance is explained by interactions among the species. Significant interactions are indicative of non-additivity, and this behavior was explored using *post hoc* analyses to determine whether the non-additivity was explained by richness and/or composition (Drake, 2003, Mikola et al., 2002). The strength of the approach is that we can first ask whether loss of a particular species is likely to affect community structure. If it does, then second we can ask whether its loss is likely to be additive or whether the consequence of its loss will be dependent (i.e. non-additive) on the presence of some or all of the other species in the community. We hypothesized that, given the gradient in initial litter quality, structure, and decomposition rate, there would be compositional effects of litter-mixing on the decomposer community, suggesting a feedback between above-ground plant communities and below-ground communities. High-quality litter (with high nutrient content and less secondary metabolites) will provide a better resource to support a larger decomposer community (Wardle et al., 2006) and low-quality litter with more structural compounds (e.g. lignin) will provide more habitat complexity to support a larger decomposer community (Hansen and Coleman, 1998). We can expect each of the four species to exert an additive individual influence in mixture with similar species, but support a synergistically larger decomposer community when species are very different from one another (Wardle, 2002).

Materials and Methods

Study site

The experiment was conducted at Coweeta Hydrologic Lab (US Forest Service) in the southern Appalachians near Otto, North Carolina (35º00'N, 83º30'W; elevation 1300 m). The mean annual rainfall is approximately 1700 mm and the mean annual temperature is 13ºC

(Heneghan et al., 1999). The study was conducted in Watershed 20 on Ball Creek, which drains into Coweeta Creek, a tributary of the Little Tennessee River.

Experimental design

The litters used were collected from the four most abundant tree species at Coweeta: *Liriodendron tulipifera* L. (tulip poplar, L), *Acer rubrum* L. (red maple, A), *Quercus prinus* L. (chestnut oak, Q), and *Rhododendron maximum* L. (rhododendron, R). The litters from these species cover a range of chemical composition and decay rate in monoculture (Table 4.1). Senesced leaves of each species were collected in October 2003 and air-dried at room temperature in paper bags in the lab for one week. Leaves were put into litterbags in each of the possible 15 combinations of the four species. Litterbags (15 cm × 15 cm) were constructed from 1 mm nylon mesh and heat-sealed at the edges. Each litterbag contained 5 g of leaves, and all species in any one combination were equally represented in mass. On November 17, 2003, one set of all 15 combinations was placed in each of four replicate blocks for each of 9 collection dates across 2.5 years: 0, 92, 181, 273, 365, 730, and 911 days. At each collection date, one set from each replicate plot was randomly chosen for processing, and litterbags were transported back to the laboratory on ice.

When bags were returned to the lab, five leaf cores were taken for each of bacterial and fungal biomass analyses. If leaf species were discernable, then disks were taken separately for each species. In order to estimate the dry weight of the punches used in these assays, five disks in each bag were also taken, and the ash-free dry mass (AFDM) was measured. The average AFDM of the disks was used to estimate the weight of the ten disks used for microbial analysis. Leaf cores for bacterial analysis were preserved in filtered (0.2 μm) solution of 3.7% formaldehyde

and stored at 4ºC until processed. Leaf cores for fungal biomass were stored in methanol at 0°C until extracted for ergosterol. After punches were removed for microbial analysis, half of the litter was then taken from each sample bag to be used in gravimetric extraction in water via Baermann funnels for 48 hours (Tarjan, 1949). Nematodes were harvested and preserved in 4% formaldehyde. Remaining litter in bags was placed on Tullgren funnels for heat extraction of arthropods for seven days (Macfadyen, 1953); arthropods (micro- and small macroarthropods) were preserved in 70% ethanol.

Bacterial cells were removed from the leaf disks by sonication (Weyers and Suberkropp, 1996), and subsamples of the suspension were stained with a 1:1 proportion of sample to 10 µg/mL DAPI (4'6-diamidino-2-phenylindole) (Velji and Albright, 1993). Samples were incubated for 10 min prior to vacuum filtration onto black 0.2 µm membrane filters (supported by a 0.45 µm backing filter), then slide mounted and stored in the refrigerator in the dark until counted. Cells were enumerated using epifluorescent microscopy (1000×) by counting ten random fields and categorizing cells into shape (coccoid or rod) and size class (small and large). Cell biovolumes were calculated using equations for the geometric shape size classes (Wetzel and Likens, 2000), and total bacterial C was estimated using the conversion factor of 5.6×10^{-13} g C μm^{-3} (Bratbak, 1985).

Leaf punches preserved for ergosterol analysis were sonicated for 30 min at 80°C in 25 ml methanol with an alcoholic base KOH (Weyers and Suberkropp, 1996). Samples were then partitioned into pentane and the pentane evaporated to dryness at 30°C under a stream of N_2 gas. Samples were redissolved in 1 ml methanol, filtered through a 0.45 µm Acrodisc filter, and stored at 0°C. Ergosterol concentration was measured on a high-pressure liquid chromatograph (HPLC) at 282 nm on a RP-10 column (Shimadzu, Columbia, Maryland, USA). Ergosterol

concentration was converted to fungal C using the conversion factor 5.5 µg ergosterol g^{-1} fungal dry $mass^{-1}$ (Gessner and Chauvet, 1993). For calculation of fungal:bacterial biomass ratios, fungal biomass was expressed as g C using a conversion of 43% C content of fungal dry mass (Baldy and Gessner, 1997, Baldy et al., 1995).

Nematodes were identified to functional feeding group (Yeates et al., 1993) and expressed as number per g AFDM. Micro and macro-arthropods were identified to the order level, or lower when possible, and expressed in the same manner. Abundance and taxa richness were recorded, and Shannon Index for species diversity was calculated using the following equations (Shannon and Weaver, 1964):

$$H' = -\Sigma (p \ln (p)), \quad (1)$$

where $p = n / N$, n = abundance for each taxonomic category, and N = total abundance for each sample;

$$H'_{max} = \ln (s), \quad (2)$$

where s = total taxa richness for each sample; and

$$\text{Evenness} = H' / H'_{max} \quad (3)$$

Data Analyses

All statistical analyses were conducted in S-Plus 7.0 for Windows using 0.05 as the critical level of alpha. Data were analyzed using time (days) as a discrete, rather than continuous, factor so that we could test whether any effects of species loss were consistent across time. Data were transformed when necessary to meet the assumptions of normality of variance. Arthropod abundance data were square root-transformed, while nematode abundance and microbial biomass data were ln(x+1)-transformed. Richness values and indices were untransformed.

Following the approach of Kominoski et al (2007), an Analysis of Variance (ANOVA), using Type I Sums of Squares (SS), was performed to test for additivity and non-additivity of species effects. Block, time, and the presence/absence of each of the four species were added sequentially as terms to the model. Block had four levels and time seven levels (for microarthropods) or four (for bacteria, fungi, and nematodes). The term representing each species had two levels: present or absent. Next, to test for non-additivity, this was then followed by a species interaction term, called SpInt. This term had 15 levels, each representing one of the specific litterbag combinations. Lastly, interactions between time and block, and then the species and interaction terms, were included.

A significant SpInt term (and/or its interaction with time) indicates a significant non-additive interaction among species, due to richness or composition that is not explained by simple presence or absence of individual species. To explore potential richness effects we replaced the SpInt term with a Richness term, composed of four levels (1 to 4 species). In the absence of a significant effect of Richness or its interaction with time, a significant SpInt term must arise through non-additive composition effects. If a Richness term is significant, a Composition term, with 15 possible levels and thereby equivalent to the SpInt term, can be added to the model, while retaining Richness, to evaluate if both non-additive richness and composition effects manifest. Non-additive composition effects could be further explored to determine which of the species were interacting. If SpInt was not significant, the model was re-run with each of the four species' presence/absence terms added first. This was done to determine which of the species had significant additive effects on decay dynamics. Since Type I SS was used, the F-values of the species terms were sensitive to the order in which they were added.

Results

Microbes

There were no significant non-additive effects on bacterial biomass, given that the SpInt term and its interaction with time were not significant, but there were significant additive effects of composition based on the presence of *R. maximum* (Table 4.1). The manner in which this species had an effect was moderately dependent on time. In general, bacterial biomass was significantly lower in the presence of *R. maximum*, but this did not develop until after 6 months of decomposition (Fig. 4.1a). Similarly, there were only significant additive effects on fungal biomass based on the presence of *R. maximum* and *L. tulipifera* (Table 4.1). These effects were also time-dependent. *L. tulipifera* generally supported greater fungal biomass than when it was absent, and *R. maximum* supported less, but for both of these species the difference was initially small, increased in the early stages of decomposition, then decreased later in time (Fig. 4.1b). The ratio of fungal:bacterial biomass was also therefore driven by additive effects, but only *R. maximum* was significant at this level, and its effect changed throughout time (Table 4.1). Fungi were always more dominant than bacteria, and initially this was more true for treatments without *R. maximum* due to higher fungal biomass but equal bacterial biomass (Fig. 4.1c). However, these differences minimized and reversed through time as treatments without *R. maximum* gained more bacterial biomass, lowering the ratio. This shows that changes in bacterial biomass driven by *R. maximum* were greater than its effect on fungal biomass.

Nematodes

Nematodes were driven by additive composition effects based on the presence (or absence) of certain species, but the responsible species varied with functional feeding group

(Table 4.1). Overall, total nematode abundance was significantly affected by all species except *A. rubrum*. This reflects the influence of those species on various different functional feeding groups. There were no effects of composition or richness on predatory (PR) nematodes, but all other feeding groups were driven by effects of composition. There were additive effects of composition on fungal feeder (FF), bacterial feeder (BF), and omnivore (OM) abundance. *L. tulipifera* was involved in all of these, and its effects were time-dependent, while those of the other two species involved were constant and could be pooled across time. Interestingly, all three species tended to decrease the abundance of each group when present, except for *L. tulipifera* in the case of OM (Fig. 4.2).

For plant-feeding nematodes, however, there were significant non-additive interactions among species that were time-dependent. While this group is often categorized as plant feeders, they have been reported to feed upon microbes and algae, which is what we believe to be true in this case. This non-additivity was determined to be due to richness, not composition (Table 4.1). Plant feeder abundance for each of the four richness levels was viewed over time, which showed that single- and four-species mixtures contained more plant feeders than two- and three-species mixtures at 365 d, but by 730 d the only difference was that 4-spp bags were significantly lower in abundance than the rest (Fig. 4.3).

We also counted two groups of biota extracted by Baermann funnels that are not often included in decomposition studies. There were no effects of composition or richness on tardigrade abundance ($P > 0.05$ for all additive and non-additive main effects), but there were additive effects on copepod abundance driven by *L. tulipifera, Q. prinus* and *R. maximum*, but the way in which *L. tulipifera* exerts its effect changes through time (Table 4.1).

Arthropods

The most abundant microarthropods found in all samples were Collembola and the three suborders of Acarina. However, 22 other taxa were found in various samples throughout the sampling dates (Appendix 6). Statistical analyses were conducted on total abundance, which includes all taxa found, and also focused on the taxa that were abundant in all samples (Acarina and Collembola). Overall, arthropod abundance was driven by additive effects, rather than non-additive (Table 4.1). *Q. prinus* and *R. maximum* had the greatest effect on arthropod abundance. *Q. prinus* increased total abundance and all of the taxa examined except the Mesostigmata mites (Fig. 4.4A). Many of these effects were time-dependent, due to a decrease in effect at days 181 and 730. *R. maximum* decreased abundance of all taxa except Oribatid mites, which were significantly more abundant in its presence. For all of these, there was initially lower abundance in the presence of *R. maximum*, but this reversed later in decomposition, only to return to this pattern by the final sampling date (Fig. 4.4B). *L. tulipifera* presence only had an additive effect on one group, the Mesostigmata, that fluctuated with time.

Both richness and the Shannon index for diversity of arthropod communities were also affected by the presence of individual species (Table 4.1). Richness was negatively affected by the low-quality *R. maximum* (though at 365 d and 730 there were small positive effects) but was positively affected by *Q. prinus*, which is also generally considered to be of fairly low quality. (Fig. 4.4b). Shannon index was most affected by the two species at opposite ends of the quality spectrum, where *L. tulipifera* increased diversity and *R. maximum* decreased it.

Discussion

While above- and belowground systems have typically been considered separately, we have shown a link between the aboveground plant community and belowground biota. The composition of the plant community affected abundance, biomass, and diversity of the biota colonizing its leaf litter. The decomposer community responded to additive effects of the presence or absence of each of the four leaf litter species, with each species exerting effects on different aspects of the community that often changed through time. Overall, the two species at opposite ends of the quality spectrum, *L. tulipifera* and *R. maximum*, affected more aspects of the biota, suggesting an important role for these two species in shaping the decomposer community. *R. maximum* decreased most of the parameters with which it was significantly associated (except at times arthropod abundance). *L. tulipifera* showed a bottom-up effect on the decomposer community, with positive effects on the lower trophic level (microbes) and higher trophic level (Mesostigmata mites, omnivorous nematodes), but decreased the abundance of taxa mid-level in the foodweb (microbivorous nematodes). The presence of *Q. rubrum* had great positive influences on arthropod communities, which could be due to habitat heterogeneity as suggested for other *Quercus* species (Hansen, 1999). The higher quality species, *L. tulipifera* and *A. rubrum*, also increased Shannon diversity (evenness), while the low quality *R. maximum* decreased it, supporting the hypothesis that higher quality species provide a better food source for a larger array of taxa (Wardle et al., 2006). *A. rubrum* did not exert much of an effect on the biota, save for arthropod diversity, suggesting only a small impact of this species in comparison to the others. Together, these data demonstrate an influence of four dominant tree species on the decomposer community . Each of these species is important for maintenance of the decomposer community (i.e. they are not redundant), as each one has an impact on some parameter of the

community, which will in turn affect organic matter processing through their feeding activity. This role of biota in decomposition allows them to exert control over nutrient availability in the soil, which will feed back to the aboveground plant community. Loss of any of the four dominant species we studied will alter the decomposer community, and thus the aboveground community, in our system.

