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ECOSYSTEM IMPACTS OF THE INVASIVE SHRUB *LONICERA MAACKII* ARE INFLUENCED BY ASSOCIATIONS WITH NATIVE TREE SPECIES

DISSERTATION

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the College of Arts and Sciences at the University of Kentucky

By

Megan Marie Poulette

Lexington, Kentucky

Co-Directors: Dr. Mary A. Arthur, Professor of Forest Ecology

and Dr. Philip Crowley, Professor of Biology

Lexington, Kentucky

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ABSTRACT OF DISSERTATION

ECOSYSTEM IMPACTS OF THE INVASIVE SHRUB *LONICERA MAACKII* ARE INFLUENCED BY ASSOCIATIONS WITH NATIVE TREE SPECIES

Invasive species are significant drivers of global environmental change, altering the stability and functioning of numerous ecosystems. The exotic shrub *Lonicera maackii* is an aggressive invader throughout much of the eastern United States. While much is known about its population and community impacts, little is known about effects on ecosystem processes.

This dissertation documents changes in ecosystem processes associated with *L. maackii* growing beneath three native tree species (*Fraxinus quadrangulata, Quercus muehlenbergii, Carya ovata*) in a savanna in Kentucky. Like many invasive plants, *L. maackii* litter decomposed and lost nitrogen (N) rapidly, especially in comparison with native tree litter. In comparison to the soils beneath the trees where the exotic shrub was absent, soils beneath *L. maackii* had a lower bulk density, elevated soil organic matter, C:N, and total soil N and a modified soil microbial community. Inorganic N deposition from spring throughfall was also altered by *L. maackii*, with higher NO_3-N deposition beneath shrubs located beneath the tree canopy relative to canopy locations without *L. maackii.*

While many exotic plant species have been shown to alter ecosystem processes, their impact is often not uniform. This variability is attributed to among-site differences (soil, climate, plant community): within site variability is often ignored. While many of *L. maackii's* alterations to ecosystem processes were uniform across the site, several were dependent upon interactions between the exotic and the native tree species. Litter from *L. maackii* decomposed and lost N more rapidly under *C. ovata* than under the other native tree species. Soils beneath *L. maackii* shrubs located under *C. ovata* also had a greater fungal:bacterial ratio and a greater abundance of the saprophytic fungal lipid biomarker 18:1ω9c.

These results demonstrate that *L. maackii*'s impact extends to ecosystem processes and suggests that invasive plants may have variable effects within a given environment depending on their interactions with the dominant native species. Identifying native species or communities that are more vulnerable to alterations of ecosystem function upon invasion may prove useful to land managers and foster a better

understanding of the role that community dynamics play in moderating or enhancing invasive species impacts.

KEY WORDS: Biological invasion, litter decomposition, *Lonicera maackii*, nitrogen, soil processes

Megan M Poulette

Student's Signature

July 12, 2012

Date

ECOSYSTEM IMPACTS OF THE INVASIVE SHRUB *LONICERA MAACKII* ARE INFLUENCED BY ASSOCIATIONS WITH NATIVE TREE SPECIES

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July 12, 2012

To my husband

Joshua John Poulette

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Chapter One

Ecosystem Impacts of the Invasive Shrub Amur Honeysuckle

Approximately 50,000 nonnative invasive species have been introduced into the United States, and a tenth of those invaders are plant species originally intended for food, fiber, and ornamental purposes that have escaped and now occupy natural ecosystems (Pimentel et al. 2005). While not all of these plants have proven problematic, many plant invasions alter ecosystem dynamics (Vitouseket al. 1997). Yet, only a handful of exotic perennial woody shrubs have shown themselves to be aggressive invaders. Woody shrubs that are commonly recognized as problem species include Japanese barberry (*Berberis thunbergii*), winged euonymus (*Euonymus alatus*), Amur honeysuckle (*Lonicera maackii*), autumn olive (*Elaeagnus umbellate*), Chinese privet (*Ligustrum sinense*), common buckthorn (*Rhamnus cathartica*), and multiflora rose (*Rosa multiflora*).

These woody shrub invaders are few in number, but they are poised to profoundly alter the structure and function of forest ecosystems in the eastern United States (Webster et al. 2006). Although the spread of these species into eastern deciduous forests has been gradual thus far, once established, they tend to form dense monotypic stands in the forest understory. As natural forest ecosystems become increasingly fragmented in the face of human population growth and associated development, their value as islands of biodiversity, wildlife habitat, aesthetic connections to nature, recreation areas, and providers of valuable ecosystem services render them ever more valuable. Therefore, it

is imperative to explore the consequences of the incursion of woody invasive shrubs into these systems. Identifying invasive species that are capable of altering the normal functioning of ecosystems, and elucidating the mechanisms responsible for these alterations could inform conservation efforts to maximize the protection of native systems (Byers et al. 2002). In this review, I will summarize the current literature on the exotic shrub Amur honeysuckle (*Lonicera maackii*), examine our current understanding of the ecological traits and community- and ecosystem-level impacts of this pervasive invader, and identify potential future research directions.

History and Habitat

Amur honeysuckle is a large, upright, multistemmed, deciduous shrub with dark green leaves. It is native to central and northeastern China, Manchuria, the Amur and Ussuri river valleys of Korea, and isolated parts of Japan (Luken and Thieret 1997, Luken et al. 1995). It was introduced to North America as early as 1896 at the Dominion Arboretum in Ottawa and arrived in the U.S. a year later at the Arnold Arboretum (Luken and Thieret 1997). A popular horticultural specimen in Europe and North America, it received awards of merit from the Royal Horticultural Society in the early 20th century and was widely circulated among botanical gardens, commercial growers, and private individuals. Reports of its escape and subsequent spread appear as early as the mid-1920s. Not surprisingly, Amur honeysuckle was cultivated and widely used for reclamation purposes by the USDA Soil Conservation Services (see Luken and Thieret (1997) for a full history of Amur honeysuckle).

In its native range, Amur honeysuckle is adapted to early-successional, frequently disturbed habitats and is commonly found growing in open forests, flood plain forests, and scrub communities (Luken et al. 1995). In its introduced range, it is a pervasive invader throughout much of the eastern United States and Ontario, Canada and has escaped to at least 30 eastern and central states (Trisel and Gorchov 1994, USDA, NRCS 2001). It preferentially invades habitats with high light availability. From there it will opportunistically radiate into forest interiors (Swab 2007). In the Eastern U.S., mixed hardwood forests are highly fragmented due to a varied history of anthropogenic disturbance (agriculture, grazing, and logging), and Amur honeysuckle opportunistically invades forest patches (Luken et al. 1995). In bottomland hardwood forests, Amur honeysuckle densities may be low due to a dense native plant understory or a preference for drier upland soils over the anaerobic lowland soils often found in bottomland sites (Gayek and Quigley 2001, Swab et al. 2008). An examination of the distribution limits of Amur honeysuckle in Ohio revealed that the shrub's distribution limit may be reached when forest cover drops below 5%, with agricultural land acting as a dispersal barrier (Hutchinson and Vankat 1998).

Landscape structure is an important feature of honeysuckle success. Amur honeysuckle can be found in high densities in urban settings like open areas, forest edges, urban riparian zones, and intact forest within cities, with densities increasing as distance to a city center decreases (Luken and Thieret 1996, Borgmann and Rodewald 2005, Trammell and Carreiro 2011). The fragmented, disturbed, and degraded habitat typical of urban areas may promote the spread of Amur honeysuckle into urban riparian systems and forests, potentially altering their ecological functioning (Pennington et al. 2009).

Disturbed plant communities in close proximity to urban centers are also highly vulnerable to invasion (Luken and Goessling 1995). Honeysuckle can spread from multiple point locations, often towns/urban areas, rather than along an advancing front like so many other invasive species (Bartuszevige et al. 2006).

Ecophysiology

General Physiological Traits

The very attributes that made horticultural shrubs like Amur honeysuckle ideal for urban planting, wildlife habitat, and erosion control have contributed to their success as invaders (Webster et al. 2006). Amur honeysuckle's multi-stemmed growth form allows it to rapidly produce dense, persistent populations in a wide range of climatic and edaphic conditions (Luken et al. 1988). Compared to native species, it has a longer photosynthetic season, expanding its leaves earlier in the spring and maintaining them well into the winter months and is highly tolerant of freezing temperatures (Trisel and Gorchov 1994, McEwan et al. 2009). This characteristic leaf phenology has been observed in urban areas, with increasing encroachment by Amur honeysuckle generating an early greenup of urban forests (Shustack et al. 2009). Early leaf out may ultimately make Amur honeysuckle easier to detect via remote sensing, allowing managers to track understory invasion (Wilfong et al. 2009).

Amur honeysuckle's origins in early-successional, frequently disturbed habitat make it likely to be shade-intolerant, preferring open areas (Luken et al. 1995). However, Luken et al. (1995) suggest that a high degree of plastic branch architecture among exotic

shrubs may allow for successful occupation of a wider range of light environments than native shrubs. So while Amur honeysuckle may exhibit greater growth in areas with more light, it can still produce multiple, fast-growing stems in shady environments (Luken et al 1995).

Seedling Establishment

Amur honeysuckle is capable of establishing seedlings in both forest edge and forest interior habitats (Luken and Goessling 1995). Amur honeysuckle seeds are characterized by a minimal delay between dispersal and germination, do not appear to have a well-developed dormancy mechanism, and germinate easily in warm, moist conditions (Luken and Goessling 1995). Light conditions don't appear to constrain seedling establishment which occurred at reduced light levels (1% of full sun), although germination rates may vary (Luken and Goessling 1995, Hidayati et al. 2000). In controlled lab experiments, non-stratified seeds germinated to 53-81% in the light and 31-55% in the darkness (Luken and Goessling 1995). Fresh seeds in another experiment germinated to 48-52% in the light and 27-31% in the darkness (Hidayati et al. 2000). While stratification was not found to be necessary for germination, warm or cold stratification increased the temperature range for germination of Amur honeysuckle, indicating that 50% of the seeds need stratification to come out of dormancy (Hidayati et al. 2000). Specifically, Hidayate et al. (2000) found that half of Amur honeysuckle seeds have nondeep simple morphophysiological dormancy, indicating that this pool of seeds

requires relatively high temperatures to germinate and has a low level of physiological dormancy. Embryo growth was also higher at warm temperatures (Hidayati et al. 2000).

The apparent advantage of this fractured dormancy-breaking/germination and temperature-dependent embryo growth pattern can be tied to production and dispersal patterns of Amur honeysuckle seeds (Hidayati et al. 2000). Fruit is typically produced between September and November; seeds dispersed in September may encounter several weeks of warm weather while seeds dispersed later in the season will encounter cold temperatures. Half of Amur honeysuckle's seeds won't germinate in autumn, but their dormancy will be broken during the winter and embryos will not grow until temperatures increase in the spring (Hidayati et al. 2000). Long-term shrub removal experiments have revealed that the shrub lacks a persistent seed bank (Luken and Mattimiro 1991). Experimental field work by Hidayati et al. (2000) confirmed this finding, with Amur honeysuckle seeds placed under oak layer or buried in the soil germinating to high percentages. Fungi may be important agents of Amur honeysuckle seed mortality but the effect appears to be density dependent. As population densities of the invasive shrub increased, so did fungal pathogen attacks on honeysuckle seeds (Orrock et al. 2012).

While light conditions don't limit seedling establishment, seedlings that establish in the heavily shaded understory of parent plants grow slowly (Luken et al. 1995). Unless seedlings are released by a disturbance event that increases light availability, heavy shade may prevent growth (Luken et al. 1995). Compared with other woody plants, Amur honeysuckle has surprisingly few apparent unique or superior traits in regards to its tolerance range or performance. Luken et al. (1995) revealed that its photosynthetic rates are comparable to those of various shrubs and trees (Harrington et al.

1989a, 1989b, Jones and McLeod 1989), and are actually lower than rates of many indigenous and nonindigenous vines (Carter et al. 1989). Seedlings are capable of acclimation and phenotypic adjustment when light levels are in excess of 25% of full sun, but this plasticity is not uncommon in other shade intolerant species (Luken et al. 1995). The dominance of Amur honeysuckle may be strongly tied to its population growth and competitive ability (Luken et al. 1995).

Population Growth

The multi-stemmed growth form exhibited by invasive shrubs like Amur honeysuckle contributes to the ability to rapidly produce dense, persistent populations (Ehrenfeld 1999). Experimental studies of mature plants often demonstrate the competitive superiority of invasive species relative to natives (D'Antonio and Mahall 1991). Light influences plant survival and growth by eliciting specific growth responses (Valladares 1999), and the branch architecture and early leaf flush of Amur honeysuckle may facilitate its competitiveness for light. Stem growth of Amur honeysuckle was found to equal or exceed that of the native spicebush (*Lindera benzoin*) across a range of light conditions (Luken et al. 1997). Amur honeysuckle maintained resprouting potential in both open (high light) and forest (low light) sites when clipped once. When stressed by repeated clipping, forest-grown shrubs often died, while open-grown shrubs were able to continuously resprout (Luken and Mattimiro 1991). Honeysuckle leaves produced in low light and then exposed to high light had higher net photosynthetic capacity than similar leaves in the shade-tolerant spicebush (Luken et al. 1997). While invasive and

native shrubs may co-exist in a closed forest setting, the ability to respond rapidly to an increase in light availability (e.g. a tree fall) can prove advantageous.

While Amur honeysuckle may exhibit greater growth in areas with more light, it can still produce multiple, fast-growing stems in shady environments. Amur honeysuckle growing under shaded conditions allocates relatively less energy to belowground structures and relatively more energy to aboveground stems. This light-seeking response may be crucial to permit shade-intolerant woody plants like Amur honeysuckle to persist in forest systems (Luken et al. 1997). In an Ohio woodland population of Amur honeysuckle, the initial number of invaders was very small and the founding population remained small for a decade (Deering and Vankat 1999). The timing of the introduction was apparently determined by the availability of seeds, perhaps from surrounding disturbed edges, rather than by a sudden change in microclimate or forest structure. The population grew slowly for 10 years before exponentially increasing, probably via reproduction of older members of the population and the introduction of additional satellite populations from nearby stands. Higher population density was not accompanied by increased mortality and no dead individuals were found in the rapidly expanding honeysuckle population. Nineteen years after the initial introduction, honeysuckle dominated the shrub layer of the site and continued to grow and spread (Deering and Vankat 1999).

Reproduction and Dispersal

Exotic shrubs that become noxious invaders tend to possess highly successful reproductive strategies and can spread in various ways (Ehrenfeld 1999). Amur honeysuckle produces both numerous shoots (Mascaro and Schnitzer 2007), as discussed in the previous section, and abundant fruit - up to 400 million berries per ha (Ingold and Craycraft 1983). Berries are bright red, globose, 3.5-8.5 mm in diameter, and contain 6.5 seeds on average (Ingold and Craycraft 1983, Luken and Theiret 1995). The fruit on invasive shrubs attracts birds and is often available in the fall, winter, and early spring when most birds are migrating, allowing for widespread seed dispersal (Miller and Albritton 2004).

Amur honeysuckle fruits ripen in September, and many are retained on the shrub until spring (McCarty et al. 2002, Bartuszevige et al. 2006). Resident and migratory birds appear to use the berries as an important winter food source but often do not take fruit in large quantities until November or December (Ingold and Craycraft 1983, Bartuszevige et al. 2006, Bartuszevige and Gorchov 2006). Temperature and precipitation appear to affect the retention and abscission rates of winter fruit, with more fruit retention evident in warmer, drier winters and more fruit abscission in colder, wetter winters (Bartuszevige et al. 2006). Greater snow cover may drive seed eating birds like sparrows, cardinals, and juncos to forage on shrubs instead of on the ground, increasing frugivory of Amur honeysuckle berries and potentially dispersing seeds further than drier years (Bartuszevige et al. 2006).

American robins (*Turdus migratorius*), cedar waxwings (*Bombycilla cedrorum*), and European starlings (*Sturnus vulgaris*) are among Amur honeysuckle's major seed dispersers, though many migrant and resident bird species also consume thefruits (Ingold and Craycraft 1983, Bartuszevige and Gorchov 2006). Berries are of poor quality with a low fat (~4.5-5%) and protein (C:N 29:1-56:1) content and tend to be consumed later in the fall and winter when higher quality sources are depleted (Ingold and Craycraft 1983). In one study, five of the 17 bird species observed consuming Amur honeysuckle fruit passed viable seeds through in their feces (Bartuszevige and Gorchov 2006). Gut passage and subsequent scarification are apparently not required for germination, but may enhance germination frequency relative to intact fruits (Bartuszevige and Gorchov 2006). Gut passage through native birds affords the seeds of Chinese tallow tree a higher germination rate (Renne et al. 2001) and bears further exploration in other woody invasives like Amur honeysuckle. Regardless of the nutritional quality or palatability of the invasives' fruit, its availability to migratory birds during winter may give the seeds a significant dispersal advantage. American robins, a resident bird, may promote the spread of Amur honeysuckle by dispersing its seeds along highly suitable woodlot edge and fencerow habitats (Bartuszevige and Gorchov 2006).