In contrast to many previous studies, we found very little evidence for significant non-additive effects on the decomposer community. Mixing of litters with different chemical and habitat structure would provide a heterogeneous resource for decomposer biota, leading to a potential for non-additive interactions among litter species on biota. The only group for which we saw this effect were lower plant-feeding nematodes, though they did not respond to richness as would be expected. Richness would be expected to increase abundance linearly, where each richness level provides greater resource heterogeneity, thus supporting more individuals with each additional richness level. However, plant feeders were higher at the lowest and highest richness levels, and lower at intermediate levels. More research is necessary to determine why this might be occurring. Beyond this, it is possible that some combinations of our leaf litter species did generate non-additive biota communities that could be detected if each mixture were analyzed individually, as many studies have done (e.g. Blair et al., 1990, Hansen, 1999). For each litter-mixture composition, these studies compare observed biota communities with those that would be expected based on the monocultures of each species involved in the mixture, where significant deviations from the expected suggest non-additive communities. However this is not practical in full-factorial combinations of higher litter diversity with so many combinations to explore. Further, the presence of non-additive interactions among a small portion of the treatments does not represent the overall effect of litter mixing. Even if occasional non-additive

interactions do occur, the statistical model showed that additive effects dominate, signifying that the vast majority of effects are driven by species identity rather than interactions among species as is suggested by many studies. The predominance of additive effects driving the decomposer community (as opposed to non-additive) will allow for potentially predictable consequences of species loss, since with additive effects we only need to know the properties of each individual species, rather than requiring new information on the interactive effects in order to predict the outcome of species loss.

Given the presence of composition effects based on the presence of each of the four species, we know that non-random species loss will greatly impact this system and the decomposer community will differ markedly if any of these four species are lost or change abundance, thus altering leaf litter decay dynamics. This is valuable given that the abundance of these dominant species is likely to change in this area. For example, the invasive hemlock woolly adelgid is projected to extirpate eastern hemlock from much of its range, and at our field site will likely be replaced by tulip poplar or rhododendron (Orwig and Foster, 1998, Ellison et al., 2005). Replacement by tulip poplar would likely have positive effects on decomposer communities whereas replacement with rhododendron would likely have negative effects. Similarly, potential declines in rhododendron caused by the invasive pathogen sudden oak death (Rizzo et al., 2002) would likely have positive effects. However, the fact that there are frequently interactions among species and time makes their effect slightly less predictable, since we clearly cannot base their effects on one sampling period.

Conclusions

These results demonstrate a link between aboveground plant communities on belowground decomposer biota through additive influences of each of four dominant tree species in this riparian area. This suggests that differences in litter quality override mixing effects. Each species exerts an influence on some parameter of the biota and plays a role in shaping the decomposer community. The groups of biota affected by each tree species, and the way in which each species drives these additive effects, depends on the characteristics of the litter produced by each. This indicates that non-random species loss, predicted to affect this system through introduced pests and pathogens, will have great effects on decomposer biota, potentially changing the way in which organic matter and nutrients are processed in the forest floor.

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References

Baldy, V. & Gessner, M. O. (1997) Towards a budget of leaf litter decomposition in a first-order woodland stream. *Comptes Rendus De L Academie Des Sciences Serie Iii-Sciences De La Vie-Life Sciences,* **320,** 747-758.

Baldy, V., Gessner, M. O. & Chauvet, E. (1995) Bacteria, fungi and the breakdown of leaf-litter in a large river. *Oikos,* **74,** 93-102.

Bardgett, R. D., Bowman, W. D., Kaufmann, R. & Schmidt, S. K. (2005) A temporal approach to linking aboveground and belowground ecology. *Trends in Ecology & Evolution,* **20,** 634-641.

Bardgett, R. D. & Shine, A. (1999) Linkages between plant litter diversity, soil microbial biomass and ecosystem function in temperate grasslands. *Soil Biology & Biochemistry,* **31,** 317-321.

Beare, M. H., Parmelee, R. W., Hendrix, P. F., Cheng, W. X., Coleman, D. C. & Crossley, D. A. (1992) Microbial and Faunal Interactions and Effects on Litter Nitrogen and Decomposition in Agroecosystems. *Ecological Monographs,* **62,** 569-591.

Blair, J. M., Parmelee, R. W. & Beare, M. H. (1990) Decay-Rates, Nitrogen Fluxes, and Decomposer Communities of Single-Species and Mixed-Species Foliar Litter. *Ecology,* **71,** 1976-1985.

Bratbak, G. (1985) Bacterial biovolume and biomass estimations. *Applied and Environmental Microbiology,* **49,** 1488-1493.

De Deyn, G. B. & Van der Putten, W. H. (2005) Linking aboveground and belowground diversity. *Trends in Ecology & Evolution,* **20,** 625-633.

De Ruiter, P., Neutel, A. M. & Moore, J. (2005) The balance between productivity and food web structure in soil ecosystems. *Biological Diversity and Function in Soils* (eds R. D. Bardgett, M. B. Usher & D. W. Hopkins), pp. 139-153. Cambridge University Press.

Drake, J. M. (2003) Why does grassland productivity increase with species richness? Disentangling species richness and composition with tests for overyielding and superyielding in biodiversity experiments. *Proceedings of the Royal Society of London Series B-Biological Sciences,* **270,** 1713-1719.

Ellison, A. M., Bank, M. S., Clinton, B. D., Colburn, E. A., Elliott, K., Ford, C. R., Foster, D. R., Kloeppel, B. D., Knoepp, J. D., Lovett, G. M., Mohan, J., Orwig, D. A., Rodenhouse, N. L., Sobczak, W. V., Stinson, K. A., Stone, J. K., Swan, C. M., Thompson, J., Von Holle, B. & Webster, J. R. (2005) Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. *Frontiers in Ecology and the Environment,* **3,** 479-486.

Gartner, T. B. & Cardon, Z. G. (2004) Decomposition dynamics in mixed-species leaf litter. *Oikos,* **104,** 230-246.

Gessner, M. O. & Chauvet, E. (1993) Ergosterol-to-biomass conversion factors for aquatic hyphomycetes. *Applied and Environmental Microbiology,* **59,** 502-507.

Gonzalez, G. & Seastedt, T. R. (2001) Soil fauna and plant litter decomposition in tropical and subalpine forests. *Ecology,* **82,** 955-964.

Grime, J. P. (1997) Ecology - Biodiversity and ecosystem function: The debate deepens. *Science,* **277,** 1260-1261.

Gross, K. & Cardinale, B. J. (2005) The functional consequences of random vs. ordered species extinctions. *Ecology Letters,* **8,** 409-418.

Hansen, R. A. (1999) Red oak litter promotes a microarthropod functional group that accelerates its decomposition. *Plant and Soil,* **209,** 37-45.

Hansen, R. A. & Coleman, D. C. (1998) Litter complexity and composition are determinants of the diversity and species composition of oribatid mites (Acari : Oribatida) in litterbags. *Applied Soil Ecology,* **9,** 17-23.

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

Heneghan, L., Coleman, D. C., Crossley, D. A. & Zou, X. M. (1999) Nitrogen dynamics in decomposing chestnut oak (Quercus prinus L.) in mesic temperate and tropical forest. *Applied Soil Ecology,* **13,** 169-175.

Hunter, M. D., Adl, S., Pringle, C. M. & Coleman, D. C. (2003) Relative effects of macro invertebrates and habitat on the chemistry of litter during decomposition. *Pedobiologia,* **47,** 101-115.

Johnson, M. T. J., Lajeunesse, M. J. & Agrawal, A. A. (2006) Additive and interactive effects of plant genotypic diversity on arthropod communities and plant fitness. *Ecology Letters,* **9,** 24-34.

Kominoski, J. S., Pringle, C. M., Ball, B. A., Bradford, M. A., Coleman, D. C., Hall, D. B. & Hunter, M. D. (2007) Nonadditive effects of leaf litter species diversity on breakdown dynamics in a detritus-based stream. *Ecology,* **88,** 1167-1176.

Lecerf, A., Dobson, M., Dang, C. K. & Chauvet, E. (2005) Riparian plant species loss alters trophic dynamics in detritus-based stream ecosystems. *Oecologia,* **146,** 432-442.

Leroy, C. J. & Marks, J. C. (2006) Litter quality, stream characteristics and litter diversity influence decomposition rates and macroinvertebrates. *Freshwater Biology,* **51,** 605-617.

Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J. P., Hector, A., Hooper, D. U., Huston, M. A., Raffaelli, D., Schmid, B., Tilman, D. & Wardle, D. A. (2001) Biodiversity and ecosystem functioning: Current knowledge and future challenges. *Science,* **294,** 804-808.

Macfadyen, A. (1953) Notes on Methods for the Extraction of Small Soil Arthropods. *Journal of Animal Ecology,* **22,** 65-77.

Mikola, J., Salonen, V. & Setälä, H. (2002) Studying the effects of plant species richness on ecosystem functioning: does the choice of experimental design matter? *Oecologia,* **133,** 594-598.

Milcu, A., Partsch, S., Langel, R. & Scheu, S. (2006) The response of decomposers (earthworms, springtails and microorganisms) to variations in species and functional group diversity of plants. *Oikos,* **112,** 513-524.

Orwig, D. A. & Foster, D. R. (1998) Forest response to the introduced hemlock woolly adelgid in southern New England, USA. *Journal of the Torrey Botanical Society,* **125,** 60-73.

Rizzo, D. M., Garbelotto, M., Davidson, J. M., Slaughter, G. W. & Koike, S. T. (2002) Phytophthora ramorum as the cause of extensive mortality of Quercus spp. and Lithocarpus densiflorus in California. *Plant Disease,* **86,** 205-214.

Saetre, P. & Baath, E. (2000) Spatial variation and patterns of soil microbial community structure in a mixed spruce-birch stand. *Soil Biology & Biochemistry,* **32,** 909-917.

Schläpfer, F., Pfisterer, A. B. & Schmid, B. (2005) Non-random species extinction and plant production: implications for ecosystem functioning. *Journal of Applied Ecology,* **42,** 13-24.

Seastedt, T. R. (1984) The Role of Microarthropods in Decomposition and Mineralization Processes. *Annual Review of Entomology,* **29,** 25-46.

Shannon, C. E. & Weaver, W. (1964) *The Mathematical Theory of Communication.* The University of Illinois Press, Urbana, IL.

Smith, M. D. & Knapp, A. K. (2003) Dominant species maintain ecosystem function with non-random species loss. *Ecology Letters,* **6,** 509-517.

St John, M. G., Wall, D. H. & Behan-Pelletier, V. M. (2006) Does plant species co-occurrence influence soil mite diversity? *Ecology,* **87,** 625-633.

Swan, C. & Palmer, M. (2006) Composition of speciose leaf litter alters stream detritivore growth, feeding activity and leaf breakdown. *Oecologia,* **147,** 469-478.

Tarjan, A. C. (1949) Utilization of the Baermann Method as a Means of Assay of Root Infection by Meadow Nematodes, Pratylenchus Spp. *Phytopathology,* **39,** 24-24.

Velji, M. I. & Albright, L. J. (1993) Improved sample preparation for enumeration of aggregated aquatic substrate bacteria. *Handbook of Methods in Aquatic Microbial Ecology* (eds P. F. Kemp, B. F. Sherr, B. F. Sherr & J. J. Cole), pp. 139-142. Lewis Publishers, Ann Arbor, MI.

Vitousek, P. M., Aber, J. D., Howarth, R. W., Likens, G. E., Matson, P. A., Schindler, D. W., Schlesinger, W. H. & Tilman, D. G. (1997) Human alteration of the global nitrogen cycle: Sources and consequences. *Ecological Applications,* **7,** 737-750.

Wardle, D. A. (2002) *Communities and Ecosystems: Linking the Aboveground and belowground components.* Princeton University Press, Princeton, NJ, USA.

Wardle, D. A. (2006) The influence of biotic interactions on soil biodiversity. *Ecology Letters,* **9,** 870-886.

Wardle, D. A., Bardgett, R. D., Klironomos, J. N., Setälä, H., van der Putten, W. H. & Wall, D. H. (2004) Ecological Linkages Between Aboveground and Belowground Biota. *Science,* **304,** 1629-1633.

Wardle, D. A., Yeates, G. W., Barker, G. M. & Bonner, K. I. (2006) The influence of plant litter diversity on decomposer abundance and diversity. *Soil Biology and Biochemistry,* **38,** 1052-1062.

Wetzel, R. G. & Likens, G. E. (2000) *Limnological Analyses.* Springer, New York.

Weyers, H. S. & Suberkropp, K. (1996) Fungal and bacterial production during the breakdown of yellow poplar leaves in 2 streams. *Journal of the North American Benthological Society,* **15,** 408-420.

Yeates, G. W., Bongers, T., Degoede, R. G. M., Freckman, D. W. & Georgieva, S. S. (1993) Feeding-habits in soil nematode families and genera - an outline for soil ecologists. *Journal of Nematology,* **25,** 315-331.

Table 4.1. Summary of the significant main effects involving the four leaf litter species, or highest order interactions of main effects, identified by the ANOVA for each parameter of the decomposer community. An effect or interaction was considered significant if $P > 0.05$, but marginally significant interactions are also included. Full ANOVA tables are listed in Appendices 1-5.

	df	SS	MS	F	*P*
Bacterial Biomass:					
R. maximum	1	0.58	0.58	5.50	0.020
*R. maximum**Day	3	0.77	0.26	2.42	0.068
Fungal Biomass:					
*L. tulipifera**Day	4	7.17	1.79	4.56	0.002
R. maximum	1	12.3	12.3	31.3	0.000
*R. maximum**Day	4	3.52	0.88	2.24	0.066
Fungi:Bacteria Ratio					
*R. maximum**Day	3	7.44	2.48	3.38	0.020
Total Nematode Abundance:					
*L. tulipifera**Day	3	9.98	3.33	5.30	0.002
Q. prinus	1	3.10	3.10	4.95	0.027
R. maximum	1	14.1	14.1	22.5	0.000
Bacterial Feeder Abundance:					
*L. tulipifera**Day	3	13.49	4.50	5.90	0.001
R. maximum	1	7.28	7.28	9.56	0.002
Fungal Feeder Abundance:					
*L. tulipifera**Day	3	12.4	4.14	3.19	0.025
*Q. prinus**Day	3	10.4	3.47	2.67	0.049
Lower Plant Feeder Abundance:					
Richness*Day	6	27.4	4.57	4.98	0.000
Omnivore Abundance:					
*L. tulipifera**Day	3	6.43	2.14	3.05	0.030
R. maximum	1	6.31	6.31	8.99	0.003
Copepod Abundance:					
*L. tulipifera**Day	3	4.19	1.40	3.40	0.019
*Q. prinus**Day	3	5.89	1.96	4.78	0.003
R. maximum	1	1.81	1.81	4.41	0.037

Table 4.1 (cont.)

	df	SS	MS	F	*P*
Total Microarthropod Abundance:					
*Q. prinus**Day	5	255	51.1	2.82	0.017
*R. maximum**Day	5	271	54.2	2.99	0.012
Oribatid Abundance:					
Q. prinus	1	44.9	44.9	4.48	0.035
*R. maximum**Day	5	150	29.9	2.99	0.012
Mesostigmata Abundance:					
*L. tulipifera**Day	5	63.7	12.74	2.67	0.023
*Q. prinus**Day	5	56.7	11.3	2.38	0.039
*R. maximum**Day	5	58.5	11.7	2.45	0.034
Collembola Abundance:					
*Q. prinus**Day	5	144	28.7	3.10	0.010
Total Microarthropod Richness:					
Q. prinus	1	10.7	10.7	4.47	0.035
*R. maximum**Day	5	37.4	7.49	3.14	0.009
Shannon Index:					
*A. rubrum**Day	5	0.40	0.08	2.20	0.055
L. tulipifera	1	0.33	0.33	8.92	0.003
R. maximum	1	0.15	0.15	4.22	0.041

Table 4.2. Summary of the two-way ANOVA results investigating the species responsible for the non-additive effects on lower plant feeder nematode abundance identified by the full model. Observed values were compared to expected values calculated as the average between the observed monoculture of each species and all of its possible interaction treatments. If "Obs/Exp" was significant ($P > 0.05$), this indicated a difference between observed and expected values and non-additive effects of that species when in mixture.

	df	**SS**	**MS**	**F**	***P***
L. tulipifera					
Days	3	585	195	241	0.000
Obs/Exp	1	13.5	13.5	16.7	0.000
Obs/Exp*Day	3	9.21	3.07	3.79	0.011
Residuals	214	173	0.81		
Total	221	781	213		
A. rubrum					
Days	3	646	215	220	0.000
Obs/Exp	1	13.0	13.0	13.3	0.000
Obs/Exp*Day	3	1.37	0.46	0.47	0.707
Residuals	214	209	1		
Total	221	870	230		
Q. prinus					
Days	3	611	204	243	0.000
Obs/Exp	1	8.56	8.56	10.2	0.002
Obs/Exp*Day	3	11.0	3.68	4.40	0.005
Residuals	214	179	0.84		
Total	221	809	217		
R. maximum					
Days	3	605	202	228	0.000
Obs/Exp	1	19.2	19.2	21.7	0.000
Obs/Exp*Day	3	8.57	2.86	3.23	0.023
Residuals	214	189	0.88		
Total	221	822	225		

Figure 4.1. Investigation of the direction of significant additive effects over time identified for (a) bacterial biomass, (b) fungal biomass, and (c) ratio of fungal:bacterial biomass. Letters refer to the genus of each of the four tree species: *L. tulipifera* (L) and *R. maximum* (R). Solid symbols represent all treatments that contained that species, and open ones include all treatments that did not. Values are means ± 1 SE; $n = 4$.

Figure 4.2. Investigation of the direction of significant additive effects identified for nematode community both for (a) cumulative effects over time and (b) effects throughout sampling for species that interacted with time. Values on the y-axis are the average difference between treatments where that species is present and those where it is not. In (a), letters above bars denote significant effects at $P > 0.05$; "c" indicates effects that were consistent over time, and "t" indicates effects that interacted with time that are represented in (b). Error bars were left out for clarity.

Figure 4.3. Investigation of the richness effects on lower plant feeder nematodes over time.

Figure 4.4. Investigation of the direction of significant additive effects identified for arthropod community both for (a) cumulative effects over time and (b) effects throughout sampling for species that interacted with time. Values on the y-axis are the average difference between treatments where that species is present and those where it is not. Hashed bars (a) and open points (b) correspond to the right y-axis. In (a), letters above bars denote significant effects at $P > 0.05$; "c" indicates effects that were consistent over time, and "t" indicates effects that interacted with time and are represented in (b). Error bars were left out for clarity.

Figure 4.1

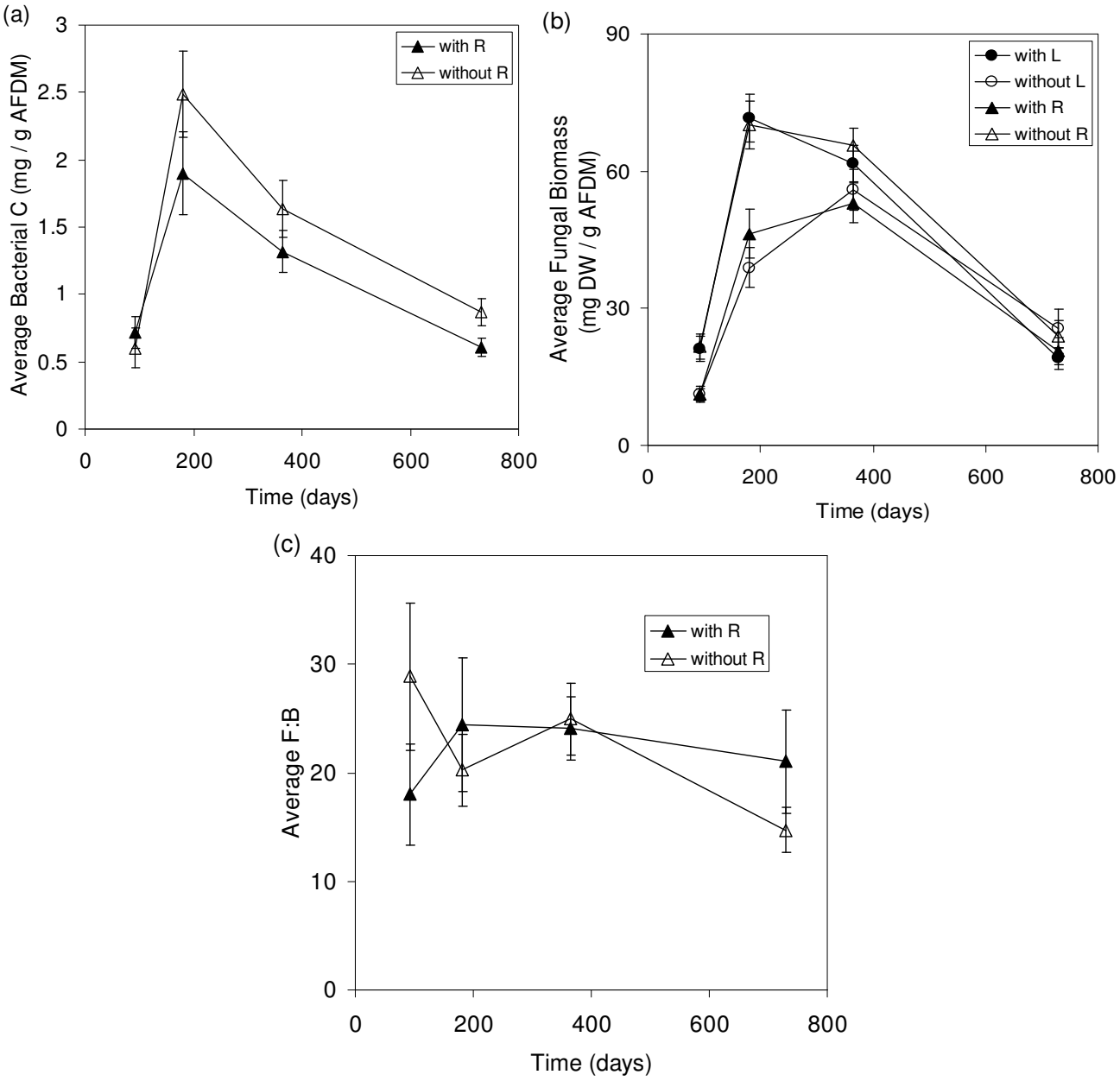

(a)
Average Bacterial C (mg / g AFDM)
Time (days)
with R
without R
(b)
Average Fungal Biomass (mg DW / g AFDM)
Time (days)
with L
without L
with R
without R
(c)
Average F:B
Time (days)
with R
without R

Figure 4.2

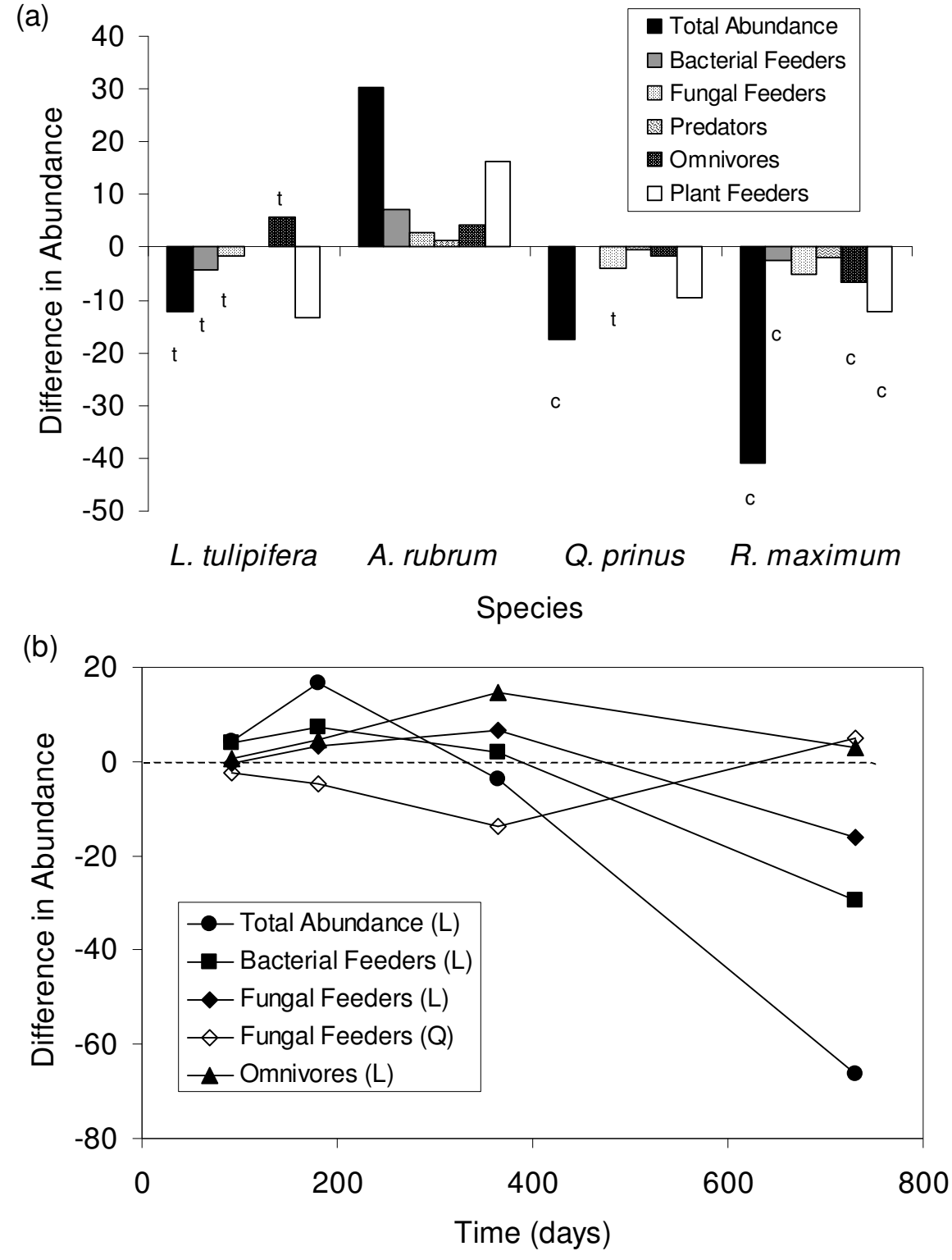

(a)
Total Abundance
Bacterial Feeders
Fungal Feeders
Predators
Omnivores
Plant Feeders
Difference in Abundance
40
30
20
10
0
-10
-20
-30
-40
-50
t
t
t
t
t
t
c
c
c
c
L. tulipifera
A. rubrum
Q. prinus
R. maximum
Species
(b)
Difference in Abundance
20
0
-20
-40
-60
-80
Total Abundance (L)
Bacterial Feeders (L)
Fungal Feeders (L)
Fungal Feeders (Q)
Omnivores (L)
0
200
400
600
800
Time (days)