Community Impact

Animal Interactions: Birds

Invasive shrubs have the potential to alter a number of animal interactions in forest ecosystems by increasing the overall architectural complexity of invaded sites

(Forseth and Innis 2004). When dense, monotypic stands of invasive shrubs replace patchy native shrubs and abundant herbaceous species, this effect may be especially pronounced. This new layer of dense understory vegetation may provide an increased number of nesting sites, benefiting some bird populations (McCusker et al. 2010). Wood thrushes (*Hylocichla mustelina*), gray catbirds (*Dumetella carolinensis*), American robins, and northern cardinals (*Cardinalis cardinalis*) readily nest in the invasive honeysuckle shrubs (Schmidt and Whelan 1999, Borgmann and Rodewald 2004, McCusker et al. 2010). A study in central Illinois tied the presence of invasive honeysuckle shrubs to changes in the summer and winter bird communities. Understory birds like American robin, northern cardinals, and gray catbirds were twice as abundant in sites with honeysuckle, while canopy birds like eastern wood-pewees (*Contopus virens*) were negatively associated with the invasive shrubs. Frugivorous overwintering species like American robins, northern cardinals, and cedar waxwings were observed in increased densities in areas with honeysuckle shrubs as well, probably in response to availability of food resources in honeysuckle stands during the winter (McCusker et al. 2010).

Amur honeysuckle's branch architecture and early leaf flush can negatively affect songbird nesting success by functioning as 'ecological traps' (Schmidt and Whelan 1999). Nest predation of American robin was directly increased through a combination of lower nest height, the absence of sharp thorns (in comparison to a native shrub), and branch architecture that may have favored predator movement. The robin's preference for early-leafing shrubs also appeared to intensify competition with wood thrushes (Schmidt and Whelan 1999). Compared to nests in native vegetation, Northern cardinal

nests in Amur honeysuckle fledged 20% fewer young over the course of the year (Rodewald et al. 2010). In an urban landscape, American robin and northern cardinal nests in Amur honeysuckle were twice as vulnerable to depredation as nests in native vegetation (Borgmann and Rodewald 2004). Artificial nest experiments revealed that 68% of predation events could be attributed to mammals (Borgmann and Rodewald 2004). In riparian forests in Ohio, nests of the Acadian flycatcher (*Empidonax virescens*) were more likely to be parasitized by brown-headed cowbirds (*Molothrus ater*) when they were surrounded by more understory stems, which was attributed to the encroachment of Amur honeysuckle into urbanizing habitats (Rodewald 2009). Amur honeysuckle invasion often increases the number of woody stems in urban landscape forests (Borgmann and Rodewald 2005) which may increase the vulnerability of associated nests to brood parasitism (Rodewald 2009).

Amur honeysuckle's role as an ecological trap appears to have the potential to uncouple honest signals of individual quality and reproductive success (Rodewald et al. 2011). Bright coloration in male Northern cardinals can indicate good condition and high levels of parental investment (Linveill et al. 1998, Jones et al. 2010). Brighter males tend to breed earlier and nest in territories with highly preferred habitat, which will be patches of early-leafing Amur honeysuckle in invaded habitats (Rodewald et al. 2011). However, brightly colored males nesting in honeysuckle in rural landscapes produced fewer young over the breeding season than their dull counterparts, dissociating male color and reproductive success (Rodewald et al. 2011).

There is some evidence that this ecological trap effect may be ephemeral, and negative consequences of honeysuckle nesting preference may be restricted to a

particular time period (Rodewald et al. 2010). Cardinal nest survival rates in Amur honeysuckle improved from a low in the spring as the breeding season continued, eventually outpacing survival rates in native substrates (Rodewald et al. 2010). Resident and short-distance-migrant bird species like cardinals that nest in Amur honeysuckle early in the breeding season may be negatively affected, but long-distance migrant bird species arriving later in the breeding season may actually benefit from nesting in the invasive shrub (Rodewald et al. 2010).

Animal Interactions: Rodents and Mesopredators

Branch architecture of the shrub may restrict the movement of animals and alter predator-prey interactions in invaded sites. Invasive shrubs like *L. maackii* can provide a low-risk refuge for native consumers, altering foraging behavior (Mattos and Orrock 2010). Antipredator and foraging behavior of the ubiquitous white-footed mouse (*Peromyscus leucopus*) was altered by the presence of the invasive shrub. Mice preferentially foraged under Amur honeysuckle when habitat and abiotic conditions provided indirect cues for predation risk (Mattos and Orrock 2010, Dutra et al. 2011). While white-footed mice eat Amur honeysuckle berries, the cover provided rather than the fruit resource appears to be the cause of this shift in foraging behavior (Dutra et al. 2011). White footed mouse population dynamics can affect community dynamics like plant recruitment and the abundance of forest insects, so Amur honeysuckle effects on one population are capable of rippling through the community in countless, cryptic ways (Jones et al. 1998, Mattos and Orrock 2010). Mesopredator foraging behavior is also

influenced by the presence of the invasive shrub, and increasing honeysuckle coverage had a positive effect on the activity of two nocturnal mammals, raccoons (*Procyon lotor*) and opossums (*Didelphis virginana*) (Dutra et al. 2011). In this case, mesopredators may be responding to the higher abundance of prey items like mice and birds in honeysuckle thickets (Schmidt and Whelan 1999, Durtra et al. 2011). Modifications to mesopredator behavior could potentially disrupt entire ecosystems via alterations of native plant and animal communities (Dutra et al. 201).

Animal Interactions: Insects

The soil arthropod community forms the base of a forest food web that supports mammals and birds (Heneghan 2003), and alterations to this community may have significant effects on other species. Amur honeysuckle reduces habitat complexity in the ground-layer, which may negatively impact ground-dwelling-spider diversity (Buddle and Higgins 2004). Generalist predators, such as spiders, are important arthropod natural enemies (Buddle and Higgins 2004), and their loss may trigger top-down effects in the food chain. Food webs may also be affected by changes in the composition of litter on the forest floor. Litter dwelling arthropods play a key role in ecosystems, linking above-ground and below-ground food webs (Schmitz 2010), altering the chemistry and mass loss of decomposing leaf litter, and ultimately affecting rates of nutrient cycling (Seastedt and Crowwley 1984, Hunter 2003). Litter from invasive plants tends to be of high quality (elevated N, lower C:N and lignin:N), and plant invasion often increases litter decomposition rates in invaded sites (Liao et al. 2007). Accelerated litter decomposition

may diminish food sources early in the year, leading to a collapse of the litter arthropod community (Heneghan et al. 2002). Amur honeysuckle's presence did not affect arthropod diversity in a forest in Ohio but did significantly affect the taxonomic composition and abundance of the litter-dwelling arthropod community (Christopher and Cameron 2012). Specifically, honeysuckle had a negative impact on the abundance of Araneae, a macrofaunal predator, and a positive impact on Acari abundance. Pre-honeysuckle arthropod assemblages rapidly returned when the invasive was removed (Christopher and Cameron 2012).

Insect herbivores may also be negatively affected by the encroachment of Amur honeysuckle. The success of invasive species is often linked to their escape from natural enemies (Knight et al. 2007), and invaders may experience less grazing pressure than co-occuring native species. Amur honeysuckle experience less herbivory than most native woody species in one study (Trisel 1994). A feeding trial revealed that gypsy moth caterpillars (*Lymantria dispar*) will not voluntarily consume Amur honeysuckle; caterpillars fed honeysuckle had 100% mortality prior to molting (McEwan et al. 2009). In another set of feeding experiments, phenolic metabolites found in leaves of Amur honeysuckle tended to deter feeding by a generalist herbivore beet armyworm (*Spodopera exigua*), although growth rate was unaffected in no-choice bioassays (Cipollini et al. 2008). When palatable native species are replaced with less palatable invasive species, insect herbivores that cannot adjust may be negatively impacted.

Animal Interactions: Amphibians and Reptiles

The appearance of a dense layer of vegetation in a previously open forest can affect microclimate conditions like light, temperature, and humidity, potentially altering the structure and composition of native amphibian communities (Watling et al. 2011b). An examination of amphibian communities in a forest in Missouri revealed altered species composition and a negative relationship between Amur honeysuckle density and species richness and evenness (Watling et al. 2011b). A shift in species composition was tied to increased numbers of green frogs (*Lithobates clamitans*) in areas dense with Amur honeysuckle, potentially due to cooler ground temperatures beneath the thick shrub cover. Otherwise, the presence of the invasive was linked to a depleted and homogenized amphibian community (Watling et al. 2011b). Greater amphibian and reptile diversity were observed in non-invaded habitat relative to an invaded habitat in a remnant old-growth forest in Ohio in the fall, but species richness and abundance did not differ (McEvoy and Durtsche 2004). Snakes were only found in habitat with Amur honeysuckle, perhaps in response to increased shelter and opportunities to ambush prey. Box turtles (*Terrapene carolina*) were only observed in non-invaded habitats. Although frogs and salamanders were found in both habitat types, some species (*Rana clamitans* and *Plethodon glutinosus*) had significantly lower body masses in sites with Amur honeysuckle (McEvoy and Durtsche 2004).

Invasive species like Amur honeysuckle often produce allelopathic compounds and can alter habitat quality via inputs of novel chemicals (Hierro and Callaway 2003, Watling et al. 2011c). Amur honeysuckle produces water-soluble phenolic compounds in its roots and leaves (Dorning and Cipollini 2006, Cipollini et al. 2008). The addition of these chemicals to aquatic environments can impact the amphibian community, as larvae

are susceptible to changes in water chemistry (Watling et al. 2011c). Lab experiments have shown a negative effect of Amur honeysuckle on some amphibian species. When reared in extracts of Amur honeysuckle, survival of American toad (*Anaxyrus americanus*) tadpoles was reduced compared to tadpoles raised in native plant extracts. However, three other amphibian species, spotted salamander (*Ambystoma maculatum*), grey tree frog (*Hyla chrysocelis/versicolor*) and green frog (*Lithobates blairi*), were unaffected. Behavior of larvae suggested that phenolics in Amur honeysuckle extracts decreased respiratory ability, such that American toad and spotted salamander tadpoles surfaced more often. The authors of the study concluded that spotted salamanders were better able to behaviorally counteract negative effects of the exotic leachate (Watling et al. 2011a). Field experiments paint a different picture of amphibian responses to Amur honeysuckle phenolics. A field study found accelerated metamorphosis by American toad larvae in pools inoculated with soil and leaf litter from dense Amur honeysuckle stands (Watling et al. 2011c). Artificial pools inoculated with leaf litter and soils from heavily invaded sites had a lower concentration of total phenolics relative to pools inoculated with native leaf litter and soils, suggesting that Amur honeysuckle may not be toxic because of total phenolic concentration but rather novel phenolic coumpounds (Watling et al. 2011c).

Plant Interactions: Impact on Biodiversity

One of the most widely recognized and visible effects of invasive species is their impact on biodiversity, whether through the suppression of a single native species or via

wholesale changes in the communities they invade. Amur honeysuckle's rapid growth and retention of foliage late into the fall and winter and tendency to leaf out earlier in the spring give it significant advantages over native plants that become dormant earlier in the fall. Shade-intolerant and early-season herbs may be the most negatively impacted by early-leafing invasive shrubs (Gould and Gorchov 2000). A comparison of three forest annuals showed that Amur honeysuckle effects were more negative for less shade tolerant species with earlier phonologies (Gould and Gorchov 2000). Three perennial herbs experienced reduced growth and reproduction in the presence of Amur honeysuckle (Miller and Gorchov 2004). Mortality was unaffected, indicating that perennial herbs may be less sensitive to competition compared to annuals and tree seedlings (Miller and Gorchov 2004). Hutchinson and Vankat (1997) showed an inverse relationship between Amur honeysuckle cover and tree seedling density, species richness of seedlings, and herb cover in forest stands in southwest Ohio. Honeysuckle basal area was linked to low species richness and densities of the native shrubs spicebush (*Lindera benzoin*) and viburnum (*Viburnum prunifolium*) and sugar maple (*Acer saccharum*) saplings in another old-regrowth forest in southwest Ohio (Medley 1997). The seed and bud bank was also depleted under honeysuckle shrubs, indicating that herb layer populations may require years to recover from an invasion (Collier and Vankat 2002). Collier and Vankat (2002) found lower species richness and abundance in plots located under Amur honeysuckle; mean species richness for all species was 53% lower and mean cover across sampling dates was 63% lower in these plots.

The extensive, shallow root system of Amur honeysuckle may reduce the availability of nutrients and water in the upper soil through competition (Collier and

Vankat 2002). However, a field experiment suggested that above-ground competition for light (via stems) appeared to be mainly responsible for reduced tree seedling density in invaded forests (Gorchov and Trisel 2003). The removal of Amur honeysuckle shoots in field experiments enhanced the survival of several native tree seedlings, including sugar maple and white ash (*Fraxinus Americana*), while root removal (via trenching) had little effect. Amur honeysuckle shoots appeared to have afforded tree seedlings some protection from deer browsing but not enough to offset increased mortality due to Amur honeysuckle cover (Gorchov and Trisel 2003).

Plant Interactions: Seed predation and pollinator services

Invasive shrubs may also suppress native plant reproduction through other means. Shrub cover, whether native or invasive, can create areas of intense seed predation, as seed predators tend to forage on a broad range of available seeds and will selectively forage in areas providing more food resources (McCormick and Meiners 2000, Meiners 2007)). This effect may be more pronounced when invasions occur in forests systems lacking a well-developed shrub layer (McCormick and Meiners 2000). A study by Meiners (2007) found that tree seeds under Amur honeysuckle and multiflora rose canopies experienced significantly greater risks of seed predation. The author concluded that the combined effects of tree-shrub competition and reduced seed regeneration may have acted together to reduce tree regeneration (Meiners 2007). Amur honeysuckle may benefit from increased foraging behavior by rodents; forming a refuge for a seed predator encourages the consumption of native-plant seeds in the immediate vicinity. This

depletion of the native seed bank within Amur honeysuckle stands can release the invasive's seeds from resource competition (Orrock et al. 2010, Mattos and Orrock 2010).

Invasive plants can also indirectly disrupt native plant reproduction via alteration of pollinator services. The floral resources provided by an invader can alter the frequency of visits to native plants, the quality of pollen delivered to native plants, or the sequence of pollinator visits (*in* McKinney and Goodell 2010). Wild geranium (*Geranium maculatum*) grown in deciduous forest plots with Amur honeysuckle received less light, fewer pollinator visits, and less conspecific pollen deposition than flowers grown in plots where honeysuckle had been removed. Geranium's fruit and seed set were also reduced in Amur honeysuckle plots, likely due to reduced pollinator services. The mechanism driving this interaction was increased understory shade rather than increased competition for pollinators. Flowers may have been less visible in lower light or obscured because of the invasive shrub (McKinney and Goodell 2010).

Plant Interactions: Allelopathy

As mentioned previously, Amur honeysuckle produces secondary chemicals that may interfere with the growth of native plants. In invaded habitats, the production of these secondary compounds may then facilitate the acquisition of habitat space and the associated resources (Hierro and Callaway 2003). In a laboratory study, aqueous leaf and root extracts of Amur honeysuckle were found to greatly inhibit the germination of three native and non-native forbs, even at very low concentrations, suggesting the potential for

inhibited germination and/or growth of competing plants in the field (Dorning and Cipollini 2006). Root and leaf extracts of Amur honeysuckle applied to soil suppressed the germination of jewelweed (*Impatiens capensis*) and the growth and reproduction of arabidopsis (*Arabidopsis thaliana*) (Cipollini et al. 2008a). However, field applications of activated carbon failed to improve the performance of jewelweed in Amur honeysuckle plots (Cipollini and McClain 2008), suggesting that further work is necessary to translate findings from the lab into a meaningful understanding of potential allelopathic effects in the field.

Leaf extracts contain two major flavones, apigenin and luteolin, their glucoside derivatives, and chlorogenic acid, though leaves sampled at different times contained different amounts of these compounds (Cipollini et al. 2008b). Generalist feeding experiments identified apigenin and chlorogenic acid as likely candidates for allelopathic activity, and apigen, but not luteolin, inhibited seed germination of arabidopsis in subsequent testing (Cipollini et al. 2008b). Amur honeysuckle fruit also appears to have germination-inhibiting compounds. Berry extracts reduced total germination of two grasses and two forbs (McEwan et al. 2009). While none of the major phenolic compounds produced by Amur honeysuckle are unique to North American flora, the high density, leaf area index in favorable light environments, and long leafing season of the invasive shrub may increase the input of these compounds into invaded habitats (Cipollini et al. 2008b). Cautious interpretations of these findings seem warranted. A study by McEwan et al. (2009) found that responses of forbs and grasses to Amur honeysuckle extracts were species-specific, tissue-specific, and not always different from

effects mediated by co-occurring native species, highlighting the complex nature of allepoathic effects and the need for further investigation.