Figure 4.3

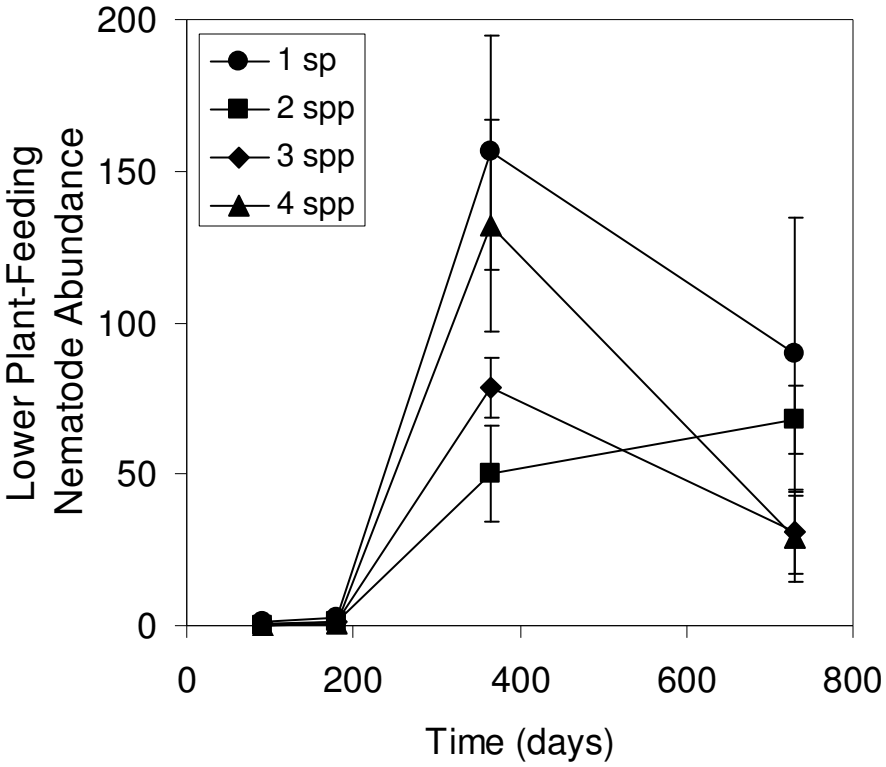
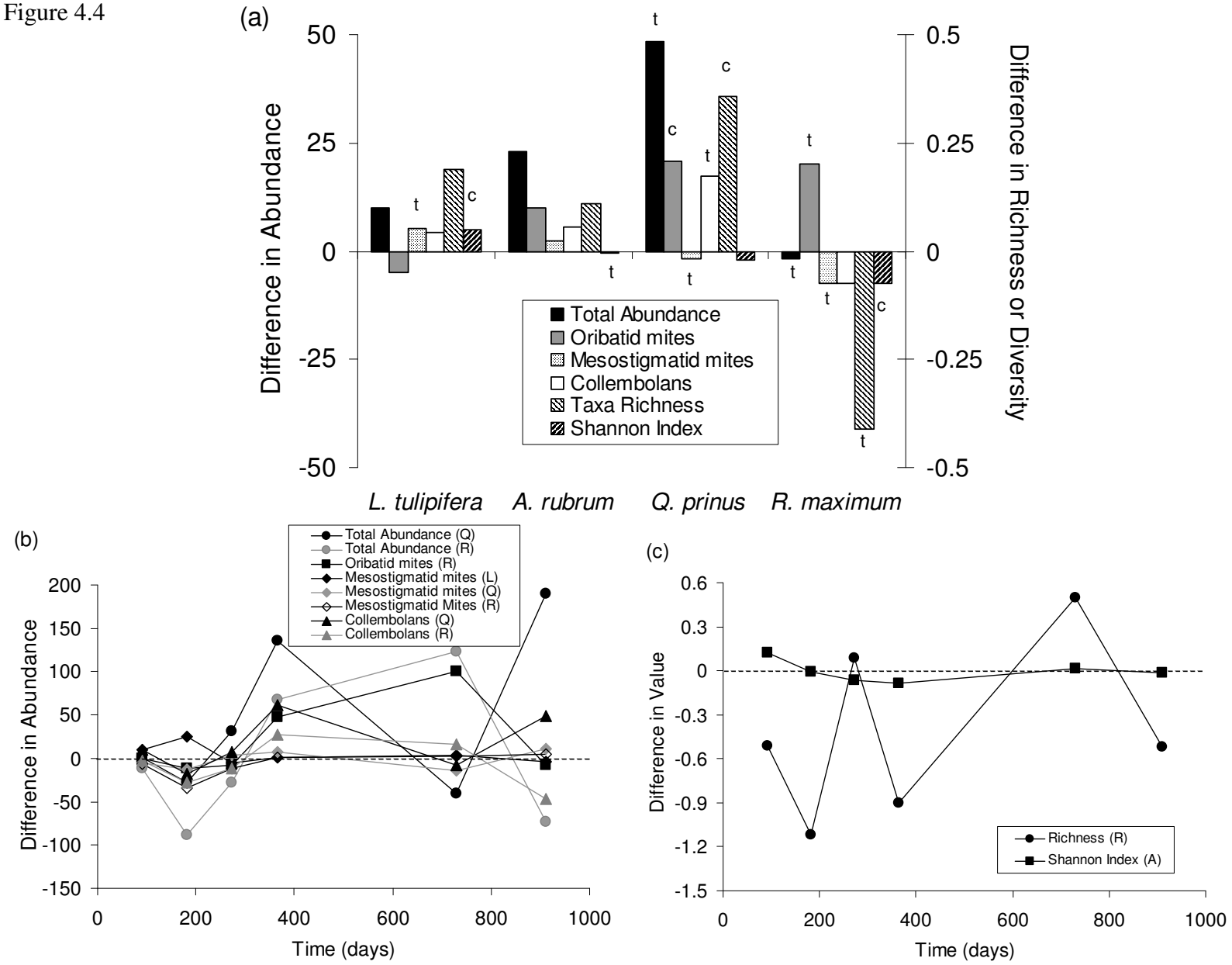

200
150
100
50
0
Lower Plant-Feeding Nematode Abundance
1 sp
2 spp
3 spp
4 spp
0
200
400
600
800
Time (days)

Figure 4.4


(a)
Difference in Abundance
Difference in Richness or Diversity
50
25
0
-25
-50
0.5
0.25
0
-0.25
-0.5
t
c
Total Abundance
Oribatid mites
Mesostigmatid mites
Collembolans
Taxa Richness
Shannon Index
L. tulipifera
A. rubrum
Q. prinus
R. maximum
(b)
Total Abundance (Q)
Total Abundance (R)
Oribatid mites (R)
Mesostigmatid mites (L)
Mesostigmatid mites (Q)
Mesostigmatid Mites (R)
Collembolans (Q)
Collembolans (R)
Difference in Abundance
Time (days)
(c)
Difference in Value
Richness (R)
Shannon Index (A)
Time (days)

Appendices

Appendix 4.1. Summary of the ANOVA's testing for additive and non-additive effects of litter mixing on microbial biomass and fungi:bacteria ratio.

	df	SS	MS	F	*P*
Bacterial Biomass:					
Block	3	1.08	0.36	3.42	0.019
Day	3	13.0	4.32	41.0	0.000
A. rubrum	1	0.03	0.03	0.24	0.626
L. tulipifera	1	0.17	0.17	1.60	0.207
Q. prinus	1	0.03	0.03	0.29	0.593
R. maximum	1	0.58	0.58	5.50	0.020
SpInt	10	0.67	0.07	0.63	0.785
Block*Day	9	7.88	0.88	8.29	0.000
*A. rubrum**Day	3	0.34	0.11	1.09	0.356
*L. tulipifera**Day	3	0.505	0.17	1.59	0.193
*Q. prinus**Day	3	0.58	0.19	1.83	0.143
*R. maximum**Day	3	0.77	0.26	2.42	0.068
SpInt*Day	30	2.98	0.10	0.94	0.560
Residuals	155	16.4	0.11		
Total	226	44.9	7.37		
Fungal Biomass:					
Block	3	1.90	0.63	1.61	0.189
Day	4	104	26.1	66.4	0.000
A. rubrum	1	0.12	0.12	0.31	0.581
L. tulipifera	1	4.10	4.10	10.4	0.001
Q. prinus	1	0.07	0.07	0.17	0.678
R. maximum	1	12.3	12.3	31.3	0.000
SpInt	10	6.68	0.67	1.70	0.083
Block*Day	12	10.8	0.90	2.28	0.010
*A. rubrum**Day	4	0.71	0.18	0.45	0.773
*L. tulipifera**Day	4	7.17	1.79	4.56	0.002
*Q. prinus**Day	4	2.43	0.61	1.55	0.191
*R. maximum**Day	4	3.52	0.88	2.24	0.066
SpInt*Day	40	19.6	0.49	1.25	0.167
Residuals	194	76.2	0.39		
Total	283	250	49.2		

Appendix 4.1 (cont.)

	df	SS	MS	F	*P*
Fungi:Bacteria Ratio					
Block	3	3.28	1.09	1.49	0.220
Day	3	9.13	3.04	4.15	0.008
A. rubrum	1	0.07	0.07	0.09	0.763
L. tulipifera	1	0.33	0.33	0.45	0.503
Q. prinus	1	0.52	0.52	0.72	0.399
R. maximum	1	1.06	1.06	1.45	0.231
SpInt	10	12.4	1.24	1.69	0.088
Block*Day	9	37.0	4.11	5.60	0.000
*A. rubrum**Day	3	0.99	0.33	0.45	0.718
*L. tulipifera**Day	3	1.25	0.42	0.57	0.638
*Q. prinus**Day	3	2.12	0.71	0.96	0.412
*R. maximum**Day	3	7.44	2.48	3.38	0.020
SpInt*Day	30	31.3	1.04	1.42	0.089
Residuals	139	102	0.73		
Total	210	209	17.2		

Appendix 4.2. Summary of the ANOVA's testing for additive and non-additive effects of litter mixing on nematode community.

	df	SS	MS	F	*P*
Total Nematode Abundance:					
Block	3	20.3	6.75	10.8	0.000
Day	3	606	202	322	0.000
A. rubrum	1	1.31	1.31	2.09	0.150
L. tulipifera	1	7.20	7.20	11.5	0.001
Q. prinus	1	3.10	3.10	4.95	0.027
R. maximum	1	14.1	14.1	22.5	0.000
SpInt	10	9.22	0.92	1.47	0.155
Block*Day	9	11.67	1.30	2.07	0.035
*A. rubrum**Day	3	0.80	0.27	0.43	0.735
*L. tulipifera**Day	3	9.98	3.33	5.30	0.002
*Q. prinus**Day	3	2.72	0.91	1.45	0.231
*R. maximum**Day	3	3.15	1.05	1.68	0.174
SpInt*Day	30	18.5	0.62	0.98	0.500
Residuals	167	105	0.63		
Total	238	813	243		
Bacterial Feeder Abundance:					
Block	3	23.9	7.96	10.45	0.000
Day	3	300	99.9	131.13	0.000
A. rubrum	1	0.53	0.53	0.70	0.405
L. tulipifera	1	3.50	3.50	4.59	0.034
Q. prinus	1	0.17	0.17	0.22	0.640
R. maximum	1	7.28	7.28	9.56	0.002
SpInt	10	8.62	0.86	1.13	0.342
Block*Day	9	12.80	1.42	1.87	0.060
*A. rubrum**Day	3	1.05	0.35	0.46	0.711
*L. tulipifera**Day	3	13.49	4.50	5.90	0.001
*Q. prinus**Day	3	2.50	0.83	1.09	0.354
*R. maximum**Day	3	4.13	1.38	1.81	0.148
SpInt*Day	30	23.86	0.80	1.04	0.414
Residuals	167	127	0.76		
Total	238	529	130		

Appendix 4.2 (cont.)