Plant Interactions: Succession

Long-term trends in productivity may be negatively affected by the ability of a forest to regenerate in the face of invading shrubs. The introduction of a dense invasive shrub layer has the potential to act as an ecological filter, effectively altering the direction of canopy tree regeneration (Gorchov and Trisel 2003). As noted in the previous section, Amur honeysuckle has been shown to reduce the survival and growth of tree seedlings. In particular, native species that expand their leaves early and depend on early season photosynthesis to remain competitive may be particularly vulnerable when invading shrubs move in (Gorchov and Trisel 2003). When suppressed seedlings represent important canopy trees, competitive effects could have a pronounced effect on successional patterns (Gorchov and Trisel 2003) and long-term trends in forest structure and NPP. The richness and density of tree seedlings decreased in forests with longer residence time of amur honeysuckle, with 100% of individual tree taxa in lower abundance below the shrub. With seed and bud banks severely depleted as well, the authors speculate that honeysuckle could alter forest succession to such a degree that some closed-canopy forests may convert to open-canopy woodlands or honeysuckle-dominated shrublands (Collier et al. 2002). An examination of successional trends along a chronosequence in Ohio found a well-established two-tiered forest system comprising overstory trees and Amur honeysuckle in long-invaded forests (Hartman and McCarthy

2008) similar to the findings of Collier et al. (2002). Long term invasion was tied to reduced herb, seedling, and sapling layer density and species richness, accompanied by a decrease in the species richness of the seed bank. Overall, invaded sites had reduced species richness, within-stratum species composition, and between-strata compositional similarity compared to non-invaded sites, suggesting a shift in species composition, structure, and successional trajectory following Amur honeysuckle invasion (Hartman and McCarthy 2008).

Ecosystem Impact

Ecosystem Impact: Productivity

Net primary productivity (NPP), the net carbon gain by vegetation, may be affected in forest systems invaded by monotypic stands of exotic shrubs. As discussed throughout, the invaders' efficient resource use coupled with biomass allocation that minimizes resource limitations could serve to maximize NPP in a system. Forest-grown populations of Amur honeysuckle had a significantly higher share of aboveground biomass in stems and a significantly lower share in leaves than open-grown populations. Mean total aboveground NPP estimates of forest-grown Amur honeysuckle populations fell within 159.1-553.3 $g \cdot m^{-2} \cdot year^{-1}$, with NPP per shrub increasing with age (Luken 1988). Aboveground NPP in temperate forests approximates 950 $g \cdot m^{-2} \cdot year^{-1}$ (Saugier et al. 2001 *in* Chapin et al. 2002), indicating that such an invasion would constitute a major component of primary production in sites where it dominates (Luken 1988). But the

degree to which dense stands of invasive shrubs would reduce primary productivity would depend on the losses of productivity in the understory following invasions.

While the impact of invasive shrubs on native herbs, shrubs, and tree seedlings is easy to understand, evaluating the impact on overstory trees is more difficult. Dendrochronological techniques have been used to evaluate the impact of Amur honeysuckle on the productivity of overstory trees in hardwood forests (Hartman et al. 2007). This approach detected a pattern of sustained, reduced tree growth following the invasion of Amur honeysuckle, with 41% of the trees in invaded stands growing more slowly. Thus Amur honeysuckle could impact both long-term inter-individual and interspecific performance, resulting in alterations in long-term canopy accession, species composition, and successional trajectories (Grime 2001, Hartman et al. 2007). Additional analyses revealed that the first significant growth reductions occurred approximately 6.25 ± 1.24 years after invasion with the greatest frequency of growth reduction occurring 20 years after stand invasion (Hartman et al. 2007). This finding underscores the need for management efforts to remove invading shrubs before long-term impacts begin.

Future Research Direction and Dissertation Objectives

A rich body of Amur honeysuckle research has emerged in the past 20 years, exploring the population, community, and ecosystem ecology of this aggressive invader. However, gaps remain in our understanding of Amur honeysuckle's effect on the soil microbial community, soil physicochemical processes, nutrient cycling, and litter

decomposition. Other invasive shrubs like Japanese barberry and common buckthorn have been shown to alter soil conditions, nutrient cycling, decomposition processes, and the soil microbial community in invaded sites (Kourtev et al. 1998, Kourtev et al. 1999, Ehrenfeld et al. 2001, Heneghan et al. 2002, Kourtev et al. 2002, Heneghan et al. 2004). There is evidence that Amur honeysuckle has rapidly decomposing litter, but this dynamic has not been fully explored. Single- and mixed-species decomposition studies in combination with field observations may help to elucidate the impact of invasive shrubs on nutrient cycling in invaded sites. Examinations of litter decomposing organisms could also shed light on decomposition dynamics. Other invasive shrubs have had dramatic effects on the soils beneath their canopy, and explorations of Amur honeysuckle effects are warranted. Inputs of litter and alterations to the soil microclimate may also affect the soil microbial community in the understory of Amur honeysuckle, which could in turn impact nutrient cycling. Evidence is emerging to suggest that invasive shrubs may facilitate their own spread via positive feedback loops in the soil (Ehrenfeld 2003). A better understanding of Amur honeysuckle effects on nutrient cycling, decomposition, and the soil microbial community is necessary to fully recognize the impact invaders may have on forest systems Also, the branch architecture and leaf surface area of invading shrubs may allow them to more successfully capture throughfall than co-occuring native species. Water conducted to the invasive shrubs via throughfall may carry high concentrations of nutrients, and no studies to date have examined the impacts of a invasive shrub on throughfall nitrogen deposition.

Finally, it is becoming increasingly clear that the impact of invasive species is often not uniform, but rather depends on the particular ecological context in which the

invasion occurs (Ehrenfeld, 2001). The scope of the invasive plant threat has accordingly become more difficult to ascertain, given mounting evidence within the invasion biology literature that individual species often have variable effects in different environments. Previous work has focused on differences between sites and how differences in factors like climate or soil characteristics can account for the variable effects of an invader. However, little research has examined species-specific interactions within a site. Invasive plants may have variable effects within a given environment depending on its interactions with the dominant native species, adding another layer of complexity to our understanding of invasive species impacts.

The objective of this dissertation work was to examine the impact of the invasive shrub *Lonicera maackii* on soil physicochemical processes, nutrient cycling, throughfall inorganic nitrogen deposition, decomposition dynamics, and the decomposer community structure under the three dominant tree species, *Fraxinus quadrangulata, Quercus muehlenbergii,* and *Carya ovata,* in a savanna in central Kentucky.

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Chapter Two

The impact of the invasive shrub *Lonicera maackii* on the decomposition dynamics of a native plant community

Introduction

Invasive species are significant drivers of global environmental change, in part through their impact on the stability and functioning of natural ecosystems (Vitousek et al. 1997). Exotic plant species have successfully invaded numerous ecosystems and have been shown to greatly alter ecosystem processes like nutrient cycling, decomposition, and disturbance regimes (Vitousek et al. 1987, Mack et al. 2001, Ehrenfeld 2003, Ashton et al. 2005, Knight et al. 2007). Despite the ability of invading plant species to alter ecosystem processes, their impact is not necessarily uniform. Rather, the impact of an invader can depend on the ecological context in which the invasion occurs (Ehrenfeld 2001). Mounting evidence within the invasion biology literature indicates that individual species may have variable effects in different environments (see review in Liao et al. 2007). This variability has been attributed to differences between sites, climate, soil characteristics, and the native plant community. Invasive plants may have variable effects within a given environment depending on their interactions with the dominant native species, adding another layer of complexity to our understanding of invasive species impacts. However, little research has examined species-specific interactions between native and invasive species within a site.

Exotic shrubs have successfully invaded numerous habitats throughout North America, and their ability to alter ecosystems may have far-ranging consequences for ecosystem processes like decomposition and nutrient cycling in invaded systems. The

nonindigenous shrub *Lonicera maackii* (L). is a pervasive invader throughout much of the eastern United States and Ontario, Canada (Luken and Thieret 1997). Its multi-stemmed growth form allows it to rapidly produce dense, persistent populations in a wide range of climatic and edaphic conditions (Luken 1988). Compared to native species, it has a longer photosynthetic season, expanding its leaves earlier in the spring and maintaining them well into the winter months, and is highly tolerant of freezing temperatures (Trisel and Gorchov 1994, McEwan et al. 2009b). It also spreads efficiently by way of its prolific berry production (Bartuszevige and Gorchov 2006). Finally, the community impacts of *L. maackii* have been well documented. Establishment of *L. maackii* reduces the density and richness of herbs and tree seedlings, the reproductive success of native herbs, and the growth of native shrubs and trees (Hutchinson and Vankat 1997, Collier et al. 2002, Miller and Gorchov 2004). The presence of the invasive has also been linked to negative impacts on songbird nest success (Schmidt and Whelan 1999), ground dwelling spider diversity (Buddle and Higgins 2004), and native forest amphibian communities (Watling et al. 2011a,b). It shares these traits with other aggressive, exotic shrubs like *Berberis thunbergii* (Japanese barberry), *Euonymus alatus* (winged euonymus), *Elaeagnus umbellate* (autumn olive), *Ligustrum sinense* (Chinese privet), *Rhamnus cathartica* (common buckthorn), and *Rosa multiflora* (multiflora rose) (Harrington et al. 1989b, Ehrenfeld 1999, Silander and Klepeis 1999, Edgin and Ebinger 2001, Swearingen et al. 2002, Miller 2003, Mascaro and Schnitzer 2007).

Few studies have examined the impact of these exotic shrubs on ecosystem processes, but *B. thunbergii* and *R. cathartica* have been shown to alter soil properties, nitrogen cycling, decomposition, and the structure and function of the soil microbial

community (Ehrenfeld et al. 2001, Heneghan et al. 2002, Kourtev et al. 2002, Heneghan et al. 2004) and there is evidence for rapid decomposition of *L. maackii* (Blair and Stowasser 2009). The recycling of carbon and nutrients during decomposition and subsequent impacts on nutrient availability and the decomposer community can influence plant growth (*in* Hattenschwiler et al. 2005), and invasive plants may co-opt these ecosystem processes in a manner that positively supports their own growth (Vitousek et al. 1987, Ehrenfeld et al. 2001).

Decomposition rates and concurrent nutrient losses are influenced by macro- and microclimate (Hobbie 1996, Aerts 1997), litter chemistry (Aber and Melillo 1980), and the decomposer community (Swift et al. 1979). Decomposition dynamics can be modified directly by invasive plant species via alterations of litter quality, microclimate, and/or the decomposer community. Invasive species often have higher litter N concentrations and lower C:N and tend to decompose more rapidly relative to native species (Vitousek and Walker 1989, Witkowski 1991, Ehrenfeld 2003, Allison and Vitousek 2004, Rothstein et al 2004, Ashton et al. 2005). Invasive plant species with higher rates of decomposition coupled with higher concentrations of leaf N can be expected to release nitrogen into the soil at a more rapid rate than native plants (Ehrenfeld et al. 2001). Invasive plants can also create microclimates that facilitate decomposition processes by increasing soil temperature and moisture (Yelenik et al. 2004). Changes to the chemical properties of the soil have also been observed beneath exotic plants (Ehrenfeld et al. 2001). These changes in resource quality and microclimate can then alter the composition, abundance, and activity of the decomposer community (Blair et al. 1990, Yeates and Williams 2001, Kourtev et al. 2002, Ravit et al. 2003),

allowing invasive species to affect decomposition indirectly via alterations to the soil biota. The increasing spread of invasive species thus has the potential to greatly alter ecosystem processes, including the influence that native species have on decomposition and the decomposer community.

The mixing of native and invasive litter may produce highly variable species-species interactions within invaded sites, and single-species dynamics often do not reliably predict decomposition patterns of mixed-species litter (see review in Gartner and Cardon 2004). Sixty-seven percent of all mixtures tested in litter decomposition experiments exhibited non-additive mass loss, with synergistic responses far outweighing antagonistic responses (Gartner and Cardon 2004). A comprehensive mixed-litter decomposition study found that mixing litters with high N concentrations often enhanced the decay rate (Wardle et al. 1997). An invasive plant with higher quality, N-rich litter could have a synergistic effect on the mass loss of native litter. In a litter mixture of invasive and native species, decomposer organisms may preferentially exploit a higher quality invasive litter, allowing nutrient transfer to the lower quality native litter, leading to a more rapid, synergistic decomposition of the entire mixture (Hattenschwiler et al. 2005). Alternatively, the novel secondary compounds often found in invasive species' litter may inhibit microbial activity and slow decomposition processes in an antagonistic manner (Ehrenfeld 2003, Rodgers et al. 2008). Several phenolic compounds found in the leaves of *L. maackii* have been shown to affect other plants and herbivorous insects (Cipollini et al. 2008b) and may influence the soil microbial community as well.

We used a savanna ecosystem in central Kentucky to examine the impact of an invasive shrub species on the leaf litter decomposition dynamics of a group of three

native tree species. Savanna trees with non-overlapping canopies offer a unique opportunity to assess changes under the canopy of individual trees that occur as a result of interactions between an invasive species and the native tree species. The three dominant, native tree species at the site were *Fraxinus quadrangulata* (Mill.) K. Koch (blue ash), *Quercus muehlenbergii* E. (chinkapin oak), and *Carya ovata* M. (shagbark hickory). Native species can be expected to have a strong influence on decomposition processes via differences in litter quality and the unique micro-environment created by single species (Hobbie 1992). Examining species-species interactions between native and invasive species will be enhanced by the incorporation of different native taxa. The genera represented by these three tree species represent a range of litter chemistry traits (Melillo et al. 1982, Li et al. 2009), allowing us to observe variable species-species interactions among a pool of native species that vary in initial resource quality and decomposition rates. The varied canopy structure among these three species (Wharton and Barbour 1973) may also create unique micro-environmental conditions that could affect the impact of *L. maackii* on decomposition dynamics. These genera occur widely throughout the midwestern and eastern United States (Wharton and Barbour 1973) in systems that are experiencing invasion by exotic plant species (Pimentel et al. 2005). More generally, oak-hickory forests have the largest range of any of the Eastern Deciduous Forest communities (Kricher 1998), and one or more of the members of each genus dominate some portion of the forest of every state east of the 100th meridian (Barrett 1995). Patterns observed at our site may apply to other taxonomically similar ecosystems.

We tested the hypothesis that *Lonicera maackii* litter would decompose more rapidly than litter from the native tree species. Because *L. maackii* litter has been observed to break down very rapidly in other ecosystems (Blair and Stowasser 2009), we predicted that it would have a high litter N content and a more favorable C:N ratio than the native litters. We further hypothesized that mixing the rapidly decomposing litter of *L. maackii* with the litter of native tree species would synergistically accelerate the decomposition of the native species, beyond the predicted additive decomposition rate, due to the postulated higher quality of *L. maackii* litter. We anticipated variation in initial litter quality among the native tree species. Accordingly, we hypothesized that the synergistic decomposition dynamics postulated for all the litter mixtures (native + invasive) would be especially strong when the presumably less recalcitrant *Fraxinus* litter was mixed with *L. maackii*. We also expected that the rapidly decomposing *L. maackii* litter would shift from N immobilization to N release earlier than the native species, and that mixing *L. maackii* with the native species would lead to more rapid release of N than predicted from additive mixing effects. Finally, to link a possible causal mechanism to observations of an alteration of decomposition dynamics, we tested the hypothesis that a greater diversity and abundance of microarthropods would be evident in litter bags containing a mixture of native tree and *L. maackii* litter.

Methods

Site Description

A litter decomposition experiment was conducted at Griffith Woods, a savanna-woodland in Harrison County, Kentucky. The savanna encompasses an approximately

80 acre area within Griffith Woods, a site that was historically used for pasture until 2005, when it was purchased by the University of Kentucky and The Nature Conservancy. A discussion of the history and origin of oak savannas in central Kentucky can be found in McEwan and McCarthy (2008). The soils at the site are predominantly moderately- to well-drained silt loams: Lowell, Faywood-Lowell, and Nicholson. These soils are classified as fine, mixed, active, mesic Typic Hapludalfs (Lowell and Faywood-Lowell) and fine-silty, mixed, active, mesic Oxyaquic Fragiudalfs (Nicholson). The savanna landscape consists of 150- to 300-year old trees surrounded by a matrix of grasses and forbs, with tall fescue (*Lolium arundinaceum*) dominating the grass matrix. The site has recently come under increased pressure from invasion by exotic plants. In particular, *L. maackii* has invaded the site and is present in the understory of many of the savanna trees, providing an opportunity to evaluate species-species interactions between *L. maackii* and the dominant native tree species in this system.

Experimental Design

Naturally senesced leaves of three tree species, *F. quadrangulata*, *Q. muehlenbergii*, and *C. ovata*, and one woody invasive shrub species, *L. maackii*, were collected bi-weekly from the understories of individual trees and shrubs. Litter was collected across the site between October and December, 2006. Litter nets were constructed from bird netting and elevated off the ground to catch litter from beneath approximately 10 members of each species. The litter of each species was pooled, mixed, air-dried, and stored at room temperature until the litter bags were constructed.