	df	SS	MS	F	*P*
Fungal Feeder Abundance:					
Block	3	5.30	1.77	1.36	0.257
Day	3	224	74.6	57.39	0.000
A. rubrum	1	0.98	0.98	0.76	0.386
L. tulipifera	1	0.23	0.23	0.17	0.678
Q. prinus	1	6.46	6.46	4.96	0.027
R. maximum	1	3.34	3.34	2.57	0.111
SpInt	10	12.9	1.29	1.00	0.450
Block*Day	9	27.8	3.09	2.37	0.015
*A. rubrum**Day	3	0.52	0.17	0.13	0.940
*L. tulipifera**Day	3	12.4	4.14	3.19	0.025
*Q. prinus**Day	3	10.4	3.47	2.67	0.049
*R. maximum**Day	3	1.42	0.47	0.36	0.779
SpInt*Day	30	32.9	1.10	0.84	0.703
Residuals	167	217	1.30		
Total	238	556	102		
Predator Abundance:					
Block	3	6.26	2.09	1.72	0.165
Day	3	98.1	32.7	26.9	0.000
A. rubrum	1	2.22	2.22	1.83	0.178
L. tulipifera	1	0.93	0.93	0.76	0.383
Q. prinus	1	0.08	0.08	0.07	0.793
R. maximum	1	2.11	2.11	1.73	0.190
SpInt	10	10.4	1.04	0.85	0.579
Block*Day	9	18.5	2.06	1.69	0.095
*A. rubrum**Day	3	2.76	0.92	0.76	0.520
*L. tulipifera**Day	3	0.19	0.06	0.05	0.984
*Q. prinus**Day	3	1.62	0.54	0.44	0.722
*R. maximum**Day	3	0.41	0.14	0.11	0.952
SpInt*Day	30	13.3	0.44	0.37	0.999
Residuals	167	203	1.22		
Total	238	360	46.5		

Appendix 4.2 (cont.)

	df	SS	MS	F	*P*
Lower Plant Feeder Abundance:					
Block	3	26.7	8.88	9.68	0.000
Day	3	609	203	221	0.000
A. rubrum	1	0.54	0.54	0.59	0.445
L. tulipifera	1	4.92	4.92	5.36	0.022
Q. prinus	1	0.34	0.34	0.37	0.546
R. maximum	1	0.37	0.37	0.40	0.526
Richness	2	12.1	6.06	6.60	0.002
Composition	8	4.5	0.56	0.61	0.766
Block*Day	9	23.4	2.60	2.83	0.004
*A. rubrum**Day	3	4.43	1.48	1.61	0.189
*L. tulipifera**Day	3	0.58	0.19	0.21	0.888
*Q. prinus**Day	3	4.45	1.48	1.62	0.187
*R. maximum**Day	3	0.75	0.25	0.27	0.846
Richness*Day	6	27.4	4.57	4.98	0.000
*Composition*Day*	24	23.3	0.97	1.06	0.398
Residuals	167	153	0.92		
Total	238	896	237		
Omnivore Abundance:					
Block	3	16.7	5.56	7.92	0.000
Day	3	461	154	219	0.000
A. rubrum	1	0.49	0.49	0.69	0.407
L. tulipifera	1	10.4	10.4	14.9	0.000
Q. prinus	1	0.20	0.20	0.29	0.594
R. maximum	1	6.31	6.31	8.99	0.003
SpInt	10	8.39	0.84	1.20	0.297
Block*Day	9	13.9	1.54	2.20	0.024
*A. rubrum**Day	3	1.70	0.57	0.81	0.492
*L. tulipifera**Day	3	6.43	2.14	3.05	0.030
*Q. prinus**Day	3	2.24	0.75	1.06	0.366
*R. maximum**Day	3	0.94	0.31	0.45	0.721
SpInt*Day	30	26.5	0.88	1.26	0.183
Residuals	167	117	0.70		
Total	238	672	184		

Appendix 4.3. Summary of the ANOVA's testing for additive and non-additive effects of litter mixing on other Baermann-extracted taxa: tardigrades and copepods.

	df	SS	MS	F	*P*
Tardigrade Abundance:					
Block	3	4.36	1.45	1.84	0.142
Day	3	32.5	10.8	13.7	0.000
A. rubrum	1	0.21	0.21	0.27	0.606
L. tulipifera	1	0.85	0.85	1.07	0.303
Q. prinus	1	0.70	0.70	0.89	0.348
R. maximum	1	2.11	2.11	2.67	0.104
SpInt	10	8.81	0.88	1.11	0.354
Block*Day	9	28.3	3.15	3.99	0.000
*A. rubrum**Day	3	0.26	0.09	0.11	0.954
*L. tulipifera**Day	3	1.32	0.44	0.56	0.645
*Q. prinus**Day	3	0.01	0.00	0.00	1.000
*R. maximum**Day	3	1.28	0.43	0.54	0.655
SpInt*Day	30	17.66	0.59	0.75	0.828
Residuals	167	132	0.79		
Total	238	230	22.5		
Copepod Abundance:					
Block	3	5.31	1.77	4.31	0.006
Day	3	51.8	17.3	42.0	0.000
A. rubrum	1	0.69	0.69	1.67	0.198
L. tulipifera	1	1.18	1.18	2.88	0.092
Q. prinus	1	4.94	4.94	12.0	0.001
R. maximum	1	1.81	1.81	4.41	0.037
SpInt	10	5.65	0.57	1.38	0.196
Block*Day	9	10.5	1.17	2.84	0.004
*A. rubrum**Day	3	1.48	0.49	1.20	0.310
*L. tulipifera**Day	3	4.19	1.40	3.40	0.019
*Q. prinus**Day	3	5.89	1.96	4.78	0.003
*R. maximum**Day	3	2.11	0.70	1.71	0.167
SpInt*Day	30	13.1	0.44	1.06	0.393
Residuals	167	68.6	0.41		
Total	238	177	34.8		

Appendix 4.4. Summary of the ANOVA's testing for additive and non-additive effects of litter mixing on arthropod community abundances.

	df	SS	MS	F	*P*
Total Microarthropod Abundance:					
Block	3	78.5	26.2	1.44	0.230
Day	5	8429	1686	93.1	0.000
A. rubrum	1	33.3	33.3	1.84	0.176
L. tulipifera	1	8.41	8.41	0.46	0.496
Q. prinus	1	109	109	6.04	0.015
R. maximum	1	2.16	2.16	0.12	0.730
SpInt	10	114	11.4	0.63	0.789
Block*Day	15	1777	118	6.54	0.000
*A. rubrum**Day	5	60.4	12.1	0.67	0.648
*L. tulipifera**Day	5	74.7	14.9	0.82	0.533
*Q. prinus**Day	5	255	51.1	2.82	0.017
*R. maximum**Day	5	271	54.2	2.99	0.012
SpInt*Day	50	987	19.7	1.09	0.329
Residuals	245	4436	18.1		
Total	352	16635	2165		
Oribatid Abundance:					
Block	3	169	56.2	5.62	0.001
Day	5	6248	1250	125	0.000
A. rubrum	1	24.7	24.7	2.47	0.117
L. tulipifera	1	4.89	4.89	0.49	0.485
Q. prinus	1	44.9	44.9	4.48	0.035
R. maximum	1	39.9	39.9	3.99	0.047
SpInt	10	95.8	9.58	0.96	0.481
Block*Day	15	1090	72.7	7.26	0.000
*A. rubrum**Day	5	36.5	7.29	0.73	0.602
*L. tulipifera**Day	5	32.9	6.58	0.66	0.656
*Q. prinus**Day	5	52.6	10.5	1.05	0.388
*R. maximum**Day	5	150	29.9	2.99	0.012
SpInt*Day	50	501	10.0	1.00	0.477
Residuals	245	2451	10.0		
Total	352	10941	1577		

Appendix 4.4 (cont.)

	df	SS	MS	F	*P*
Mesostigmata Abundance:					
Block	3	34.2	11.4	2.39	0.069
Day	5	692	138	29.1	0.000
A. rubrum	1	1.12	1.12	0.24	0.628
L. tulipifera	1	22.5	22.5	4.72	0.031
Q. prinus	1	0.000	0.000	0.000	0.995
R. maximum	1	17.6	17.6	3.69	0.056
SpInt	10	47.1	4.71	0.99	0.454
Block*Day	15	523	34.8	7.31	0.000
*A. rubrum**Day	5	12.93	2.59	0.54	0.744
*L. tulipifera**Day	5	63.7	12.74	2.67	0.023
*Q. prinus**Day	5	56.7	11.3	2.38	0.039
*R. maximum**Day	5	58.5	11.7	2.45	0.034
SpInt*Day	50	209	4.17	0.88	0.707
Residuals	245	1167	4.77		
Total	352	2905	277.87		
Prostigmata Abundance:					
Block	3	13.2	4.41	2.21	0.088
Day	5	609	122	61.0	0.000
A. rubrum	1	0.67	0.67	0.34	0.562
L. tulipifera	1	0.79	0.79	0.39	0.531
Q. prinus	1	4.04	4.04	2.02	0.156
R. maximum	1	0.0002	0.0002	0.0001	0.992
SpInt	10	8.48	0.85	0.42	0.934
Block*Day	15	67.9	4.53	2.26	0.005
*A. rubrum**Day	5	18.7	3.75	1.87	0.099
*L. tulipifera**Day	5	2.59	0.52	0.26	0.935
*Q. prinus**Day	5	14.2	2.85	1.42	0.216
*R. maximum**Day	5	13.7	2.74	1.37	0.237
SpInt*Day	50	84.0	1.68	0.84	0.766
Residuals	245	490	2.00		
Total	352	1328	150.7		
Collembola Abundance:					
Block	3	83.0	27.7	2.99	0.032
Day	5	2788	558	60.2	0.000
A. rubrum	1	5.39	5.39	0.58	0.446
L. tulipifera	1	10.3	10.3	1.11	0.292
Q. prinus	1	54.3	54.3	5.86	0.016
R. maximum	1	8.03	8.03	0.87	0.353
SpInt	10	62.2	6.22	0.67	0.750
Block*Day	15	708	47.2	5.10	0.000
*A. rubrum**Day	5	35.9	7.18	0.78	0.569
*L. tulipifera**Day	5	25.2	5.04	0.54	0.743
*Q. prinus**Day	5	144	28.7	3.10	0.010
*R. maximum**Day	5	86.4	17.3	1.87	0.101
SpInt*Day	50	584	11.7	1.26	0.129
Residuals	245	2269	9.26		
Total	352	6863	796		

Appendix 4.5. Summary of the ANOVA's testing for additive and non-additive effects of litter mixing on arthropod community richness and diversity.

	df	SS	MS	F	*P*
Total Arthropod Richness:					
Block	3	30.0	9.99	4.18	0.007
Day	5	752	150	62.97	0.000
A. rubrum	1	0.45	0.45	0.19	0.663
L. tulipifera	1	4.08	4.08	1.71	0.192
Q. prinus	1	10.7	10.7	4.47	0.035
R. maximum	1	10.0	10.0	4.19	0.042
SpInt	10	41.6	4.16	1.74	0.072
Block*Day	15	76.5	5.10	2.14	0.009
*A. rubrum**Day	5	6.32	1.26	0.53	0.754
*L. tulipifera**Day	5	18.7	3.73	1.56	0.171
*Q. prinus**Day	5	5.57	1.11	0.47	0.801
*R. maximum**Day	5	37.4	7.49	3.14	0.009
SpInt*Day	50	161	3.23	1.35	0.072
Residuals	245	585	2.39		
Total	352	1740	214		
Shannon Index:					
Block	3	1.33	0.44	12.16	0.000
Day	5	5.50	1.10	30.10	0.000
A. rubrum	1	0.01	0.01	0.15	0.702
L. tulipifera	1	0.33	0.33	8.92	0.003
Q. prinus	1	0.00	0.00	0.00	0.947
R. maximum	1	0.15	0.15	4.22	0.041
SpInt	10	0.34	0.03	0.93	0.510
Block*Day	15	1.18	0.08	2.16	0.008
*A. rubrum**Day	5	0.40	0.08	2.20	0.055
*L. tulipifera**Day	5	0.28	0.06	1.55	0.176
*Q. prinus**Day	5	0.29	0.06	1.58	0.168
*R. maximum**Day	5	0.27	0.05	1.48	0.196
SpInt*Day	50	1.92	0.04	1.05	0.390
Residuals	245	8.95	0.04		
Total	352	20.9	2.47		

Appendix 4.6. All taxa found in heat-extracted litter, the total number of individuals found for each throughout the entire experiment, and the taxonomic level at which each taxa sits. Taxonomic levels are Order (O), Suborder (sO), and Family (F).

Taxa	No. Individuals	Level
Oribatida	19599	sO
Collembola	12886	O
Mesostigmata	5395	sO
Prostigmata	1426	sO
Homoptera	1121	sO
Protura	1003	O
Diptera	716	O
Araneae	356	O
Pauropoda	189	O
Enchytraeidae	159	O
Pseudoscorpionida	110	O
Formicidae	103	F
Julidae	89	O
Lithobiomorpha	88	O
Psocoptera	88	O
Symphyla	74	O
Coleoptera	50	O
Gastropoda	42	C
Thysanoptera	31	O
Geophilomorpha	25	O
Scolopendromorpha	12	O
Hymenoptera	9	O
Diplura	4	O
Lepidoptera	2	O
Ephemeroptera	1	O
Isopod	1	O

CHAPTER 5

DOES MICROBIAL BIOMASS CONFOUND LITTER-MIXING EFFECTS ON MASS LOSS?[4]

[4] Ball, B.A., M.D. Hunter, and M.A. Bradford. To be submitted to *Soil Biology & Biochemistry.*

Abstract

Plant litter decomposition is an ecosystem process fundamental to nutrient cycling and hence ecosystem sustainability. Much of our knowledge about litter decomposition is derived from studies that quantify the loss of litter mass across time. Litter inherently includes microbial biomass that colonizes and decays plant litter, and mass loss of the litter substrate alone is rarely quantified. Given concerns about declining biodiversity and/or whether our knowledge of litter decomposition can explain how litters decay in multi-species plant communities, an increasing number of studies are testing whether decomposition dynamics in litter mixtures are predictable from monocultures. It is feasible that decay dynamics in mixtures could be obscured by the separate dynamics of the microbial biomass and the substrate, potentially leading to misinterpretations about whether decomposition in mixtures is predictable (additivity) or not predictable (non-additivity) from single species decay dynamics. To test whether substrate mass loss responses of litter mixing differ when the microbial biomass is quantified, we conducted a full-factorial litterbag study using four dominant tree species in a temperate rainforest across 2 years. Total litter carbon and microbial carbon were measured and used to calculate the carbon content of the substrate alone. We found that additivity explained litter and microbial carbon dynamics. However, the magnitude of these effects differed when the substrate was considered alone and the microbial biomass response to species presence/absence differed to that of the substrate mass. Our results demonstrate that while additive or non-additive decay dynamics are not obscured by the microbial biomass response to litter mixing, more can be revealed about

mass loss dynamics in multi-species litter layers when the substrate is analyzed separately from the microbial biomass colonizing it.

Key Words: Ecosystem function, decomposition, litter mixtures, species diversity, species composition, biodiversity, litter quality, microbial biomass, random species loss, non-random species loss

Short Communication

Decomposition of plant litter is a fundamental ecological process, integral to nutrient cycling, energy flow in foodwebs, and the structure and dynamics of ecosystems (Swift et al., 1979, Moore et al., 2004). Decomposition is most often studied through mass loss of litter. However, the mass of litter inherently includes all of that associated with the microbial biomass colonizing the litter, which can be a significant component of the total litter mass remaining (up to 50% or more, e.g. Swift, 1973). As carbon (C) is lost from the litter substrate, it is processed by the microbial community and either lost from the litter or assimilated into microbial biomass. Thus it is the sum of microbial C and substrate C that is measured as total litter C (e.g. Fig. 5.1a).

Classically, much of our knowledge about decomposition dynamics comes from studies of individual plant species, though most ecosystems consist of mixed communities. To determine whether decomposition dynamics differ in multiple-species litter mixtures, studies have investigated the effects of litter composition and richness on mass loss (reviewed by Hättenschwiler et al., 2005, Gartner and Cardon, 2004). Additive decay dynamics would result from the independent influence of individual species on mass loss, where diverse litter mixes have a faster decay rate due to increased probability of including species with faster decay rates

(Johnson et al., 2006). If decay dynamics in mixtures are the sum of their parts, decay dynamics of single litters can used to predict decay dynamics in multi-species litter layers. Alternatively, if species' decay rates in mixture are dependent on other litter species (giving rise to non-additive dynamics), research on decay rates of mixtures would be required for us to understand decomposition in multi-species systems. To date, results have varied, where non-additive dynamics occur in some cases (e.g. McTiernan et al., 1997, Leroy and Marks, 2006, Swan and Palmer, 2004, Wardle et al., 1997, Briones and Ineson, 1996), but not in others (Hansen, 1999, Blair et al., 1990).

Litter-mixing studies that specifically measure the response of microbes to litter mixing have often shown non-additive effects on microbial biomass (Bardgett and Shine, 1999, Blair et al., 1990, Wardle et al., 1997). This suggests that there may be significantly more or less retention of mass in the litter than expected as it is decomposed, masking the dynamics of the substrate. For example, if two species are mixed, one that supports a larger microbial biomass and has faster total decay (Fig. 5.1a) and one with lower microbial biomass and slower decay (Fig. 5.1b), the resulting effects could be additive or non-additive. If the effects of mixing are additive for microbial biomass and substrate mass loss, they would be the average of the two component species in monoculture (Fig. 5.1c). This would generate an additive overall mass loss of the litter (substrate + microbes). Alternatively, it is possible that a positive non-additive interaction could occur on the microbial biomass and substrate mass loss (Fig. 5.1d). Though substrate is being lost at a faster rate, the microbial biomass is also growing faster, showing a net additive change in total litter mass loss, masking the non-additive interactions that are occurring. Microbial biomass could also mask effects of mixing when overall non-additive interactions are observed for total litter mass loss. In such an instance, if the microbial biomass responds non-

additively, the rate of substrate mass loss may actually be additive (Fig. 5.1e). If microbial biomass were significantly lower (or higher) than expected, the resulting total litter mass remaining would also appear proportionally significantly lower (or higher). There is also the possibility that both microbial biomass and substrate mass loss respond non-additively, but not proportionately with one another, generating total litter mass loss dynamics that are non-additive but of a different relative magnitude to the actual effects of mixing on substrate mass loss. Given this potential for microbial biomass to confound the results of litter mixing studies, it is necessary to separately examine the dynamics of the total litter and its component microbial and substrate biomass.

To test whether litter substrate mass loss responds differently to litter mixing when analyzed separately from microbial biomass, we conducted a full-factorial decomposition study at Coweeta Hydrologic Laboratory in the southern Appalachian Mountains, USA (35º00'N, 83º30'W). The litters were collected from the four most abundant tree species: *Liriodendron tulipifera* L. (tulip poplar, L), *Acer rubrum* L. (red maple, A), *Quercus prinus* L. (chestnut oak, Q), and *Rhododendron maximum* L. (rhododendron, R). Leaves were put into litterbags in each of the possible 15 combinations of the four species. Each litterbag contained 5 g of leaves, and all species in any one combination were equally represented in mass. In November 2003, one set of all 15 combinations was placed in each of four replicate blocks for each of 5 collection dates across two years: 0, 92, 181, 365, and 730 days. At each collection date, one set from each replicate plot was randomly chosen for processing. Litter was dried, ground, and the carbon (C) content was determined by combustion in a Carlo Erba Elemental Analyzer (Carlo Erba, Milan, Italy).

Microbial biomass was estimated as the sum of bacterial and fungal biomass from subsamples of the litter. Bacterial cells were stained with DAPI (4'6-diamidino-2-phenylindole) and vacuum filtered onto 0.2 µm membrane filters (Weyers and Suberkropp, 1996, Velji and Albright, 1993). Cells were enumerated by cell shape using epifluorescent microscopy (1000×). Biovolumes were calculated using equations for the geometric shape (Wetzel and Likens, 2000), and total bacterial C was estimated using the conversion factor 5.6×10^{-13} g C μm^{-3} (Bratbak, 1985). Fungal biomass was estimated from ergosterol concentration, which was extracted in methanolic KOH, partitioned into pentane, evaporated, and redissolved in methanol. (Weyers and Suberkropp, 1996). Ergosterol concentration was measured on a high-pressure liquid chromatograph (HPLC) at 282 nm on a RP-10 column (Shimadzu, Columbia, Maryland, USA) and was converted to biomass using the conversion factor 5.5 µg ergosterol g fungal dry $mass^{-1}$ (Gessner and Chauvet, 1993). This was then expressed as g C using previous conversions of 43% C of dry weight (Baldy and Gessner, 1997, Baldy et al., 1995).

All statistical analyses were conducted in S-Plus 7.0 for Windows. Data were analyzed using time as a discrete factor to test whether effects were consistent across time. Microbial biomass data were natural log-transformed to meet assumptions of normality of variance; all other data were un-transformed. An Analysis of Variance (ANOVA), using Type I Sums of Squares (SS), was performed to test for additivity and non-additivity of species effects. Block, time, and the presence/absence of each of the four species were added sequentially to the model. Next, to test for non-additivity, this was followed by a species interaction term (SpInt). Lastly interactions between time and block, and then the species terms and finally the interaction term, were included. A significant SpInt term (and/or its interaction with time) indicates a significant non-additive interaction among species, due to richness or composition, that is not explained by

simple presence or absence of individual species. If the SpInt term was not significant, the model was re-run with each of the four species' presence/absence terms added first. This was done because Type I SS were used, and the F-values of the species terms were sensitive to the order in which they were added.

Litter mixing generated only additive effects on total litter C loss, given that the SpInt term and its interaction with time were not significant ($P > 0.05$). These additive effects of composition were based on the presence/absence of each of the four species (Table 5.1). Given that time did not interact with three of four of the species terms (Table 5.1), data were pooled across all sampling dates. This revealed that there was significantly more C remaining when *Q. prinus* and *R. maximum* were present and less with *L. tulipifera* and *A. rubrum* (Fig. 5.2a). The effects of *R. maximum* were time-dependent, as the difference between its presence and absence decreased through time (Fig. 5.2b). Microbial C also responded additively to litter mixing and only two of the species, *L. tulipifera* and *R. maximum*, had significant effects on this variable (Table 5.1b). Specifically, there was significantly less microbial C when *R. maximum* was present and more when *L. maximum* was present (Fig. 5.2c), though this effect diminished over time for *L. tulipifera*. When microbial biomass was removed from the C content of total litter, giving only the C of the substrate, there were additive effects of the presence of all four species, and the effect of *R. maximum* was again time-dependent (Table 5.2). Pooling these substrate data across time revealed similar effects as with total litter C (Fig. 5.1d). However, the magnitude of these additive effects differed slightly and were more pronounced for substrate than total litter C for some species (e.g. *Q. prinus*) and less for others (e.g. *A. rubrum*).

Given the additive effects of litter mixing on total litter, substrate and microbial C, our results are most accurately represented by the hypothetical scenario shown in Fig. 5.1c.

However, by removing the microbial biomass, we were able to detect different magnitudes of these effects, reflecting the magnitude and direction of the effects of each species on microbial biomass. If microbial biomass is increased or decreased by the presence of a species, and this change is not proportional to the change in C loss from the litter, then the magnitude of a species effect on substrate C can differ from that for total litter C.

The results of our study show that additive effects of litter mixing on litter substrate decomposition were not obscured by the dynamics of the microbial biomass colonizing that litter. This means that additive or non-additive effects detected in other studies may represent true effects of mixing on litter substrate decay. However, when substrate and microbial C were quantified separately, we were able to detect different magnitudes of additive composition effects of mixed litter that might affect interpretation of the consequences of the loss of these species. This is valuable given changing distribution and abundances of species in this area. For example, the invasive hemlock woolly adelgid is projected to extirpate eastern hemlock from much of its range, and at our field site will likely be replaced by tulip poplar or rhododendron (Orwig and Foster, 1998, Ellison et al., 2005). Similarly, the invasive pathogen sudden oak death will potentially cause declines in rhododendron (Rizzo et al., 2002). Given the additive influence of the four species, changes in abundance of the any of these species will influence substrate mass loss in a predictable manner, while only the two species at opposite ends of the quality spectrum will influence microbial biomass. While microbial biomass may be resilient to loss of the other two mid-quality species, it would be interesting to know whether the foodwebs depending on these microbes are as well. Notably, microbial biomass was only a small percentage of overall litter C, which could prevent it from having a large masking effect on substrate dynamics. Larger microbial communities, such as those growing on woody litter

(Swift, 1973), may be more likely to obscure substrate dynamics. A different scenario may be detected if litter mixtures included woody material, but most decomposition work is done with leaf litter and therefore accurately represented by this study.

In conclusion, our study shows that the effects of different species' litters on decomposition dynamics in our system are largely independent of the presence/absence of other species. This suggests that effects of species loss in our system will likely be predictable from knowledge about single species. Notably, loss of any of the four dominant species we studied will alter litter decomposition dynamics in our system, but microbial biomass dynamics appeared more resistant, being sensitive only to the loss of two of the four species. While microbial biomass dynamics in the litter layer are strongly tied to nutrient release and immobilization, in our study they do not necessarily respond similarly to species presence/absence as mass loss. There is a need for future litter mixing investigations to quantify microbial biomass separately to gain a complete understanding of litter layer dynamics.

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References

Baldy, V. & Gessner, M. O. (1997) Towards a budget of leaf litter decomposition in a first-order woodland stream. *Comptes Rendus De L Academie Des Sciences Serie Iii-Sciences De La Vie-Life Sciences,* **320,** 747-758.

Baldy, V., Gessner, M. O. & Chauvet, E. (1995) Bacteria, fungi and the breakdown of leaf-litter in a large river. *Oikos,* **74,** 93-102.

Bardgett, R. D. & Shine, A. (1999) Linkages between plant litter diversity, soil microbial biomass and ecosystem function in temperate grasslands. *Soil Biology & Biochemistry,* **31,** 317-321.

Blair, J. M., Parmelee, R. W. & Beare, M. H. (1990) Decay-Rates, Nitrogen Fluxes, and Decomposer Communities of Single-Species and Mixed-Species Foliar Litter. *Ecology,* **71,** 1976-1985.

Bratbak, G. (1985) Bacterial biovolume and biomass estimations. *Applied and Environmental Microbiology,* **49,** 1488-1493.

Briones, M. J. I. & Ineson, P. (1996) Decomposition of eucalyptus leaves in litter mixtures. *Soil Biology and Biochemistry,* **28,** 1381-1388.

Ellison, A. M., Bank, M. S., Clinton, B. D., Colburn, E. A., Elliott, K., Ford, C. R., Foster, D. R., Kloeppel, B. D., Knoepp, J. D., Lovett, G. M., Mohan, J., Orwig, D. A., Rodenhouse, N. L., Sobczak, W. V., Stinson, K. A., Stone, J. K., Swan, C. M., Thompson, J., Von Holle, B. & Webster, J. R. (2005) Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. *Frontiers in Ecology and the Environment,* **3,** 479-486.