Fiberglass litter bags were constructed with a mesh size of 1 mm^2 and interior dimensions of 10 x 10 cm. Litter bags were divided into three treatments: single-species bags of *L. maackii*, single-species bags of a native tree species, and a mixed-species bag containing *L. maackii* and the native tree litter. Single species bags were filled with 10 grams of a single tree litter or *L. maackii* litter. Mixed species bags were filled with 5 grams each of the following combinations of native and exotic litter: *F. quadrangulata-L. maackii, Q. muehlenbergii-L. maackii,* or *C. ovata-L. maackii*. Litter was gently mixed before being placed in the bag. Subsamples of each litter were oven dried at 60°C for air-dry to oven-dry conversions, ground, and analyzed for initial lignin, carbon (C), and nitrogen (N) concentrations.

Litter bags were placed under five trees of each species for a total of 15 trees. To enter the pool of trees for possible selection, trees had to have an area approximately 1 m from the bole of the tree facing northwest that was large enough to accommodate the litter bags. The area had to have tree canopy cover over the plot without having any *L. maackii* canopy within three meters from the edge of the space that would be suitable for litterbags. The five trees of each species were randomly selected from this pool of candidate trees. Individual tree and mixed-species bags (tree species- *L. maackii)* were only placed in plots that corresponded with the overstory tree (e.g. *F. quadrangulata* litter under *F. quadrangulata* trees). In total, 18 bags were placed in the plots beneath each of the trees: 6 bags containing tree litter specific to the overstory species, 6 bags containing *L. maackii* litter, and 6 bags with a 1:1 mix of the two litters. To parse out the effects of litter and microclimate on decomposition dynamics, five plots were also established in the open savanna. Plots in open locations were located at least 10 meters from the

dripline of any tree and contained all of the individual-tree (n = 18) and mixed-species (n = 18) bag combinations in addition to the *L. maackii* (n = 6) bags for a total of 42 bags. Bags within each treatment were assigned at random to plots and to locations within the plots beneath each tree and in the open. Any plant material in the plots was clipped to the ground and removed so that litterbags could be affixed directly to the soil surface. Litterbags were placed in the field in late March, 2007.

One litterbag of each litter type was collected from under each tree and in the open plots at 0, 6, 16, 32, 56, and 104 weeks. Litter was dried in a 60°C oven and weighed to determine mass loss. Each bag of litter was then ground and analyzed for C and N using a Leco CN 2000 analyzer. Subsamples of the ground litter were analyzed for percent ash-free dry mass for correction of litter mass data. Immediately after bag retrieval, microarthropods were extracted from each of the bags collected in week 6 using Tullgren funnels. The mesh size in the bottom of the collection bowl matched the size of the litterbag mesh to minimize the loss of fine litter. Approximately 0.01-0.04 g of fine litter was lost through the mesh when litter was placed in the Tullgren funnels. Light was applied to the samples for 76 hours, and specimens were stored in ethanol-filled scintillation vials for later identification. Microarthropods from the six-week samples were sorted to Class, Order, and, when possible, family-level (Triplehorn and Johnson 2005). Taxa included: Arachnida (Orbitida and others), Chilopoda, Diplopoda, Hexopoda (Coleoptera, Collembola [Smithuridae and others], and Hymenoptera [Formicidae and others]), and Malacostraca (Isopoda).

Light was measured directly over decomposition plots using a line-integrating quantum sensor (LI-191SA, Li-cor) in July, 2008. Measurements were taken under clear

skies ± 1hour of solar noon over the course of two days. Soil was sampled under each tree to a depth of 12 cm in May, July, and October, 2007 and again in May, 2008. Samples were returned to the laboratory for analysis of moisture content using standard gravimetric techniques, pH (1:1 slurry in distilled deionized water), and KCl-extractable NO_3^- and NH_4^+ (determined colorimetrically with a Bran-Luebbe Autoanalyzer 3). Additional sampling in January, 2009 measured soil organic matter (SOM%), via loss on ignition after 8 hrs at 500°C (analyzed at the University of Kentucky Regulatory Services, Lexington, KY, USA).

Statistical Analyses

Differences in initial litter lignin, N, and C concentrations were tested across species (4 litter types, fixed) using a one-way ANOVA in Proc MIXED (SAS 9.2 Institute, Cary, North Carolina, USA). Changes in litter mass and N content were compared across litter types, plot location (tree species/open), and time using a repeated measure (tree = repeated unit) mixed model ANOVA in Proc MIXED. Decomposition (percent mass remaining) and litter N content (percent N remaining) in the open plots were compared across litter type (seven levels: all single- and mixed-species litters, fixed), collection (6 pickup times, fixed), and replication (random). To examine species-species interactions, data for the three individual native tree litters were separately compared across litter type (two levels: single or mixed, fixed), location (two levels: under the tree or in the open, fixed), collection (6 pickup dates, fixed), and replication (random). Finally, data for *L. maackii* were compared separately for location (four levels: under *F. quadrangulata, Q. muehlenbergii,* or *C. ovata* or in the open, fixed),

collection (6 pickup dates, fixed), and replication (random). The abundance of litter microarthropods at 6 weeks was analyzed using the same techniques, but without time as a factor. Litter microarthropod data were log-transformed for analysis. Decay curves were created using nonlinear models. Proc NLIN from SAS was used to generate k values using a single negative exponential decay function (Olson 1963). Means generated by Proc MIXED and NLIN were compared using the LSMEANS procedure.

Predicted litter decomposition (% mass remaining) and litter N (% N remaining) for mixed-species litterbags were calculated using data from single-species litterbags. For example, mean predicted percent mass remaining for the *F. quadrangulata-L. maackii* mixed litter at 6 weeks was calculated as:

$$(F1 + L1)/2,$$

where F1 = percent mass of *F. quadrantulata* litter remaining at 6 weeks and L1 = percent mass of *L. maackii* litter remaining at 6 weeks (Blair 1990). Predicted values for litter N content were calculated in the same way. Predicted and observed percent litter and N remaining were compared using paired *t* tests over all collection dates (n = 6) in Proc TTest (SAS Institute, Cary, North Carolina).

Results

Litter Quality and Decomposition

The invasive *L. maackii* had the highest initial litter N concentration (P = <0.0001), the lowest initial C:N ratio (P = <0.0001), and a lower initial lignin content (P = <0.0001) compared to all of the native species except *F. quadrangulata* (Table 1). As expected based on initial litter N and lignin content, *L. maackii* also decomposed more

rapidly than any of the other species (Table 1). Of the tree species, *F. quadrangulata* and *C. ovata* had the highest initial litter N concentrations and the lowest initial C:N ratios, while *Q. muehlenbergii* and *C. ovata* had the highest initial lignin concentrations (Table 1). The invasive shrub's litter was almost completely decomposed by the 1-year collection period, whereas the tree litters all had mass remaining at the 2-year collection point (Figure 1a). Decay rate constants calculated from the single negative exponential decay model (Table 1) showed that the tree species litters decomposed from fastest to slowest: *F. quadrangulata* > *C. ovata* > *Q. muehlenbergii*.

The exotic shrub's decomposition rate was affected by the location in which its litter was placed (Table 2, $F = 5.3$, $P = 0.002$); *L. maackii* litter decomposed more rapidly under the canopy of *C. ovata* trees than it did under the other tree species or in the open savanna (Figure 2a). The decomposition rate of the single-species tree litters did not differ by location (under tree vs open), with all species breaking down at approximately the same rate (Table 1) under their respective tree canopies as they did in the open savanna (Table 1, $P > 0.05$ for all comparisons).

The litter nitrogen dynamics of single-species litter followed a similar pattern shown by decomposition rate. The invasive *L. maackii* lost N more rapidly than any of the other species, with no period of N immobilization captured between the initiation of the study and the first litter pick-up at 6 weeks (Figure 1b). All three tree species immobilized N early on, with *Q. muehlenbergii* showing N immobilization on the 6-week collection date and ultimately losing less N than any of the other species by the end of the incubation. The other two tree species immobilized N between the 6-week and 12-week collection dates only (Figure 1b). By 24 weeks, N was lost by all of the tree species

litter. Decay rate constants again showed tree species' N losses proceeding from fastest to slowest: *F. quadrangulata* > *C. ovata* > *Q. muehlenbergii* (Table 1).

The invasive *L. maackii* also lost N from its litter at a different rate based on its location during decomposition (Table 2, $F = 3.51$, $P = 0.02$). As with mass loss, *L. maackii* litter lost N more rapidly under the canopy of *C. ovata* trees than it did in any other location (Figure 2b). The location of the decomposing litter, under the respective tree canopy or in the open, did not significantly affect the loss of litter N from *F. quadrangulata* or *Q. muehlenbergii*. However, *C. ovata* litter did lose N more rapidly under its canopy than it did in the open ($F = 4.7$, $P = 0.05$).

Mixed-species litter bags followed the same general trends exhibited by single-species tree litter, with decay rates proceeding from fastest to slowest for both mass loss and litter N loss in the following order: *F. quadrangulata-L. maackii* > *C. ovata-L. maackii* > *Q. muehlenbergii-L. maackii* (Table 1). None of the mixed-species litter combinations decomposed more rapidly or lost more N under their respective tree canopies than they did in the open savanna (Table 1). Predicted decay rates, calculated from the component species mass remaining data, were not significantly different from observed litter mass and N loss for any of the species (Table 3). An examination of individual collection dates (n = 6) revealed no differences in mass loss between the expected and observed values during the decomposition process for any of the mixed-species bags, under the respective tree species (Figure 3a) or in the open, with one exception: *Q. muehlenbergii –L. maackii* litter in the open lost less mass than predicted at 1 year. However, mixed-species litter bags released N more rapidly than expected under their respective tree species. Nitrogen was lost more rapidly in *C. ovata-L. maackii* bags

than predicted at 6-weeks, in *F. quadrangulata-L. maackii* bags at 12-weeks, and in *Q. muehlenbergii-L. maackii* bags at 24-weeks (Figure 3b). These differences between observed and expected N loss were not present in mixed-species bags placed in the open, with one exception: *Q. muehlenbergii-L. maackii* bags lost more N than expected at 1 and 2 years.

Abiotic Conditions

Physical properties rarely differed in the soils beneath the canopies of the native tree species where the decomposition plots were located. The soils beneath *F. quadrangulata* trees were significantly more acidic (Table 4, $F = 5.28$, $P = 0.03$) than the soils of the other two species. Although the branching and canopy structure of the native trees species were visibly different, no significant differences in the light environment under the canopies was observed (Table 4, $F = 1.19$, $P = 0.34$). Soil moisture fluctuated throughout the growing season, but significant differences among the tree species were only evident in the summer, with lower soil moisture observed under *F. quadrangulata* compared to the other species (Table 4, $F = 7.43$, $P = .003$). Soil organic matter content and soil NO_3^- and NH_4^+ concentrations (Table 4) were not significantly different among tree species.

Biotic Community

Litter microarthropod abundances were highly variable, likely due to the small sample size in this study. Oribatei dominated the samples to such an extent that we were unable to quantitatively measure diversity. Given the small sample size, an ANOVA

yielding a $P < 0.1$ was considered significant. No differences in microarthropod abundance were observed among tree species' litter or among *L. maackii* litter located under the different tree species. Significant differences in abundance in single and mixed *F. quadrangulata* litter were observed among decomposition locations, with more litter microarthropods present in single- and mixed-litter bags under *F. quadrangulata* canopies than in the open (Table 5, $F = 12.1$, $P = 0.01$). The same trend was evident in single- and mixed *C. ovata* litter (Table 5, $F = 7.85$, $P = 0.04$). Location did not have an effect on microarthropod abundance in *Q. muehlenbergii* litter, but litter type did. Mixed species *Q. muehlenbergii-L.maackii* litter had higher numbers of arthropods than *Q. muehlenbergii* litter both under the canopy and in the open (Table 6, $F = 6.52$, P 0.06). Overall, *C. ovata-L. maackii* litter located under *C. ovata* canopies had the most litter microarthropods (Figure 4).

Discussion

Litter Quality and Decomposition

Our finding that leaf litter from the invasive *L. maackii* decomposed and released nitrogen more rapidly than the litter of the native tree species (Fig. 1) is in keeping with much of the invasive species literature (Ehrenfeld 2003, Rothstein et al. 2004, Ashton et al. 2005). A meta-analysis by Liao et al. (2008) revealed that on the whole, plant invasion increased litter decomposition rates by 117% in invaded ecosystems. They attributed this increase to elevated plant and litter N concentrations and a lower litter C:N and lignin:N ratio in the invaders compared to native species (Liao et al. 2008). This is consistent with the litter traits of *L. maackii* relative to the native litter in this study

(Table 1). Specifically, the decomposition dynamics of *L. maackii* closely mirrored those of other woody invasive shrubs in deciduous systems, with litter losing on average 97% of its initial mass and 96% of its initial N within the first year of the study. For example, in a New Jersey study, *B. thunbergii* decomposed rapidly, losing as much as 90% of its initial mass within a year; the N-rich litter also lost 83% of its N in the first year of the study, as compared to 50% N loss from the native litter (Ehrenfeld et al. 2001). Similarly, N-rich *R. cathartica* litter decomposed far more rapidly than the litter of native tree species in an Illinois woodland (Heneghan et al. 2002). Finally, in a deciduous forest in Long Island, a mixture of exotic woody species, including the N-rich exotic shrubs *Lonicera morrowii* and *R. multiflora,* decomposed and lost N more rapidly than native woody species (Ashton et al. 2005).

Our results also indicate that *L. maackii* has a variable effect on decomposition dynamics depending on its association with particular native species. Previous examinations of invasive species have focused on impacts on decomposition across entire sites; none to our knowledge has examined interactions between invaders and different native species within a site. Litter from *L. maackii* decomposed and released N more rapidly when it was located under the canopy of *C. ovata* rather than under the other two native tree species or in the open savanna (Figure 2). Litter decomposition dynamics are primarily driven by litter quality, climate factors like temperature and precipitation, and local microenvironmental factors like soil temperature and moisture regimes (Meentemeyer 1978, Hobbie 1996). With one exception, none of the soil physicochemical or microenvironmental effects varied significantly among the native tree species. Soil moisture was highest under *C. ovata* trees in July (Table 4, F =7.43, P =

0.003) of 2007. This corresponds with the time period within which *L. maackii* litter experienced accelerated mass and N loss under *C. ovata* trees (Figure 2).

Contrary to our predictions, mixing the litter of the invasive *L. maackii* and the native tree species did not result in any synergistic or antagonistic mass loss effects for any of the species combinations (Figure 3a). We expected that the high quality *L. maackii* litter would have a synergistic effect on the mass loss of native litters, especially when mixed with the native tree litter containing the highest litter N concentration, *F. quadrangulata.* The other two native tree species in our study, *Q. muehlenbergii* and *C. ovata,* are species with high litter lignin concentrations (Table 1). Inhibitory secondary compounds like tannins or phenolics released from recalcitrant *Quercus* litter (McArthur et al. 1994) and trace amounts of juglone found in *C. ovata* litter (Borazjani et al. 1985) may have slowed decay rates via suppression of decomposers. It is possible that the inhibitory aspects of these litters cancelled out any potentially synergistic effects of the N-rich *L. maackii* litter on decomposition. Microenvironmental factors did not appear to influence mixed litter dynamics as non-additive effects were also absent in the mixed litterbags in the open savanna.

Mixing the invasive *L. maackii* litter with the native tree species' litter did result in synergistic N losses at times that varied among the tree species (Figure 3b). Gartner and Cardon (2004) found non-additive effects on nutrient dynamics in 67% of studies reviewed, with both increases and decreases in litter nutrient content observed. Decreased immobilization and increased total N mineralization were found most often in litter mixtures of broad-leaved species (Blair et al. 1990, Gartner and Cardon 2004). Given the rapid N loss of *L. maackii* litter, none of our mixtures were expected to

immobilize N when predicted litter N loss was calculated; rather N loss was predicted to proceed more slowly between 6 weeks and 12 weeks (Figure 3b). Only *F. quadrangulata-L. maackii* litter lost N more rapidly than expected in this interval, perhaps due to the favorable mixing of two N-rich litters. The other two species mixtures lost N at a slower rate than expected, with the *Q. muehlenbergii-L. maackii* mixture immobilizing N between 6 and 12 weeks. However, following this period of immobilization, the *Q. muehlenbergii-L. maackii* mixture lost N at a faster rate than predicted, with significantly less litter N remaining at 24 weeks. In the *C. ovata-L. maackii* mixture, significantly less N remained in the litter than expected at 6 weeks. Analyses of component species from litter mixtures suggest that there is a transfer of nutrients between mixed litter types, with nutrient concentrations increasing in lower quality litter and decreasing in higher quality litter (Briones and Ineson 1996, McTiernan et al. 1997). Ultimately, mixing these two recalcitrant litters with *L. maackii* resulted in periods of synergistic N loss during the course of the experiment. Synergistic N losses of litter mixed with *L. maackii* were not observed in the open savanna, indicating that the microenvironment or soil decomposer community associated with the native tree species played a part in the variable decomposition dynamics. None of the physicochemical or environmental variables that we measured could readily account for seasonal differences observed in synergistic N loss among litter mixtures, suggesting some other underlying cause.

It is important to note that *L. maackii* leaves drop later in the season than most of the native species in its invaded range (McEwan et al. 2009, Wilfong et al. 2009), including the native tree species at our site. Mixed-litter decomposition studies do not

take into account inter- or intra-annual temporal differences in leaf drop among component species, and these differences may have an effect on decomposition rates. The native species' leaves typically fall to the ground prior to *L. maackii* leaf drop, potentially altering the microclimate into which the invasive's leaves will drop. Other woody invasive species like *B. thunbergii* and *R. cathartica* also retain their leaves late into the fall/early winter (Harrington et al. 1989a, Silander and Klepeis 1999) in comparison to native species. Conventional mixed litter experiments can still inform decomposition dynamics at invaded sites, but moving forward it may be necessary to examine these dynamics in the context of temporal differences in leaf drop and microclimate effects.

Biotic Community

Significant effects of litter mixtures on microarthropod abundance and diversity at 6 weeks were not readily apparent due to small sample sizes and the resultant high variability; however, trends did emerge (Figure 4). Litter mixtures containing *C. ovata* and *L. maackii* located in the understory of *C. ovata* trees tended to contain the most microarthropods at six weeks, which corresponded with a synergistic increase in N loss from the mixed litter under *C. ovata* trees. It is possible that the litter conditions at this point favored the colonization of *C. ovata-L. maackii* litter over the other single-species and mixed-species litters. Unknown microenvironmental conditions under *C. ovata* trees may have also played some role: microarthropod abundance on mixed *C. ovata-L. maackii* litter was significantly higher under *C. ovata* than in the open (Figure 4, F = 7.85, P = 0.04). In a woodland in Illinois, microarthropods colonized N-rich, invasive *R.*

cathartica litter more rapidly than native litter (Heneghan et al. 2002). We only observed a rapid colonization of *L. maackii* litter relative to the native tree litter under *Q. muehlenbergii* trees (Figure 4, $F = 6.52$, $P = 0.06$), perhaps due to the unpalatable nature of the *Q. muehlenbergii* litter. Though statistically weak, as is common in microarthropod data from field studies (Gartner and Cardon 2004), our results suggest that further investigation of the microarthropod community may be warranted, albeit with larger sample sizes.

It is possible that the soil microbial communities fostered by the tree microenvironments and litter played a role in observed differences in litter mass and N loss, as both the overstory native and the invasive litters could be expected to affect the soil microbiota. In contrast to the other two species, the understory of *C. ovata* is carpeted with coarse woody debris and nuts containing small, varying concentrations of juglone (Borazjani et al. 1985). The presence of juglone in the soils beneath *C. ovata* may influence the microbial populations and subsequent pool of decomposers. The presence of invasive *L. maackii* litter could also affect the microbial population. A review by Ehrenfeld (2003) found that invasive plants increased microbial biomass N in eight out of the ten studies reviewed. The invasive shrub *B. thunbergii* has been linked to alterations in the composition of the soil microbiota in a deciduous forest in New Jersey (Kourtev et al. 2002). Other studies have linked accelerated rates of litter decomposition with more complex faunal communities (Bradford et al. 2002) and it would be pertinent to include other groups of animal micro- and macrofauna in future examinations of the decomposer community. Future studies could incorporate measurements of nematode

populations, macrofauna like Annelids, bacteria, lengths of fungal hyphae in the litter, respiration, and/or microbial biomass.

Conclusion

Our results indicate that variable interactions between the invasive *L. maackii* and the dominant, native tree species at our site may have a subtle but potentially significant influence on decomposition dynamics. The invasive *L. maackii* litter lost mass and N more rapidly when it was located under *C. ovata* trees. Further, synergistic N loss was observed in *C. ovata-L. maackii* mixtures under *C. ovata* trees at 6 weeks in April. Like other woody invasive shrub species, *L. maackii* can leaf out as much as a month or more before native tree species (Harrington et al. 1989a, Trisel and Gorchov 1994, and Silander et al. 1999). At our site, *L. maackii* puts on leaves in May, before the overstory native trees (McEwan et al. 2009), and an extra pulse of N in the soil from decomposing litters could benefit a buildup of leaf mass and photosynthetic enzymes and associated compounds. In *B. thunbergii* stands in five sites within a New York preserve, barberry has two leaf flushes to acclimate to the dynamic light regimes in forest systems (Xu et al. 2007). The first flush of leaves displayed sun-leaf characteristics, with *B. thunbergii* showing a peak in photosynthetic capacity before upper canopy closure, effectively utilizing high spring irradiance to build a carbon subsidy. Upon canopy closure, a second flush of leaves developed on newly elongated branches and maintained shade-leaf characteristics, with low maintenance, throughout the remainder of the growing season (Xu et al. 2007). If *L. maackii* shrubs utilize spring conditions in this fashion, mixed *L. maackii* and native litter under *F. quadrangulata* and *Q. muehlenbergii* may provide

similar pulses of N to growing *L. maackii* shrubs, but at a less advantageous time. This could preferentially facilitate the growth and subsequent spread of *L. maackii* to a greater extent under *C. ovata* trees as compared to the other dominant, native species. The composition of mixed litter in the field will naturally differ from experimental mixed litter combinations, but these results can still reveal the potential effects of variable species interactions on decomposition dynamics (Blair et al. 1990).

To properly manage invasive species, it is necessary to understand the mechanisms through which a particular invasive plant species may alter an invaded ecosystem. If associations with native plant species within a site variably affect the impact an invader has on ecosystem processes, this might provide insights into management. Identifying native species or communities that are more vulnerable to alterations of ecosystem function upon invasion could provide managers with information that helps them better focus their efforts. To advance the study of invasive plant species as a whole, it would be useful to link common mechanisms through which invaders affect their own success. Though inconclusive, there is evidence that exotic plants may create positive feedback loops that facilitate their growth and general invasiveness (*in* Ehrenfeld 2003). To fully explore the potential for such positive feedbacks, it is germane to consider the spatial scale of ecosystem alterations and subsequent feedbacks. The overall impact of an invader may be masked if species-species interactions with native residents of a given environment are ignored. Invasive shrubs like *L. maackii* may be able to alter decomposition processes to increase nutrient availability to an extent that hinges on associations with native species. It will be useful to further explore the mechanisms through which different invaders may affect their own success and the scale at which they

do so. Linking the ecology of common invasive species and the mechanisms through which they impact ecosystem functions like decomposition is one way to more closely examine the response of ecosystems to invasion and the manner in which invasive plants may be able to facilitate their own success.

Table 1. Initial litter nitrogen concentration, C:N, lignin concentration, and decay rates for single- and mixed-species litterbags.

Litter type	Initial N (%)	Initial C:N	Initial lignin (%)	Mass Loss k (1/yr)	
				Under trees	In the open
Single-species					
F. quadrangulata	1.27 ± 0.02^{A}	36.6 ± 0.37^{A}	17.0 ± 0.44^{A}	2.01 ± 0.09^{A}	2.01 ± 0.09^{A}
Q. muehlenbergii	1.02 ± 0.04^{B}	48.8 ± 2.01^{B}	25.4 ± 1.84^{B}	0.85 ± 0.05^{B}	0.89 ± 0.05^{B}
C. ovata	1.25 ± 0.03^{A}	35.2 ± 0.65^{A}	27.4 ± 1.06^{B}	1.45 ± 0.09^{C}	1.35 ± 0.08^{C}
L. maackii	1.44 ± 0.01^{C}	30.0 ± 0.23^{C}	17.6 ± 0.91^{A}	3.65 ± 0.08^{D}	3.31 ± 0.19^{D}
Mixed-species					
F. quadrangulata-L. maackii	1.39 ± 0.02^{A}	32.6 ± 0.38^{A}		2.97 ± 0.31^{A}	2.55 ± 0.16^{A}
Q. muehlenbergii-L. maackii	1.22 ± 0.02^{B}	38.4 ± 0.63^{B}		1.82 ± 0.08^{B}	1.52 ± 0.11^{B}
C. ovata-L.maackii	1.34 ± 0.03^{A}	32.3 ± 0.48^{A}		2.49 ± 0.08^{C}	2.31 ± 0.05^{A}

Notes: Single- and mixed-species litter bags were placed in plots located under tree canopies (n = 5 trees of each species) and in the open savanna (n = 5 plots). Litterbags containing *L. maackii* were present in all of the under-tree plots. Decay rate for *L. maackii* is the average across all three species locations. All values are means ± 1 SE and letters indicate significant differences in initial litter quality and decay rates among single- and mixed-species litterbags.

Table 1, Cont.

Litter type	N Loss k (1/yr)	
	Under trees	In the open
Single-species		
F. quadrangulata	1.34 ± 0.08[A]	1.47 ± 0.11[A]
Q. muehlenbergii	0.42 ± 0.08[B]	0.40 ± 0.07[B]
C. ovata	0.76 ± 0.09[C]	0.62 ± 0.06[C]
L. maackii	2.88 ± 0.10[D]	2.24 ± 0.18[D]
Mixed-species		
F. quadrangulata-L. maackii	1.94 ± 0.22[A]	1.88 ± 0.18[A]
Q. muehlenbergii-L. maackii	1.19 ± 0.12[B]	1.02 ± 0.09[B]
C. ovata-L.maackii	2.65 ± 0.09[C]	1.56 ± 0.09[A]

Table 2. ANOVA results for decomposition (mass loss) and N loss of litter bags containing only *L. maackii* litter.

	Mass Loss			N Loss		
Source	df	*F*	*P*	df	*F*	*P*
Location	3	5.28	**0.002**	3	3.51	**0.002**
Time	5	2336.19	**<0.0001**	5	1047.11	**<0.0001**
Location*time	15	1.25	0.25	15	1.69	0.07

Notes: Litter bags were placed in four locations (under all three tree species' canopies and in the open savanna). Time refers to bag pick-up dates over the course of 2 years. Boldface type indicates significant differences.

Table 3. Comparison of observed and predicted decay rates of dominant tree species litter decaying with *L. maackii* litter.

Litter type	Mass Loss k (1/yr) Under Trees		Mass Loss k (1/yr) in the Open	
	Observed	Predicted	Observed	Predicted
F. quadrangulata-L. maackii	2.97 ± 0.31	2.64 ± 0.08	2.55 ± 0.16	2.54 ± 0.12
Q. muehlenbergii-L. maackii	1.82 ± 0.08	1.76 ± 0.04	1.52 ± 0.11	1.79 ± 0.11
C. ovata-L.maackii	2.49 ± 0.08	2.42 ± 0.10	2.31 ± 0.05	2.23 ± 0.12

Notes: Predicted decay rates were based on the mass loss of the component species: a tree species and *L. maackii.* Mixed species bags contained 50% tree litter and 50% *L. maackii* litter and were located in plots under a corresponding overstory tree species (n = 3 species, n = 5 of each species) and in the open savanna (n = 5 plots). Decay rates were calculated from a single negative exponential model. All values are means ± 1 SE.

Table 4. Soil physicochemical and light conditions in the understory of the native tree species.

Characteristics	*P*	*F. quadrangulata*	*Q. muehlenbergii*	*C. ovata*
pH	**0.02**	5.29 ± 0.15[A]	6.01 ± 0.11[B]	5.85 ± 0.16[B]
Light (µmol/m2/s)	0.34	171.5 ± 79.0	146.7 ± 50.2	56.2 ± 22.8
Organic matter (%)	0.72	10.2 ± 0.86	9.47 ± 0.57	9.64 ± 0.32
Moisture (%)				
May	0.63	24.5 ± 1.74	24.4 ± 1.80	22.1 ± 1.48
July	**0.003**	21.8 ± 1.20[A]	27.5 ± 1.82[B]	30.3 ± 1.01[B]
October	0.44	16.2 ± 2.31	16.7 ± 3.29	18.3 ± 1.71
NO_3- (ug/g soil)				
May	0.45	2.17 ± 0.99	2.09 ± 0.21	2.21 ± 0.65
July	0.2	0.33 ± 0.17	1.08 ± 0.62	2.12 ± 0.92
October	0.69	4.41 ± 1.58	3.91 ± 1.01	4.73 ± 1.46
NH_4+ (ug/g soil)				
May	0.34	6.25 ± 1.42	4.37 ± 0.77	4.30 ± 0.84
July	0.79	2.66 ± 0.32	2.86 ± 0.30	3.11 ± 0.43
October	0.51	3.53 ± 0.46	9.10 ± 6.23	4.12 ± 1.07

Notes: All values are means ± 1 SE. Significant differences are indicated with boldface type and letters indicate significant differences in physicochemcical and light conditions across tree species.

Table 5. ANOVA for total microarthropods per gram of litter in single-tree and mixed-species (tree + *L. maackii*) litterbags at six weeks.

	F. quadrangulata			*Q. muehlenbergii*			*C. ovata*		
Source	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Location	1	12.1	**0.01**	1	0.21	0.67	1	7.85	**0.04**
Litter type	1	1.75	0.23	1	6.52	**0.06**	1	0.27	0.63
Location*litter type	1	0.04	0.84	1	3.36	0.14	1	0.02	0.88

Notes: Litter bags were placed in plots located under the tree canopy that corresponded with their litter (n = 3 species, n = 5 plots per species) and in the open savanna (n = 5 plots). Location refers to the placement of litter bags under the trees' canopies or in the open savana. Boldface type indicates significant differences.

Figure 1. Decomposition (percentage of initial litter mass remaining) and nitrogen dynamics (percentage of initial litter nitrogen remaining) of single-species litter bags under tree canopies over a two year period. Tree litter was only placed in plots located under the canopy of the corresponding tree species (n = 3 species, n=5 trees of each species), while *L. maackii* was located in plots under all of the tree species' canopies. Error bars are ± 1 SE.

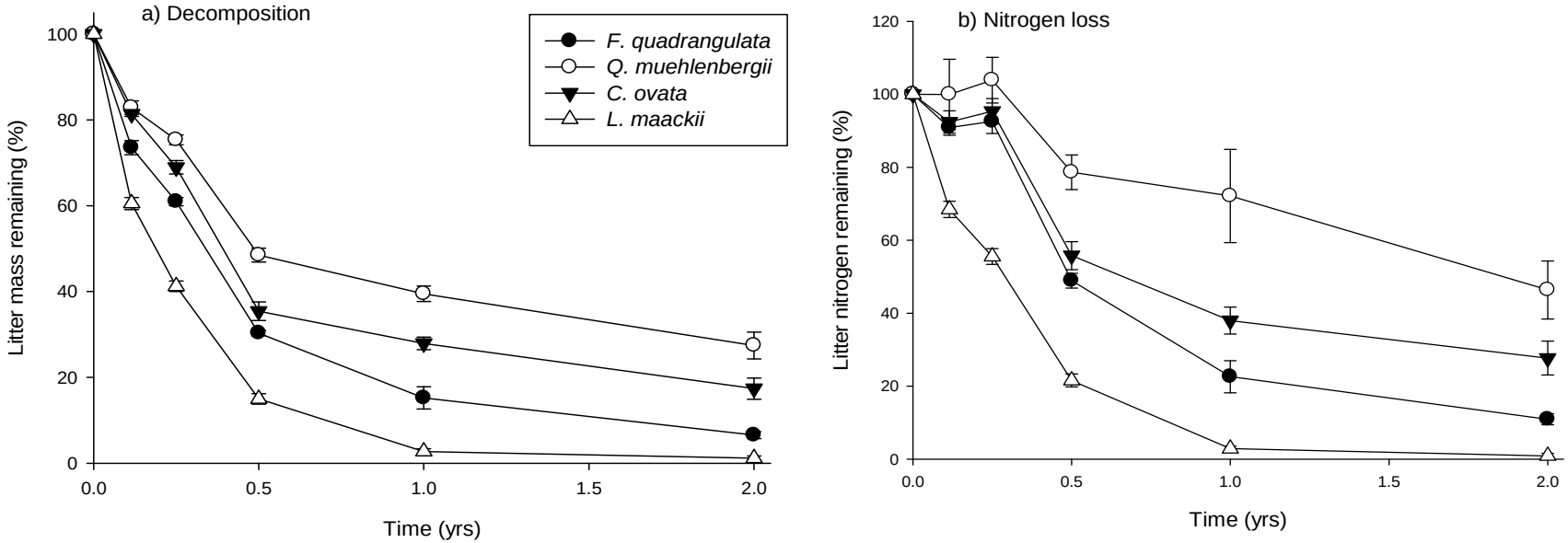


Figure 2. Comparison of decomposition (percentage of initial litter mass remaining) and nitrogen dynamics (percentage of initial litter nitrogen remaining) of single-species *L. maackii* bags located in plots under different tree canopies (n = 3 species, n = 5 trees per species) and in the open savanna over a two year period. Data is only shown for the first year as *L. maackii* litter was almost completely decomposed at this point. Error bars are ± 1 SE.