Gartner, T. B. & Cardon, Z. G. (2004) Decomposition dynamics in mixed-species leaf litter. *Oikos,* **104,** 230-246.

Gessner, M. O. & Chauvet, E. (1993) Ergosterol-to-biomass conversion factors for aquatic hyphomycetes. *Applied and Environmental Microbiology,* **59,** 502-507.

Hansen, R. A. (1999) Red oak litter promotes a microarthropod functional group that accelerates its decomposition. *Plant and Soil,* **209,** 37-45.

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

Johnson, M. T. J., Lajeunesse, M. J. & Agrawal, A. A. (2006) Additive and interactive effects of plant genotypic diversity on arthropod communities and plant fitness. *Ecology Letters,* **9,** 24-34.

Leroy, C. J. & Marks, J. C. (2006) Litter quality, stream characteristics and litter diversity influence decomposition rates and macroinvertebrates. *Freshwater Biology,* **51,** 605-617.

McTiernan, K. B., Ineson, P. & Coward, P. A. (1997) Respiration and nutrient release from tree leaf litter mixtures. *Oikos,* **78,** 527-538.

Moore, J. C., Berlow, E. L., Coleman, D. C., de Ruiter, P. C., Dong, Q., Hastings, A., Johnson, N. C., McCann, K. S., Melville, K., Morin, P. J., Nadelhoffer, K., Rosemond, A. D., Post, D. M., Sabo, J. L., Scow, K. M., Vanni, M. J. & Wall, D. H. (2004) Detritus, trophic dynamics and biodiversity. *Ecology Letters,* **7,** 584-600.

Orwig, D. A. & Foster, D. R. (1998) Forest response to the introduced hemlock woolly adelgid in southern New England, USA. *Journal of the Torrey Botanical Society,* **125,** 60-73.

Rizzo, D. M., Garbelotto, M., Davidson, J. M., Slaughter, G. W. & Koike, S. T. (2002) Phytophthora ramorum as the cause of extensive mortality of Quercus spp. and Lithocarpus densiflorus in California. *Plant Disease,* **86,** 205-214.

Swan, C. M. & Palmer, M. A. (2004) Leaf diversity alters litter breakdown in a Piedmont stream. *Journal of the North American Benthological Society,* **23,** 15-28.

Swift, M. J. (1973) The estimation of mycelial biomass by determination of the hexosamine content of wood tissue decayed by fungi. *Soil Biology and Biochemistry,* **5,** 321-332.

Swift, M. J., Heal, O. W. & Anderson, J. M. (1979) *Decomposition in Terrestrial Ecosystems.* University of California Press, Los Angeles, CA.

Velji, M. I. & Albright, L. J. (1993) Improved sample preparation for enumeration of aggregated aquatic substrate bacteria. *Handbook of Methods in Aquatic Microbial Ecology* (eds P. F. Kemp, B. F. Sherr, B. F. Sherr & J. J. Cole), pp. 139-142. Lewis Publishers, Ann Arbor, MI.

Wardle, D. A., Bonner, K. I. & Nicholson, K. S. (1997) Biodiversity and plant litter: Experimental evidence which does not support the view that enhanced species richness improves ecosystem function. *Oikos,* **79,** 247-258.

Wetzel, R. G. & Likens, G. E. (2000) *Limnological Analyses.* Springer, New York.

Weyers, H. S. & Suberkropp, K. (1996) Fungal and bacterial production during the breakdown of yellow poplar leaves in 2 streams. *Journal of the North American Benthological Society,* **15,** 408-420.

Table 5.1. Summary of the ANOVA's testing for additive and non-additive effects of litter mixing on total litter carbon (C) and microbial C.

	df	SS	MS	F	*P*
Total Litter C Remaining:					
Block	3	0.07	0.02	4.58	0.004
Day	3	3.99	1.33	278	0.000
A. rubrum	1	0.10	0.10	20.9	0.000
L. tulipifera	1	0.10	0.10	21.9	0.000
Q. prinus	1	0.05	0.05	9.42	0.003
R. maximum	1	0.17	0.17	35.0	0.000
SpInt	10	0.05	0.005	1.05	0.406
Block*Day	9	0.31	0.03	7.18	0.000
*A. rubrum**Day	3	0.009	0.003	0.65	0.583
*L. tulipifera**Day	3	0.002	0.001	0.11	0.954
*Q. prinus**Day	3	0.03	0.01	2.24	0.086
*R. maximum**Day	3	0.04	0.01	2.67	0.050
SpInt*Day	30	0.07	0.002	0.48	0.990
Residuals	142	0.68	0.005		
Total	213	5.67	1.84		
Microbial C:					
Block	3	85.0	28.3	0.40	0.756
Day	3	16858	5619	78.6	0.000
A. rubrum	1	16.7	16.7	0.23	0.630
L. tulipifera	1	1296	1296	18.1	0.000
Q. prinus	1	0.000	0.003	0.000	0.995
R. maximum	1	1685	1685	23.6	0.000
SpInt	10	570	57.0	0.80	0.631
Block*Day	9	911	101	1.42	0.187
*A. rubrum**Day	3	19.8	6.61	0.09	0.964
*L. tulipifera**Day	3	2419	806	11.3	0.000
*Q. prinus**Day	3	334	111	1.56	0.203
*R. maximum**Day	3	459	153	2.14	0.098
SpInt*Day	30	1913	63.8	0.89	0.631
Residuals	142	10155	71.5		
Total	213	36722	10017		

Table 5.2. Summary of the ANOVA testing for additive and non-additive effects of litter mixing on substrate C. Substrate C was calculated as the difference between total litter C and microbial C.

	df	**SS**	**MS**	**F**	***P***
Block	3	0.07	0.02	4.62	0.004
Day	3	3.98	1.33	275	0.000
A. rubrum	1	0.10	0.10	20.5	0.000
L. tulipifera	1	0.11	0.11	23.8	0.000
Q. prinus	1	0.05	0.05	9.58	0.002
R. maximum	1	0.18	0.18	37.7	0.000
SpInt	10	0.05	0.005	1.03	0.424
Block*Day	9	0.32	0.04	7.30	0.000
*A. rubrum**Day	3	0.009	0.003	0.63	0.595
*L. tulipifera**Day	3	0.003	0.001	0.21	0.892
*Q. prinus**Day	3	0.03	0.01	2.22	0.088
*R. maximum**Day	3	0.04	0.01	2.79	0.043
SpInt*Day	30	0.07	0.002	0.51	0.984
Residuals	142	0.68	0.005		
Total	213	5.69	1.86		

Figure 5.1. Hypothetical dynamics of plant litter carbon content (C) during decomposition. Through time, C is lost from the substrate (gray) as it is processed by the microbes and either lost from the litter layer (white) or incorporated into the microbial biomass (black). Total C loss may be greater for (a) faster decomposing litters than (b) slower ones. In mixture, resulting decay dynamics may be (c) additive for substrate and microbial C, (d) non-additive for both, or (e) non-additive only for one of the two. Depending on the responses of the microbes and substrate, the resulting total litter C loss may appear (c-d) additive or (e) non-additive, possibly misrepresenting the actual dynamics of substrate C.

Figure 5.2. Investigation of the direction of significant additive effects that were identified for (a) fraction C remaining in the total litter (substrate + microbes), (c) microbial C and (d) fraction C remaining in the substrate alone. Effects of most species were significant, but those that were not are denoted with "ns". Letters on the x-axis refer to the genus of each of the four tree species: *L. tulipifera* (L), *A. rubrum* (A), *Q. prinus* (Q), and *R. maximum* (R). Bars represent pooled values across time. Species whose effects interacted with time (denoted with a "t" in (a), (c), and (d)) are shown in (b). Solid bars or symbols represent all treatments that contained the particular species under consideration, and open ones include all treatments that did not. Values are means ± 1 SE; $n = 4$.

Figure 5.1

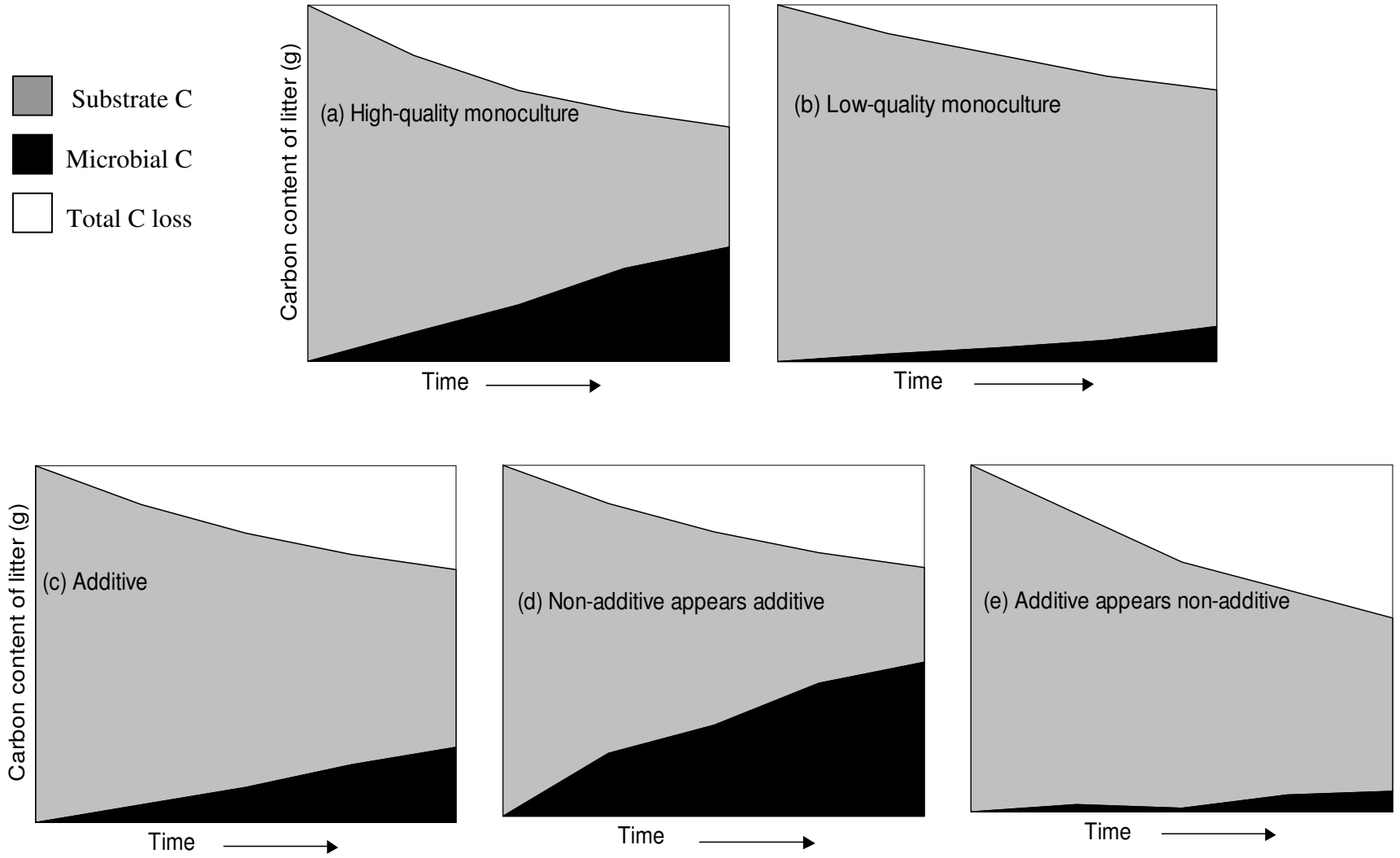
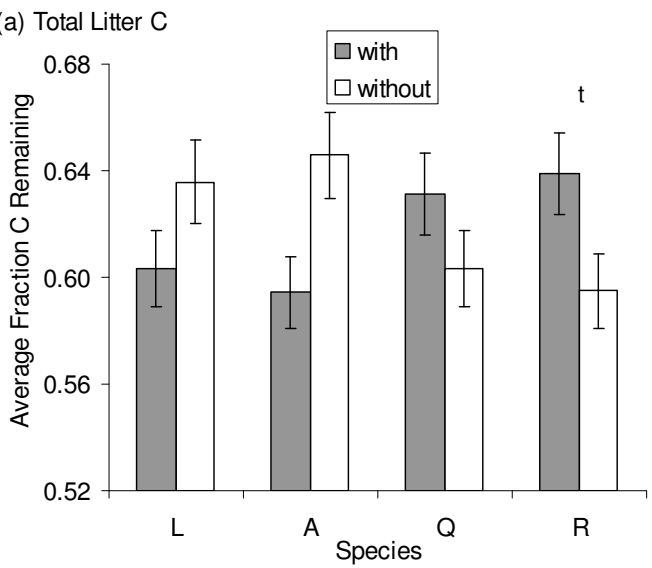
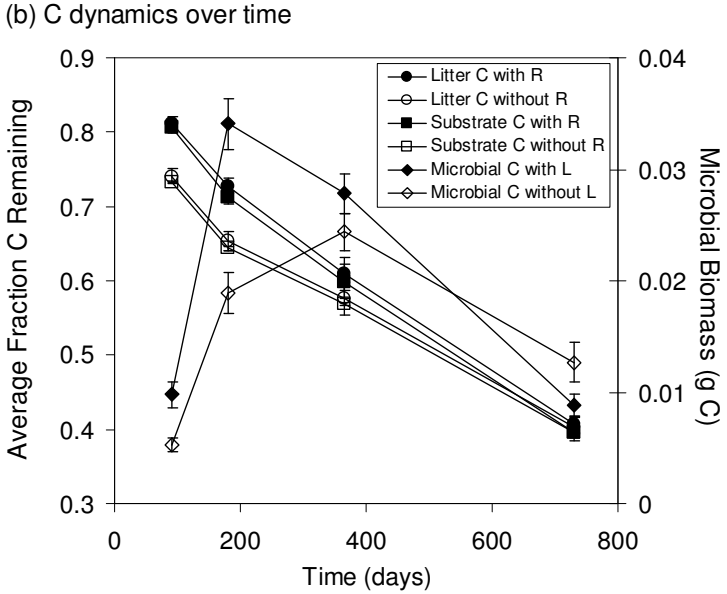
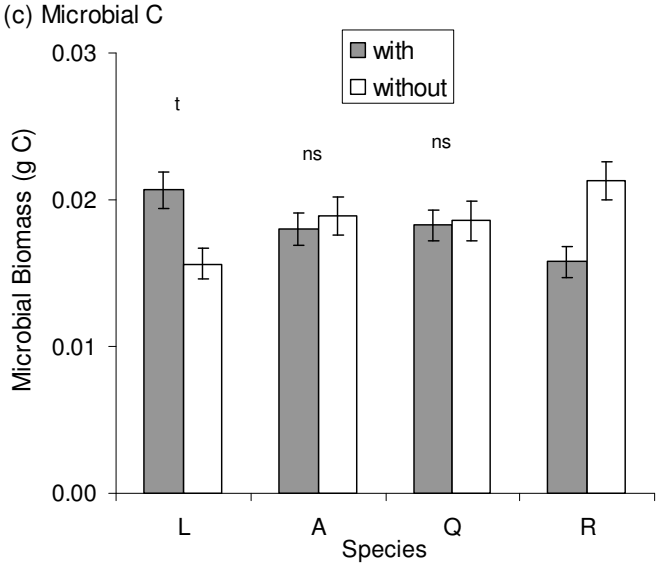
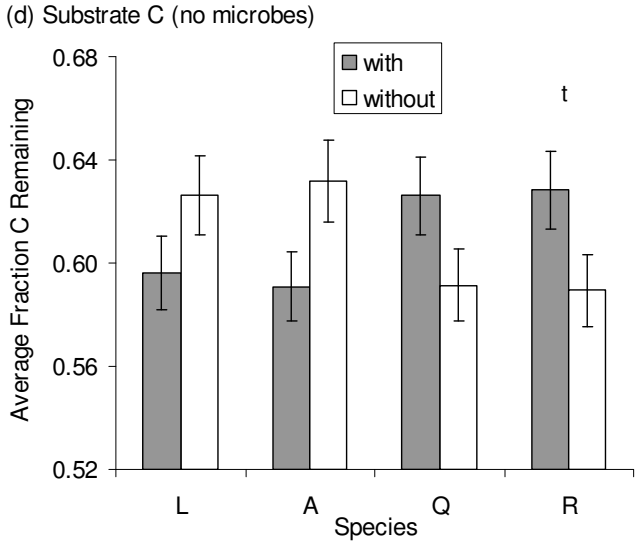