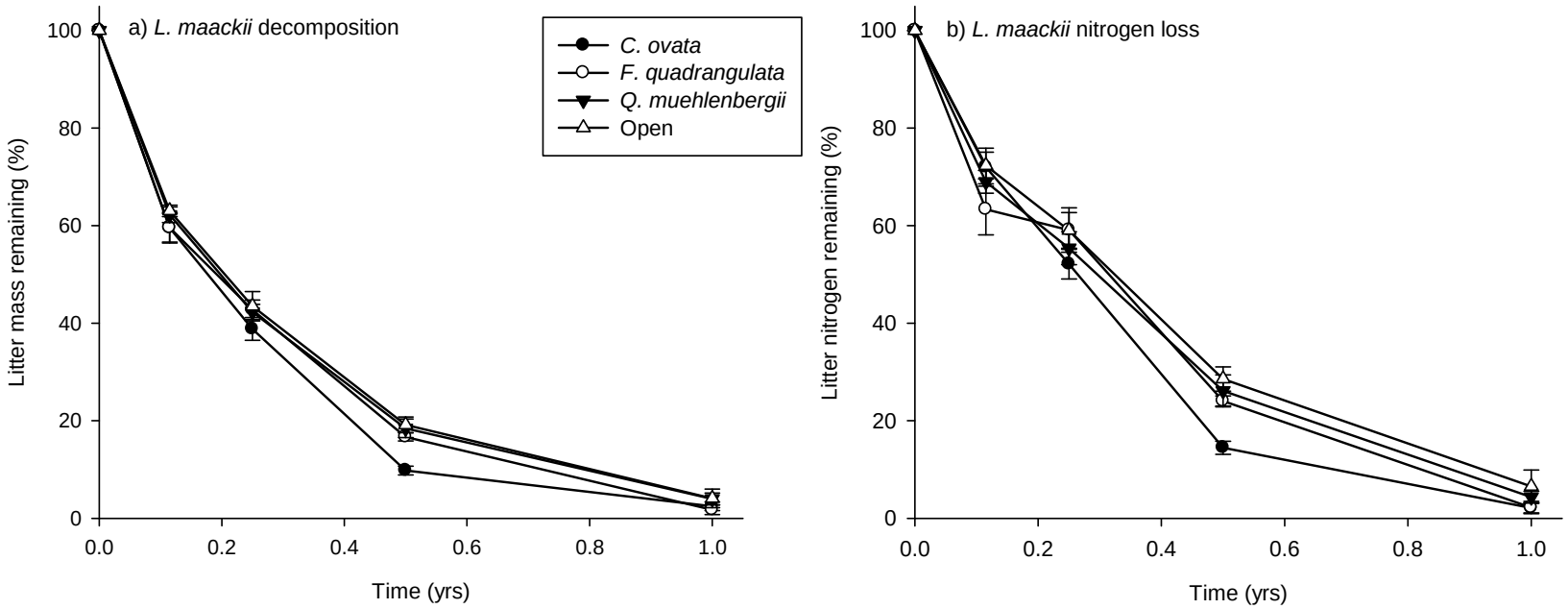


Figure 3. Comparison of (a) observed and predicted decomposition (percentage of initial litter mass remaining) and (b) N remaining (percentage of initial litter nitrogen remaining) in mixed-species litter bags located in plots under tree canopies for a period of two years. Predicted values were calculated using component species values measured in single-species litter bags. Predicted and observed decay rates (k) are given in Table 3. Mixed-species litter bags were placed in plots under the canopy of the tree that corresponded with the tree litter in the bag. Error bars are ± 1 SE, while asterisks indicate significant differences between values ($P < 0.05$).

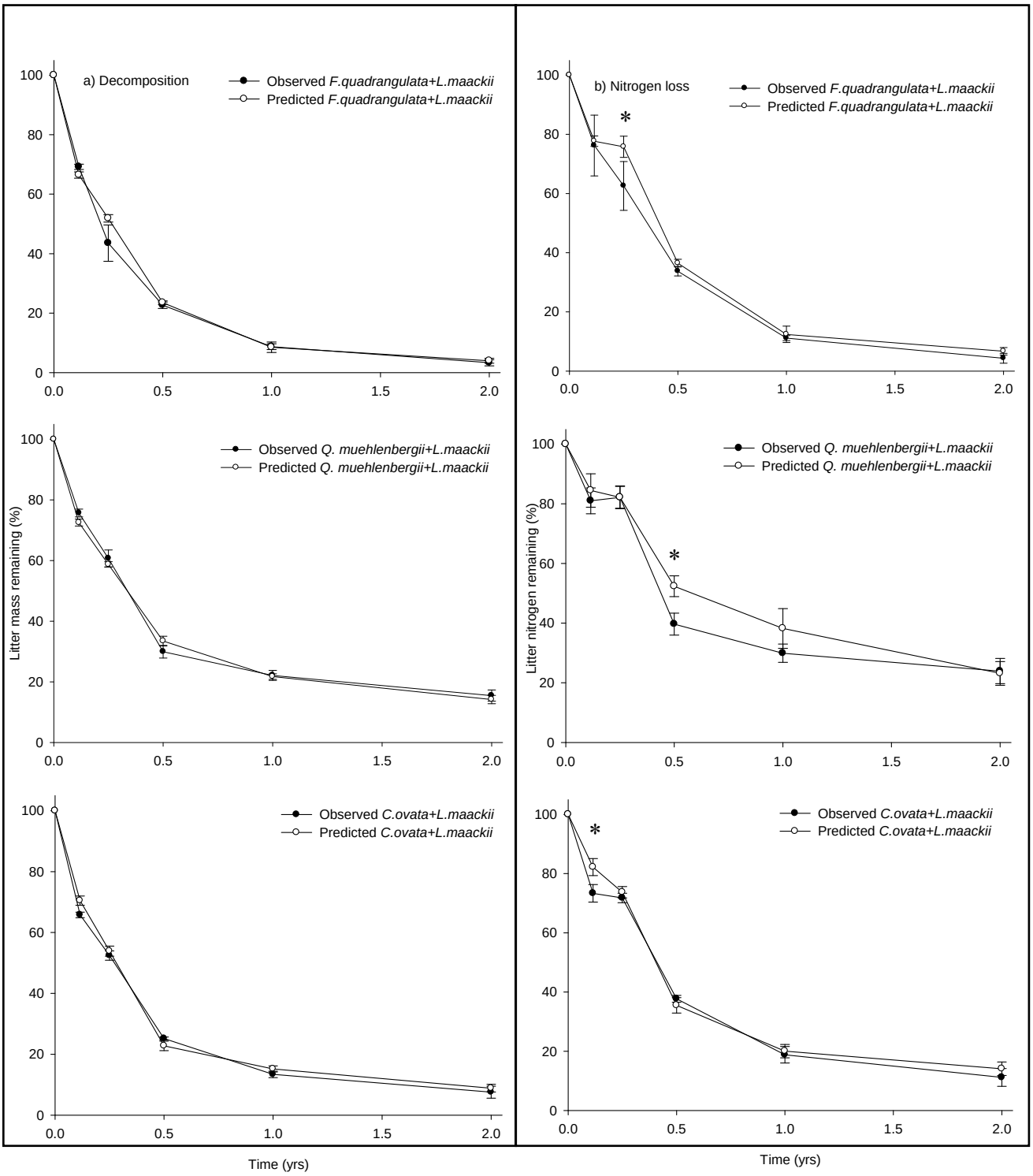
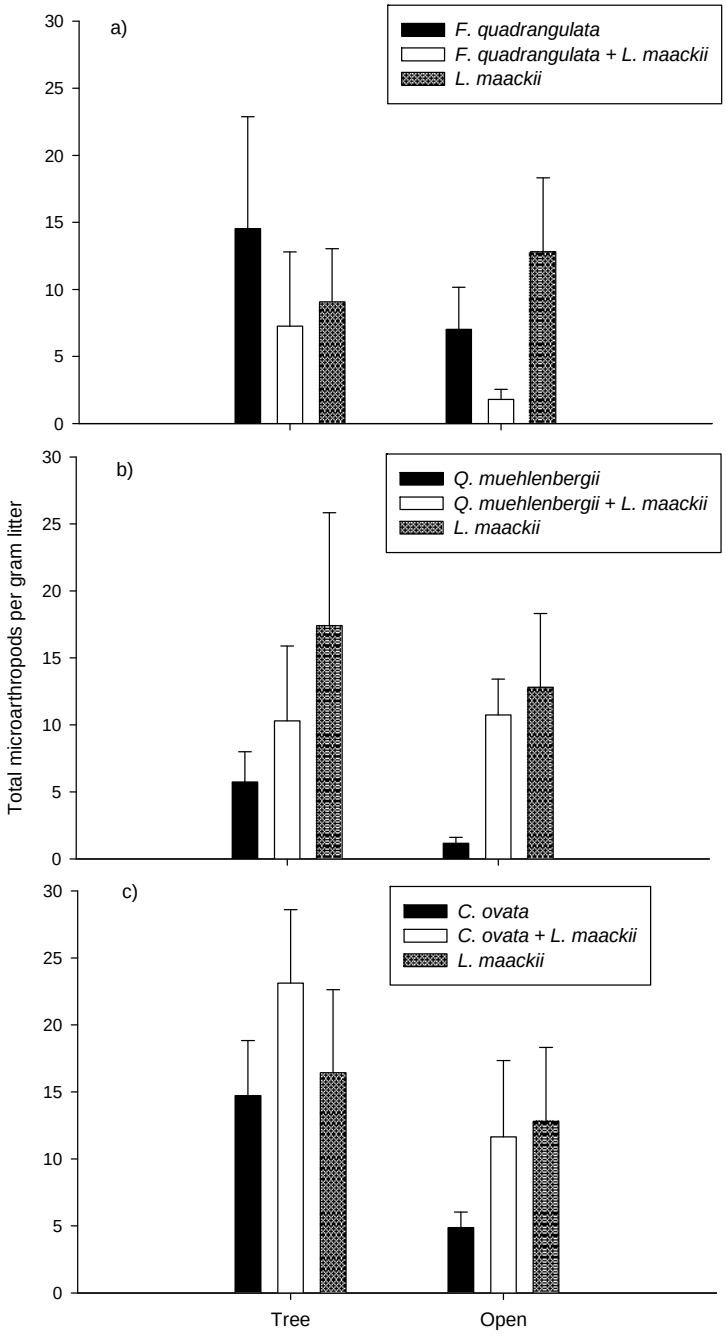

a) Decomposition
Observed F.quadrangulata+L.maackii
Predicted F.quadrangulata+L.maackii
b) Nitrogen loss
Observed F.quadrangulata+L.maackii
Predicted F.quadrangulata+L.maackii
Observed Q. muehlenbergii+L.maackii
Predicted Q. muehlenbergii+L.maackii
Observed Q. muehlenbergii+L.maackii
Predicted Q. muehlenbergii+L.maackii
Observed C.ovata+L.maackii
Predicted C.ovata+L.maackii
Observed C.ovata+L.maackii
Predicted C.ovata+L.maackii
Litter mass remaining (%)
Litter nitrogen remaining (%)
Time (yrs)
Time (yrs)

Figure 4. Total microarthropods found per gram of litter in single-tree species (n = 3 species), mixed-species (tree + *L. maackii)*, and single-*L. maackii* species litter bags at 6 weeks. Litter bags were placed in plots located under the tree canopy that corresponded with their litter (n = 3 species, n = 5 plots per species) and in the open savanna (n = 5 plots). Single-species *L. maackii* bags were placed in plots under all three tree species' canopies (n = 15) and in the open savanna plots (n = 5). Panels show comparisons of a) *F. quadrangulata* litter, b) *Q. muehlenbergii* litter, and c) *C. ovata* litter to their respective mixed litters and *L. maackii* litter in the two different environments. Error bars are ± 1 SE. ANOVA results are listed in Table 5.



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Chapter Three

Ecosystem impacts of the invasive shrub *Lonicera maackii* depend on associations with native tree species

Introduction

Few natural systems are likely to remain free of invasion by exotic species and the ecosystem consequences of these invasions have been well documented (Levine et al. 2003, Ehrenfeld 2010). It has been suggested that invasive plants may facilitate their own success by creating feedback mechanisms that alter N cycling and other soil processes (Ehrenfeld et al. 2001). Plant invaders have been shown to alter soil features like pH (Ehrenfeld et al. 2001), bulk density (Throop and Archer 2008), and soil temperature and moisture (Yelenik et al. 2004). Root penetration, organic matter deposition, and other rhizosphere inputs affect soil aggregate structure and macropore formation, soil aeration, and water movement and storage in the soil, potentially resulting in positive feedback effects on plant growth (Angers and Caron 1998). Nitrogen dynamics and controls over the cycling of nitrogen are of particular interest given N limitations in temperate-zone terrestrial ecosystems (Vitousek and Howarth 1991). Invasive plants may enhance soil N availability, leading to positive feedbacks between N cycling and plant growth (Ehrenfeld 2003). Invasive species have also been shown to alter the structure and function of the microbial community (Kourtev et al. 2002, Hawkes et al. 2005, Holly et al. 2009). In eight out of ten studies examining linkages between the microbial community and invasive plants, Ehrenfeld (2003) found increases in microbial biomass N. Similarly, a meta-analysis found an overall increase of 26% in microbial N

pools in invaded systems (Liao et al. 2008). Despite a growing body of research documenting the ecosystem impacts of numerous plant invaders and evidence for increases in biogeochemical pool sizes, accelerated flux rates, and alterations to soil biological and physical attributes, such effects vary across ecosystem types and are often site-specific and temporally variable (Liao et al. 2008, Ehrenfeld 2010).

Moving forward, it seems prudent to link our mechanistic understanding of invasive species impacts with an examination of the ecological context in which invasions are occurring. Linking these two facets of invasive plant research could serve to explain why exotic plant species variably affect the systems they invade. Some of the observed site and temporal variability of invasive plant effects may reflect differences in relative abundances of community dominants. Failure to take specific members of the native plant community and their interactions with invaders into account may mask or exaggerate the impact of an invasive plant.

To examine these potentially variable native-invasive species effects, we used a savanna ecosystem in Kentucky that has been invaded by the invasive shrub *Lonicera maackii* (L). A savanna-like ecosystem offers an ideal context for testing hypotheses regarding the potentially variable relationship between native and invasive species and their relationship to nutrient inputs and cycling, soil properties, and the soil biotic community. Single tree species are known to modify patterns of chemical and physical soil properties relative to the surrounding understory through the deposition of above- and belowground litter, leachates, and root exudates (Zinke 1962, *in* Boettcher and Kalisz 1990; Rhoades 1997, Eviner and Chapin 2003). The resultant small-scale patterns in soil properties have a significant impact on nutrient cycling, soil physicochemical properties,

and the composition of the underlying soil biotic community. Invasive plant species can influence these small-scale patterns and the impacts of an invasive plant in the understory of a native tree species may be enhanced or muted by interactions with the native species. Because individual savanna trees and their canopies are typically non-overlapping, the spatial 'arrangement' of canopy trees permits evaluation of the species-specific nature of interactions between native trees and an invasive species in their understory.

Teasing out invasive-native species interactions at our savanna site could provide a better understanding of invasive species effects on ecosystem processes and may prove especially pertinent to forest communities that are dominated by several native tree species. The savanna system we studied is dominated by *Fraxinus quadrangulata* (Mill.) K. Koch (blue ash), *Quercus muehlenbergii* E. (chinkapin oak), and *Carya ovata* M. (shagbark hickory). Although the dominant, native tree species at our site are specific to a particular region, the genera they represent are common and abundant throughout forest systems in the eastern United States (Barrett 1995, Kricher 1998). Exotic shrubs are prevalent in the Eastern United States and common taxa are well represented at our site, thus interactions between native and invasive species observed at our site may be present in ecosystems dominated by a similar suite of tree species.

Recent estimates indicate that ecosystems in the eastern U.S. have been invaded by at least 129 exotic shrub species (Fridley 2008). A native of China, *L. maackii* has naturalized in at least 27 states/provinces in the eastern United States and Canada since its introduction to North America in the late 19th century (NRCS, Luken and Thieret 1997). The exotic *L. maackii* is an upright, multistemmed, deciduous shrub with a shallow root system (Luken and Thieret 1997, Luken 1988). It shares a number of traits

with other aggressive, exotic shrubs like *Berberis thunbergii* DC. (Japanese barberry), *Rhamnus cathartica* L. (common buckthorn), *Ligustrum sinense* Lour. (Chinese privet), and *Elaeagnus umbellata* Thunb. (autumn olive). Like many other woody invasive shrubs, *L. maackii* is highly plastic in growth and biomass allocation across different environments, allowing it to form dense, monotypic stands (Luken 1988). It has an extended leaf phenology relative to most native species, leafing out earlier in the spring and retaining its leaves late into the fall and early winter and is considerably more tolerant of freezing temperatures (McEwan et al. 2009b). It produces copious amounts of bright red berries that remain on the shrub well into the winter.

The negative effect of *L. maackii* on the density and richness of herbs and tree seedlings, the reproductive success of native herbs, and the growth of native shrubs and trees have been well documented (Hutchinson and Vankat 1997, Collier et al. 2002, Miller and Gorchov 2004, Hartman and McCarthy 2008). Despite a rapidly expanding literature, studies of effects on ecosystem processes are few. A concurrent experiment examining the effect of *L. maackii* on decomposition dynamics revealed that litter from the invasive shrub is higher in nitrogen (N) and breaks down and releases N rapidly in comparison with native tree species, although it does so at a different rate depending on its association with different native species (Poulette and Arthur 2012). Mixing litter from *L. maackii* with the native species litter resulted in a synergistic loss of N from the litter, but this was again temporally dependent on the native species in the mixture (Poulette and Arthur 2012).

Building on this previous work, we broadly hypothesized that *L. maackii* would alter soil processes, nutrient cycling, and the soil microbial community under the

dominant native trees. However, we expected that these effects would vary depending on the identity of the associated native tree species. The following hypotheses generally address *L. maackii* effects, but we anticipated that the magnitude of these effects would vary beneath different native trees. We hypothesized that (1) *L. maackii* would alter the soil physicochemical properties beneath its canopy. Given *L. maackii*'s dense, shallow root system, N-rich litter, and rapid litter turnover, we anticipated that the soils beneath the invader would have a lower bulk density, higher soil organic matter (SOM) content, altered C:N ratio, and larger total N pool relative to the soils beneath the tree species where *L. maackii* was not present. Due in part to *L. maackii*'s N-rich, rapidly decomposing litter we further hypothesized that (2) *L. maackii* would significantly increase rates of N transformation in the soils beneath its canopy. We anticipated that this effect might be strongest in the understory of *C. ovata* given our previous findings of accelerated *L. maackii* litter N and mass loss under this tree species (Poulette and Arthur 2012). Finally, to link a causal mechanism to anticipated observations of altered N transformations, we hypothesized that (3) the structure of the soil microbial community would differ in response to vegetation-mediated differences in the soil physicochemical properties and nutrient availability.

Methods

Study Site and Tree Characteristics

Research was conducted at Griffith Woods, a savanna woodland in Harrison County, Kentucky. See McEwan and McCarthy (2008) for a detailed description of oak savannas in central Kentucky. Griffith Woods lies within the Inner Bluegrass Region,

which is defined largely by its underlying Ordovician limestone (Wharton & Barbour 1991). Soils in this region tend to be deep, well-drained, phosphate-rich silt loams (Wharton and Barbour 1991). At our site, the soils are classified as fine, mixed, active, mesic Typic Hapludalfs (Lowell and Faywood-Lowell) and fine-silty, mixed, active, mesic Oxyaquic Fragiudalfs (Nicholson). The regional climate is characterized as temperate, humid, and continental (Wharton and Barbour 1991). The mean annual temperature at the nearby Cynthiana, Kentucky weather station (located at 38.4456 latitude, 84.1615 longitude) is 12.2°C and mean precipitation is 108 cm (http://www.sercc.com). The savanna encompasses approximately 80 acres with 150- to 300-year old trees widely scattered in a matrix of grasses and forbs. Tall fescue (*Lolium arundinaceum* (Schreb.)) dominates the grass matrix, and until 2005 the site was historically used for pasture. *L. maackii* has invaded the site and shrubs of varying size are located primarily in the understory of the savanna trees and in the adjacent woodland. Although *L. maackii* only grows beneath the trees in the savanna, its establishment in the understory of the savanna trees appears to follow no discernable pattern, occurring regardless of cardinal direction, overstory branching pattern, or any other apparent attribute.

A survey of the trees in the savanna was conducted at Griffith Woods in spring, 2005. The dominant, native trees at the site were identified as *Fraxinus quadrangulata, Quercus muehlenbergii,* and *Carya ovata.* Fifteen trees of each species were selected for inclusion in the study for a total of 45 trees. The following criteria were used to select study trees: (1) at least one *L. maackii* shrub was present under the canopy of the tree, (2) *L. maackii* did not inhabit the entire understory of the tree canopy, (3) the canopy of the

tree did not overlap with the canopy of another tree, and (4) the tree had canopy coverage over both the *L. maackii* shrub in its understory and in an area free of *L. maackii* (lightning damage left some of the trees with only half their canopy). Within a species, when more than 15 trees fit these criteria, trees were selected at random for inclusion in the study. In the case of *C. ovata*, only 15 trees were available, so all were included in the study. All the trees used in the study, including *C. ovata,* were well distributed throughout the savanna. After initial sampling in 2005, a work crew mistakenly removed some of the *L. maackii* shrubs beneath several of the study trees resulting in some reduction in the number of experimental units for future sampling.

Experimental Design

The objective of this research was to examine the synergistic impacts of the invasive shrub *L. maackii* on soil processes, nutrient cycling, and the microbial community under three dominant, native tree species in a savanna in Kentucky. Over 5 years, we examined soil physicochemical properties (2009), net N mineralization rates (2005-2008), and soil microbial biomass and microbial community structure (2006). Throughout the duration of the study, samples were collected from two microsites under each individual tree (n = 45 trees, n = 3 species): under the tree canopy and under an *L. maackii* shrub under the tree canopy (n = 90 samples). Preliminary sampling revealed little or no O horizon present, and so the A horizon of approximately 12 cm was sampled to represent the depth at which most nutrient cycling and microbial activity occur in these soils. Under each tree, all samples were taken at 12 cm depth and 1 m from the bole of the tree.

Microsite and Soil Physicochemical Properties

Light was measured under the trees and *L. maackii* shrubs using a line-integrating quantum sensor (LI-191SA, Li-cor) in July, 2008. Soil samples were collected for bulk density and organic matter in January, 2009 1 m from the bole of the tree at the two microsites around each individual tree (see above). Samples were collected using a soil-core sampler with a slide hammer (internal diameter of 3.8-cm), placed in plastic bags, and transported to the lab. Samples were weighed and passed through a 2mm sieve to remove fine root material. The total volume of stones and any organic material (e.g. hickory nuts) > 2mm was measured by water displacement. The volume of these solids was subtracted from the total volume of the core. Each sample was dried at 105°C and weighed to determine soil bulk density. Soil organic matter (SOM%) was measured in soil subsamples via loss on ignition after 8 hrs at 500°C. In other soil subsamples, gravimetric moisture content and soil pH (1:1 slurry in distilled deionized water) were assessed. A final subsample was pulverized in a ball grinder to determine total C and N content on a Leco CN analyzer (University of Kentucky Regulatory Services, Lexington, KY, USA).

Net N Mineralization

Soil samples were collected from 2005-2008 to assess species-specific effects on seasonal variation in net rates of N mineralization. Samples were again collected from two microsites around each individual tree (under the tree canopy, under tree + *L. maackii*) to assay soil nutrient availability and nitrogen mineralization. Samples in 2005

were incubated in July/August, and samples in 2006-2008 were incubated in May/June, July/August, and October/November. To quantify plant-available N, two techniques were used: the intact-core method (Raison et al. 1987), used from 2005-2007, and the soil core-resin bag technique (DiStefano and Gholz 1986), used in 2008. Resin bags were constructed using an equal mix of a Lewatit Lanxess MonoPlus S100 resin (H^+ form) (Siemens) and an Ionac Sybron ASB-1P resin (OH^- form) (Siemens). Approximately five grams of each resin were mixed together and sealed into a nylon stocking.

In the field, soils (0-12 cm) were sampled and placed in polyethylene bags, held on ice, and returned to the lab. PVC soil incubation tubes were driven 12 cm into the ground and loosely covered with duct tape (2005-2007). In 2008, incubation tubes were carefully removed after initial insertion, and resin bags were placed in the bottom of the tubes beneath the soil. Tubes were gently placed back in their associated hole and remained open to the elements. In both cases, incubation tubes were left in place for 28 days, collected, and returned to the lab. Initial and incubated samples were stored in the lab at 4°C and processed within 2 days of collection. Soils were sieved through a 2 mm mesh sieve to remove any plant material or debris and to homogenize the samples. A 10g subsample of both initial and incubated soils were assayed using a 1N KCl extraction; field resins were shaken in 50 mL 1 N KCl for 60 minutes and allowed to sit overnight. Filtered samples extracted from both the soils and resins were analyzed colorimetrically for NH_4^+ and NO_3^- on an autoanalyzer (Bran-Luebbe Autoanalyzer 3; Technicon Industrial Systems, Tarrytown, New York, USA). Net rates of nitrification, ammonification, and total N mineralization (μg N x (g soil)$^{-1}$ x day^{-1}) were calculated from the changes in NO_3-N and NH_4^+-N per gram dry soil over the 28-day incubation.

Microbial Community

Soil microbial biomass and community structure were determined using phospholipid fatty acid (PLFA) analysis (Findlay and Dobbs 1993) according to the methods of D'Angelo et al. (2005). Samples were collected from two microsites around each individual tree (under the tree canopy, under tree + *L. maackii*) in May 2006. Four to five soil cores per microsite were collected to a depth of 12 cm using a soil auger (2 cm diameter). Samples were bulked by microsite and immediately placed on dry ice in the field. Samples were returned to the lab and stored at -80°C until analysis. In the lab, soils were thawed, passed through a 2mm sieve, and immediately extracted. See Weand et al. (2010) for a detailed description of the PLFA procedure. Previously published PLFA biomarker data (Vestal and White 1989, Frostegard et al. 1993, Zelles 1997, Green and Scow 2000), were used to group PLFAs into separate taxonomic groups. Gram-negative bacteria biomarkers were identified as monounsaturated fatty acids, and Gram-positive bacteria biomarkers were identified as terminally branched fatty acids. Fungal biomarkers were identified as fatty acid 18:1ω9, all polyunsaturated fatty acids, and fatty acids having 20 or more C atoms. Actinomycete biomarkers were defined as mid-chain, branched, saturated fatty acids, and sulfate-reducing (SRB) and other anaerobic bacteria markers were defined as branched monounsaturated fatty acids (Weand et al. 2010). The total extractable PLFA quantities (nmol g^{-1} soil) were summed to calculate microbial biomass. Total bacterial % mole abundance (normal saturated C_{12}-C_{18} fatty acids, terminal and mid-chain branched fatty acids, branched monosaturated fatty acids, and all monounsaturated fatty acids excepting 18:1ω9) and total fungal % mole abundance

(fungal biomarkers) were calculated to generate a fungal to bacterial ratio (F:B). Common PLFA biomarkers (% mole abundance > 1%) were identified for further analysis.

Statistical Analysis

Differences in understory light, soil physicochemical properties, microbial biomass, and concentrations of the common PLFA biomarkers were tested across tree species (n = 3) and sampling location (n = 2, under tree and under *L. maackii*) using a mixed model ANOVA in Proc Mixed of SAS version 9.3 (SAS Institute Inc., Cary, North Carolina, USA). Tree species (n = 3) and sampling location (under tree, under *L. maackii*) were used as fixed main effects and tree number (replication) was used as a random effect. Differences in net N mineralization were analyzed within a repeated measures (tree = repeated unit) mixed model ANOVA with time, tree species (n = 3 species), and location (under tree, under *L. maackii*) as fixed main effects and tree number (replication) as a random effect. The seasonal and multi-year nature of the net N mineralization data resulted in a covariance matrix that was too large for the repeated measures analysis to handle with sufficient power. As a result, this analysis was broken into two specific time components: differences across seasons (all years included) or differences within years (all seasons). Data were then analyzed separately using the above description with time defined as a particular season (spring, summer, or fall) or a particular year (2006, 2007, or 2008). For significant main-effects differences ($p < 0.05$), least squares means were compared using the LSMEANS procedure.

Results

Microsite and Soil Physicochemical Properties

Light availability was significantly affected by both tree species (p=0.004) and the presence of *L. maackii* (p = 0.03, Figure 5a). The canopy of *F. quadrangulata* allowed the most light to penetrate, while *C. ovata* allowed the least amount of light beneath its canopy. Light readings were further reduced under *L. maackii* shrubs located under the canopies of *F. quadrangulata* and *Q. muehlenbergii*, but there was no significant further reduction under *L. maackii* that were beneath *C. ovata* (Figure 5a). The invasive *L. maackii* had no effect on soil pH, but tree species did. The soils beneath *F. quadrangulata* were significantly more acidic (p = 0.0004) than the soils of the other two species (Figure 5b). Bulk density was significantly lower in soils beneath *L. maackii* than in soils beneath the native tree species (p = 0.04, Figure 5c), but this trend was most apparent in the soils beneath *L. maackii* shrubs located under *C. ovata* (Figure 5c). The presence of *L. maackii* had a significant positive effect on soil organic matter (%) (p = 0.006), C:N (p = 0.0003), and total soil N (%) (p = 0.02) (Figure 5d, e, and f). These effects were most apparent in soils beneath *L. maackii* located under *F. quadrangulata* and *Q. muehlenbergii* relative to soils under *L. maackii* beneath the canopy of *C. ovata* (Figure 5d, e, and f), but species x location interactions were not significant for any of these parameters.

Net N Mineralization

Soils were assessed in July of 2005 and over the course of the growing season (May, July, October) from 2006-2008 to test for the effects of both tree species and the

presence of the invasive *L. maackii* on extractable $NO_3^-$and NH_4^+ and on rates of nitrification, ammonification, and net N mineralization. Both tree species and the presence of *L. maackii* significantly affected N cycling at varying times throughout the experiment. However, consistent trends were difficult to discern and effects were often subtle, seasonal, and varied between years (Table 6).

Extractable NO_3^- and NH_4^+ concentrations were affected by tree species and location (the presence of *L. maackii*) at various times throughout the experiment (Table 6). In the summer, NO_3^- concentrations tended to be higher in the soils under *Q. muehlenbergii* (July 2005, p = 0.009; July 2006, p = 0.001), while NH_4^+ concentrations tended to be higher in the soils under *F. quadrangulata* (July 2005, p = 0.04; July 2006, p = 0.03). In May of 2006, both NO_3^- and NH_4^+ concentrations were lower in soils beneath *L. maackii* (p = 0.001 and p = 0.002 respectively).

Net nitrification also varied across seasons and years and was influenced by tree species and the presence of *L. maackii* (Table 6). At various times, rates were lower in the soils beneath *F. quadrangulata* trees (July 2008, p = 0.05; 2008) and higher in the soils beneath *C. ovata* trees (July 2006, p = 0.05). In two instances, the presence of *L.* maackii decreased rates of net nitrification (May 2007, p = 0.002; October 2007, p = 0.03). The contribution that nitrification made to overall N mineralization rates (nitrification fraction) was largely influenced by tree species. At various times, the soils beneath *C. ovata* had a greater % nitrification (July 2006, species p = 0.04), while the soils beneath *F. quadrangulata* had significantly less % nitrification (May 2007, p = 0.05).

Results varied across both seasons and years, but net ammonification rates were primarily affected by tree species (Table 6). Soils beneath *F. quadrangulata* trees had higher rates of net ammonification compared to the other tree species (October 2006, $p = 0.02$; May 2008, $p = 0.03$; July 2008, $p = 0.02$; 2008). Sampling location also had an effect on rates of net ammonification at times. In July of 2005, soils beneath *L. maackii* had higher rates of ammonification ($p = 0.03$). In May of 2006, rates were variably affected by the presence of *L. maackii* with increased rates of net ammonification in the soils beneath *L. maackii* in the understory of *C. ovata* but not of the other two species ($p = 0.02$).

Net N mineralization rates were affected by tree species, the presence of *L. maackii*, and the interaction between the two at times throughout the experiment (Table 6). Notably, rates were variably affected by the presence of *L. maackii* in the understory of the native trees. Net mineralization rates in the soils beneath the canopy of *F. quadrangulata* were lower under *L. maackii* relative to soils not under *L. maackii* in July, 2007 ($p = 0.01$). In contrast, the presence of *L. maackii* in the understory of *C. ovata* increased rates of N mineralization in the soil, while rates were lower in soils that were beneath *L. maackii* located beneath the other two tree species (July 2005, $p = 0.009$; May 2006, $p = 0.005$). In October 2007, soils beneath *F. quadrangulata* had higher rates of N mineralization compared to soils beneath the other 2 tree species ($p = 0.03$). In May of 2007, soils beneath *L. maackii* tended to have lower rates of net N mineralization ($p = 0.04$)

Microbial Community

The total PLFA biomass was calculated using 96 PLFAs ranging in contribution from 108 nmol g^{-1} to 555 nmol g^{-1}. There were no significant differences in total PLFA biomass, bacterial PLFA biomass, or fungal PLFA biomass by tree species or location (beneath tree or beneath *L. maackii*) (Figure 6a-c). However, the fungal:bacterial PLFA biomass was significantly affected by the interaction of species and location ($F = 6.77$, $p = 0.02$), with a greater fungal:bacterial ratio in the soils beneath *L. maackii* located under *C. ovata* trees. Soils beneath *L. maackii* located under *F. quadrangulata* and *Q. muehlenbergii* had lower fungal:bacterial ratios than the soils beneath their associated native trees (Figure 6d).