Substrate C
Microbial C
Total C loss
Carbon content of litter (g)
(a) High-quality monoculture
Time
(b) Low-quality monoculture
Time
Carbon content of litter (g)
(c) Additive
Time
(d) Non-additive appears additive
Time
(e) Additive appears non-additive
Time

Figure 5.2


(a) Total Litter C
with
without
Average Fraction C Remaining
0.68
0.64
0.60
0.56
0.52
t
L
A
Q
R
Species


(b) C dynamics over time
Litter C with R
Litter C without R
Substrate C with R
Substrate C without R
Microbial C with L
Microbial C without L
Average Fraction C Remaining
Microbial Biomass (g C)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.04
0.03
0.02
0.01
0
0
200
400
600
800
Time (days)


(c) Microbial C
with
without
Microbial Biomass (g C)
0.03
0.02
0.01
0.00
t
ns
ns
L
A
Q
R
Species


(d) Substrate C (no microbes)
with
without
Average Fraction C Remaining
0.68
0.64
0.60
0.56
0.52
t
L
A
Q
R
Species

CHAPTER 6

CONCLUSIONS

To demonstrate a possible link between aboveground plant communities and belowground processes, we sought to determine if there were additive or non-additive effects of litter diversity, through richness or composition, on leaf litter mass loss, nutrient dynamics, and the decomposer community in a southern Appalachian riparian zone. We were primarily interested in the relative importance of additive and non-additive effects in order to assess potential consequences of non-random species loss, which has not yet been addressed for decomposition and its related processes. Given the variation in characteristics represented by the four species, we expected additive effects of litter mixing based on species identity. This was confirmed for mass loss and decomposer biota, where there were significant effects of the presence/absence of each of the four species. Given previous work (reviewed by Hättenschwiler and Gasser, 2005, Gartner and Cardon, 2004), we also expected non-additive effects due to the large difference in litter quality between some of the species (Seastedt, 1984). Indeed, this was the case for nutrient dynamics, for which there were non-additive effects of both composition and richness.

Our results suggest a potentially large impact of non-random species loss on this system. Effects of litter composition and/or richness were identified for each of the decomposition parameters measured, and we were able to identify the species driving these effects and explore the direction and magnitude of each. By addressing additive effects in addition to the non-

additive effects, we were able to detect impacts of composition that may otherwise have been considered idiosyncratic or absent when analyzed by the methods of previous work. Together, these data yield valuable information about the consequences of non-random species loss for decomposition in this system.

It appears that differences in litter quality override mixing effects for mass loss and most aspects of the decomposer community, suggesting that consequences will be predictable based on the individual influence of each of the four species. The presence of high-quality litter species, such as *L. tulipifera* and *A. rubrum*, support larger, more diverse decomposer communities, which is reflected by an increased rate of mass loss. Low-quality species, such as *R. rubrum*, tend to decrease decomposer abundance and diversity and thus slow the rate of mass loss in mixture. *Q. prinus*, which is generally considered to be low quality due to high structural compound content, had positive effects on arthropod decomposers, though this was not reflected in the lower trophic levels or litter mass loss. Interestingly, while the microbes are responsible for the majority of decomposition (Chapin et al., 2002), their additive responses to species were not reflected in the mass loss. There is a need for future investigation to quantify microbial biomass separately from substrate mass to gain a complete understanding of this.

Mass loss and the decomposer community did not respond to litter-mixing in the same way as the nutrient dynamics with which they are often associated, as has been noted by others (Prescott, 2005). There are non-additive effects on nutrient dynamics driven by each species, causing the effects of species loss to be less predictable. We do know, however, that transfer of nutrients from high-content species to low-content species in mixture created a difference in overall release between single- and multiple-species mixtures. Each of the four species are involved in driving these non-additive interactions, so there will be significant impacts on

nutrient dynamics if any of them are lost. When this information was applied at the ecosystem level, the resulting predictions of net nutrient release were much smaller than would have been predicted based on monocultures, as is done for most estimates of ecosystem-level nutrient dynamics. However, this transfer of nutrients is not reflected in the abundance or biomass of the biota, as there are no non-additive effects of richness on any group, save the lower plant-feeding nematodes (and the response to increasing richness was not the same). Additional research is necessary to determine the mechanisms that cause these related processes to respond to litter mixing differently.

Together, each species' influence on additive effects and non-additive interactions suggests large effects of non-random species loss on nutrient dynamics at the ecosystem level, with large repercussions on the decomposer community, the rate of organic matter turnover, the amount and availability of mineral nutrients and the length of time for which they are stored in the litter layer. The relationship between aboveground plant communities on belowground processes, through litter input, may then feed back on aboveground plant communities through energy and nutrient availability.

Future directions

To allow broader conclusions of the consequences of non-random species loss on decomposition processes, the next step is to determine how these patterns compare across systems. We have only examined responses in the litter layer, where the biota are directly consuming the plant litter, so responses to species loss may be faster and greater than other systems. Other systems of interest will include the soil and aquatic systems. Traditionally, litter and soils systems, as well as aquatic and terrestrial systems, are studied separately. However,

they undergo similar processes and are closely linked through the leaf litter inputs that represent a significant addition of energy and nutrients (Odum, 1971). However, due to different environmental constraints, responses among systems may differ.

For example, the soil system processes only fragments of this litter input, and may be buffered from or respond differently to changes in the aboveground plant community. Effects of litter quality, diversity, and interactions with the decomposer community may differ from those seen in the litter layer. Investigation into the magnitude and direction of responses to litter mixing in the soil compared to the litter layer is necessary to fully understand how species loss in aboveground plant communities will affect belowground communities and processes (Carrillo, unpublished manuscripts). Also, the linkages between litter quality and soil biota in determining function have not been addressed by many studies, and the findings of the few studies of their interactive effects (e.g. Couteaux et al., 1991, Bradford et al., 2002) are inconsistent (reviewed by Smith and Bradford, 2003). Therefore, the extent and strength of this interaction, and how it may differ between the litter and soil system, needs to be addressed.

Additionally, riparian plant communities not only influence the terrestrial system, but also the aquatic systems with which they are associated. As with terrestrial systems, the composition of riparian forests determines the allochthonous input into streams that form the basis of the food chain (Allan, 1995). To bridge the gap between aquatic and terrestrial research, this experiment was replicated in the associated stream, using comparable methods, to explore how patterns may differ between the systems of the effects of species diversity on decomposition (Kominoski et al., 2007). For example, it is possible that species composition will play a more important role in the relationships between litter mixtures and measured variables, such as decay rate and microbial biomass, for aquatic systems due to the fact that abiotic factors will have a

greater impact on aquatic decomposition than on terrestrial. Additionally, chemical properties of litter will follow the same pattern over time, but on vastly different time scales. Comparison of the patterns between the terrestrial and aquatic system will allow for broader conclusions of the consequences of species loss on this riparian system, allowing organic matter turnover and nutrient dynamics to be estimated for the entire ecosystem, rather than just the terrestrial system.

Finally, there is interest in how these patterns may compare across ecosystems. Different ecosystems are subjected to different climatic factors, potentially driving differences in relationships between aboveground plant communities and decomposition processes. We are specifically interested in determining how the trajectory of litter chemistry, which is so often associated with decomposition and used as a measure for decomposition rate, may behave throughout decomposition, and whether it behaves the same for a variety of systems. We will follow this study with a cross-site comparison of litter chemistry from both terrestrial and aquatic systems over tropical, sub-tropical, and temperate ecosystems to determine whether the chemical drivers of decomposition and role of initial chemistry are constant across systems.

References

Allan, J. D. (1995) *Stream Ecology: Structure and Function of Running Waters.* Kluwer Academic Publishers, Boston, MA.

Bell, D. T., Johnson, F. L. & Gilmore, A. R. (1978) Dynamics of Litter Fall, Decomposition, and Incorporation in Streamside Forest Ecosystem. *Oikos,* **30,** 76-82.

Bradford, M. A., Tordoff, G. M., Eggers, T., Jones, T. H. & Newington, J. E. (2002) Microbiota, fauna, and mesh size interactions in litter decomposition. *Oikos,* **99,** 317-323.

Chapin, F. S., Matson, P. A. & Mooney, H. A. (2002) *Principles of Terrestrial Ecosystem Ecology.* Springer-Verlag New York, Inc., New York, NY.

Couteaux, M. M., Mousseau, M., Celerier, M. L. & Bottner, P. (1991) Increased Atmospheric Co2 and Litter Quality - Decomposition of Sweet Chestnut Leaf Litter with Animal Food Webs of Different Complexities. *Oikos,* **61,** 54-64.

Gartner, T. B. & Cardon, Z. G. (2004) Decomposition dynamics in mixed-species leaf litter. *Oikos,* **104,** 230-246.

Hättenschwiler, S. & Gasser, P. (2005) Soil animals alter plant litter diversity effects on decomposition. *Proceedings of the National Academy of Sciences of the United States of America,* **102,** 1519-1524.

Kominoski, J. S., Pringle, C. M., Ball, B. A., Bradford, M. A., Coleman, D. C., Hall, D. B. & Hunter, M. D. (2007) Nonadditive effects of leaf litter species diversity on breakdown dynamics in a detritus-based stream. *Ecology,* **88,** 1167-1176.

Odum, E. P. (1971) *Fundamentals of Ecology.* W.B. Saunders Company, Philadelphia, PA.

Prescott, C. E. (2005) Decomposition and mineralization of nutrients from litter and humus. *Nutrient Acquisition by Plants* (ed H. BassiriRad), pp. 15-41. Springer-Verlag, Berlin Heidelberg.

Seastedt, T. R. (1984) The Role of Microarthropods in Decomposition and Mineralization Processes. *Annual Review of Entomology,* **29,** 25-46.

Smith, V. C. & Bradford, M. A. (2003) Litter quality impacts on grassland litter decomposition are differently dependent on soil fauna across time. *Applied Soil Ecology,* **24,** 197-203.

Uowolo, A. L., Binkley, D. & Carol Adair, E. (2005) Plant diversity in riparian forests in northwest Colorado: Effects of time and river regulation. *Forest Ecology and Management,* **218,** 107-114.