We examined both percentage molar abundance (% mole) and biomass of PLFAs pooled into their respective taxonomic groups (see methods), but only the former was affected by tree species or by the presence of *L. maackii*. On a % mole abundance basis, gram-negative bacteria represented the most abundant group and protozoa the least, averaged across all treatments (Figure 7). Actinomycetes were the only taxonomic group affected by species or location: abundance was lower under *F. quadrangulata* trees than the other two tree species (Figure 7e, $F = 4.49$, $p = 0.04$). Location was not significant for any of the taxonomic groups. From the original 96 PLFAs identified, we individually examined the most common PLFAS for responses to tree species or presence of *L. maackii* on a finer scale. We identified16 PLFAs having on average > 1% mole abundance (Table 7). Tree species significantly affected cy17 (Gram-negative bacteria) and 10Me16 (Actinomycetes), which together represented 13.4% mole of the total biomass. The abundance of cy17 was high while the abundance of 10Me16 was low in

the soils beneath *F. quadrangulata* compared to soils beneath the other tree species. The abundance of i17(Gram-positive bacteria) was negatively affected by the presence of *L.maackii,* while the abundance of 18:1ω7c (Gram-negative bacteria) was positively affected by the invasive, regardless of associations with tree species. Together, these PLFAs represented 13.4% mole of the total biomass. The fungal group 18:1ω9c, representing 6.2% mole of the total biomass, was the only PLFA that was affected by an interaction between tree species and the presence of *L. maackii*. The abundance of the group was highest in the soils beneath *L. maackii* located under the canopy of *C. ovata*. Under the other two tree species, the abundance of 18:1ω9c was lower in the soils beneath *L. maackii* in comparison to the soils beneath the trees alone.

Discussion

Invasive species effects

Elevated SOM, C:N, and total soil N, and decreased light availability and bulk density in soils beneath *L. maackii* shrubs supported our hypothesis that the invasive shrub would alter the physicochemical properties beneath the native trees (Figures 5, 8). The rapidly decomposing, N-rich litter of *L. maackii* could be expected to create 'islands of fertility' beneath the shrubs as soils are enriched in organic matter and mineral nutrients (Ehrenfeld et al. 2005). Islands of fertility are often seen in arid and semiarid ecosystems, but the evidence for a buildup of organic matter and N in the soils beneath invasive shrub species in deciduous-forest systems is mixed. A meta-analysis of the invasive plant literature revealed that relative to native systems, invaded ecosystems had higher soil N (19%) and soil C (7%) pools (Liao et al. 2008). An examination of soils

beneath the invasive shrub *R. cathartica* detected twice the percent nitrogen, a 78% increase in total C, and a lower C:N ratio relative to soils in an open area of woodland free of the invasive (Heneghan et al. 2004), although a follow-up study failed to find differences in C:N (Heneghan et al. 2006). In contrast, soils beneath *B. thunbergii* had a lower organic matter content and lower total N content, although there was some spatial variability among study sites (Kourtevet al. 1999). The extensive, shallow root system of *L. maackii* coupled with its rapidly decomposing litter explains, in part, observed differences in the soil structure. Soils beneath *L. maackii* had lower bulk densities and may be more favorably structured to facilitate aeration, water movement, and the cycling of nutrients. This altered soil structure beneath the invasive shrub may positively influence its growth via the storage and turnover of organic matter, contributing to plant available nutrients (Angers and Caron 1998).

In light of physicochemical changes in the soil beneath *L. maackii* shrubs, we were surprised to find no differences in overall microbial, bacterial, or fungal biomass. Numerous studies have linked invasive plants and alterations to the soil microbial community biomass (see reviews in Ehrenfeld 2003 and Liao et al. 2008). The presence of *L. maackii* did have an effect on the structure of the soil microbial community, albeit subtle. The gram-negative lipid biomarker 18:1ω7c was more abundant in the soils beneath *L. maackii*, while the gram-positive lipid biomarker i17 was less abundant. The microbial community was sampled in the spring, and the presence of easily decomposable materials in the soils beneath *L. maackii* in early spring may account for these differences, which should favor bacterial dominance. However, given the significant differences in litter quality between *L. maackii* and the dominant tree species

(Poulette and Arthur 2012) it was surprising that more significant alterations in the microbial community were not observed.

Plant available N in the soils beneath *L. maackii* was seasonally variable. When significant location effects were detected, soils under *L. maackii* tended to have lower N cycling and availability than uninvaded canopy spaces (e.g. net nitrification - May and October 2007, net N mineralization - May 2007, and lower NH_4^+-N and NO_3^--N - May 2006), although higher rates of net ammonification (July 2005) were also observed. A meta-analysis of the invasive plant literature revealed that relative to native systems, invaded ecosystems had higher rates of net N mineralization (52%), and nitrification (53%), and increases in soil NH_4^+ (30%) and NO_3^- (17%) concentrations (Liao et al. 2008). However, in accord with our data, observed differences are often spatially and temporally variable, especially among invasive shrubs. For example, soils directly beneath the invasive shrub *B. thunbergii* had higher nitrification and net N mineralization rates than soil beneath common native shrubs, although the effect on N mineralization was temporally variable (Ehrenfeld et al. 2001). Soils beneath *R. cathartica* in a woodland in Illinois had lower NH_4-N availability and greater total N relative to soils in an open woodland area free of the invasive, but nitrification and total N mineralization rates varied across study sites with lower rates under the invasive at one site and lower ammonification rates under the invasive at another (Heneghan et al. 2004, Heneghan et al. 2006).

Invasive species effects are influenced by associations with native tree species

These results also suggest that *L. maackii*'s effect was not uniform across the landscape, and the strength of the effects appears to vary depending on associations between *L. maackii* and the native tree species (Figure 8). Soils beneath *L. maackii* in the understory of *C. ovata* had a greater fungal:bacterial ratio relative to the soils beneath the native tree. It is important to note that this effect was dependent on the overstory tree species, such that only the communities beneath *L. maackii* in the understory of *C. ovata* were altered (Figure 6d); the fungal:bacterial ratio in the soils beneath the invasive shrub in the understory of the other two tree species were not significantly different from the ratio in soils beneath their respective trees. The increase in the fungal:bacterial ratio beneath *L. maackii* located in the understory of *C. ovata* was primarily driven by a lipid biomarker thought to represent saprophytic fungus (18:1ω9c) (Table 7). Relative to soils beneath the native trees, this biomarker was only present in greater amounts under *L. maackii* growing in the understory of *C. ovata* (species x location effect). A concurrent decomposition study found that *L. maackii* litter lost both mass and N more rapidly when decomposing under *C. ovata* as compared to the other two native tree species (Poulette and Arthur 2012). If soils beneath *L. maackii* shrubs located in the understory of *C. ovata* foster a more abundant saprophytic fungal community, this may facilitate a more rapid breakdown of the invasive shrub's litter.

As mentioned previously, soils beneath *L. maackii* had elevated SOM content, C:N, and total soil N in comparison to adjacent soils beneath the native tree species with no invasive shrub, but this effect was not as apparent under *L. maackii* shrubs located in the understory of *C. ovata* (Figure 5d, e, f). Greater SOM and total N in the soils beneath *L. maackii* in the understory of *F. quadrangulata* and *Q. muehlenbergii,* coupled with a

lack of observed increases in N cycling, may suggest that these soils are storing C and N in response to the abundance of N-rich litter from the invasive without immediately making litter inputs of N available for plant uptake. A more robust community of saprophytic fungi enhancing litter decomposition under *L. maackii* shrubs associated with *C. ovata* trees may account for these observed differences. While bacteria tend to utilize more labile pools of SOM, fungi can access the relatively stable, recalcitrant pools of SOM (Billings and Ziegler 2008). The fungi in the soils beneath *L. maackii* in the understory of *C. ovata* may contribute to a SOM mineralization priming effect, such that enzymatic activity created by the labile C source rapidly breaks down both the fresh, labile C and recalcitrant SOM, depleting soil C and N pools (Norton et al. 2004). This priming effect may further result in a rapid exploitation of nutrients as they are mineralized, stimulating both plant and microbial uptake of C and N and facilitating plant growth (Norton et al. 2004). Soils beneath *L. maackii* located in the understory of *C. ovata* experienced periods of greater N mineralization and ammonification rates relative to the soils beneath *C. ovata* at times throughout the experiment (July 2005 and May 2006). Resultant increases in plant available N in the soils beneath *L. maackii* in the understory of *C. ovata* trees at variable times throughout the growing season may create a positive feedback effect not seen under *L. maackii* shrubs occupying the understory of the other native tree species. These possibilities remain to be further explored.

Tree species effects

The identity of the native tree species also had a significant effect on soil physical properties, N cycling, and the soil microbial community. The native tree *F.*

quadrangulata appeared to drive most of these differences and it is important to note that these differences are identified as those that occurred in the understory of both the native and the understory of *L. maackii* shrubs located under the native (Figure 8). Regardless of the presence or absence of *L. maackii*, soils beneath *F. quadrangulata* tended to be more acidic, and their canopy allowed for greater light penetration. The abundance of the Actinomycete community as a whole and the specific Actinomycete group 10Me16 was suppressed, while the lipid biomarker for cy17 (gram negative) was significantly more abundant in the soils beneath *F. quadrangulata* compared to *C. ovata* and *Q. muehlenbergii*. Actinomycetes are important decomposers that are more active at a higher pH, so the acidic soil conditions under *F. quadrangulata* may account for observed differences. Actinomycetes are also well suited to decompose recalcitrant compounds like lignin, but *F. quadrangulata* litter is relatively low in lignin in comparison to the other two native tree species (Poulette and Arthur 2012). Much like the soils beneath *L. maackii*, the relatively labile nature of *F. quadrangulata* litter may favor gram negative bacteria like cy17 over gram positive bacteria like the Actinomycetes.

At times throughout the experiment, the soils beneath *F.quadrangulata* had higher rates of net N mineralization (October 2007) and ammonification (October 2006, May 2008, and July 2008) and lower rates of net nitrification (May 2007) compared to soils beneath the other native tree species. This is consistent with the literature, as higher rates of net N mineralization have been observed beneath *Fraxinus americana* (white ash) in a mixed-species forest in Ohio (Boerner and Koslowsky 1989) and Connecticut (Finzi et al. 1998). Litter from *F. quadrangulata* has a higher initial N concentration, lower initial

C:N ratio and initial lignin concentration, and loses litter mass and N more rapidly than the other two native tree species (Poulette and Arthur 2012). This more rapid delivery of N via litter decomposition may account for observed differences in N cycling beneath *F. quadrangulata*. Regardless of the mechanism, increases in plant available N, however temporary, extended to the soils beneath *L. maackii* located in the understory of *F. quadrangulata*. Just as *L. maackii*-mediated alterations to the soil physicochemical structure and plant available N that occurred may facilitate a positive feedback effect, so may alterations dictated by the overstory tree species. If *L. maackii* located in the understory of *F. quadrangulata* experience periods of increased N availability due to the influences of the native tree, it may result in a period of increased growth and productivity that invasive shrubs in the understory of the other native tree species may not encounter.

Conclusion

We observed two distinct scenarios by which soil conditions beneath *L. maackii* may have promoted the growth of the invasive in a variable manner as influenced by associations with overstory native tree species (Figure 8). Firstly, soils beneath *L. maackii* in the understory of *C. ovata* experienced periods of greater N mineralization and ammonification compared to both the soils beneath *C. ovata* and the soils beneath *L. maackii* located under the other tree species. The buildup of SOM and total N in these soils was less apparent than in the soils beneath *L. maackii* located in the understory of the other two native tree species. We also observed a higher relative abundance of a

saprophytic fungal lipid biomarker. Taken together with evidence of evidence of enhanced *L. maackii* litter decomposition and N loss in the understory of *C. ovata* (Poulette and Arthur 2012), these observations may indicate a tendency for these soils to make more N available for plant uptake at the expense of the longer term storage seen under *L. maackii* in the understory of the other two native trees. Magnifying potential positive feedback effects under *L. maackii* in the understory of *C. ovata* could facilitate its fitness to a greater extent than *L. maackii* shrubs located in the understory of other native species. Secondly, regardless of the presence or absence of *L. maackii,* soils beneath *F. quadrangulata* experienced periods of higher N mineralization and ammonification, receive a rapidly decomposing, N-rich litter, and may favor gram-negative bacterial-decomposers relative to the other tree species. *L. maackii* shrubs growing in the understory of *F. quadrangulata* may also benefit from these conditions. Both scenarios underscore the importance of considering the interactions of invasive and native plants, in particular overstory native tree species. Future examinations of the potential for positive feedbacks and the impact of invasive species on ecosystem processes should be carefully structured to take the associated native species into account.

Table 6. Summary of significant N-cycling ANOVA results and trends from each sampling period. Sampling was conducted in July of 2005 and in May, July, and October of 2006-2008. 'Effect' identifies the treatment variable that is significantly effecting the particular N response variable: native tree species ('Species'), sampling location ('Location'), or the interaction between the two (Sp*Loc). NS indicates that the effect was not significant ($p > 0.05$).

	NO_3-N			
	Effect	F	p	Trend
Jul-05	Species	5.25	0.009	Higher under oak
May-06	Location	7.58	0.001	Lower under HS
Jul-06	Species	3.19	0.05	Higher under oak
Sep-06	None			
May-07	None			
Jul-07	None			
Aug-07	None			
May-08	None			
Jul-08	None			
Aug-08	None			

Table 6, Cont.

	NH_4-N			
	Effect	F	p	Trend
Jul-05	Species	3.57	0.04	Higher under Ash
May-06	Species	3.76	0.03	Higher under Ash
	Location	11.67	0.002	Lower under HS
Jul-06	None			
Sep-06	None			
May-07	None			
Jul-07	None			
Aug-07	None			
May-08	None			
Jul-08	None			
Aug-08	None			

Table 6, Cont.

	Nitrification			
	Effect	F	p	Trend
Jul-05	None			
May-06	None			
Jul-06	Species	3.23	0.05	Higher under Hickory
Sep-06	None			
May-07	Location	10.65	0.002	Lower under HS
Jul-07	None			
Aug-07	Location	3.9	0.03	Lower under HS
May-08	None			
Jul-08	Species	3.2	0.05	Lower under Ash
Aug-08	None			

Table 6, Cont.

	Percent Nitrification			
	Effect	F	p	Trend
Jul-05	None			
May-06	None			
Jul-06	Species	3.63	0.04	Higher under Hickory
Sep-06	None			
May-07	Species	3.22	0.05	Lower under Ash
Jul-07	None			
Aug-07	None			
May-08	None			
Jul-08	None			
Aug-08	None			

Table 6, Cont.

	Ammonification			
	Effect	F	p	Trend
Jul-05	Location	4.87	0.03	Higher under HS
May-06	Sp*Loc	4.32	0.02	Elevated under HS-Hick not other species
Jul-06	None			
Sep-06	Species	4.55	0.02	Higher under Ash
May-07	None			
Jul-07	None			
Aug-07	None			
May-08	Species	3.72	0.03	Higher under Ash
Jul-08	Species	4.24	0.02	Higher under Ash
Aug-08	None			

Table 6, Cont.

	Mineralization			
	Effect	F	p	Trend
Jul-05	Sp*Loc	5.44	0.009	Elevated under HS-Hick not other species
May-06	Sp*Loc	6.16	0.005	Elevated under HS-Hick not other species
Jul-06	None			
Sep-06	None			
May-07	Location	4.59	0.04	Lower under HS
Jul-07	Sp*Loc	5.11	0.01	Depressed under HS-Ash, not other species
Aug-07	Species	4.14	0.03	Highest under Ash
May-08	None			
Jul-08	None			
Aug-08	None			

Note: Samples were taken from the soils beneath native savanna trees (n = 3 species, n = 15 trees per species) in two locations (under an *L. maackii* shrub in the understory of a native tree species or in a spot in the tree understory that was free of *L. maackii*).

Table 7. Mean % mole abundance of the most common microbial phospholipid fatty acids (PLFAs) found in the soils beneath the native tree species and the invasive *L. maackii* shrubs in their understory. PLFAs are grouped by taxa.

	% mole abundance					
	Ash		Hickory		Oak	
PLFA	Tree	HS	Tree	HS	Tree	HS
16:1ω7c	6.5	6.8	6.7	6.8	6.9	7.2
cy17	**3.1**[ab]	**3.2**[b]	**2.8**[a]	**2.9**[a]	**2.9**[ab]	**3.1**[ab]
18:1ω7c	**1.2**[ab]	**1.2b**	**1.1**[a]	**1.2**[ab]	**1.1**[a]	**1.2**[ab]
18:1ω5	1.2	1.1	0.9	0.9	1.1	1.1
cy19	6.6	6.1	5.9	6.3	5.1	5.1
i14	0.6	0.9	0.9	0.7	0.9	1.2
i15	5.2	5	5.9	5.8	4.9	5.3
i16	2.2	2.1	2.5	2.3	1.9	1.8
i17	**1.6**[abc]	**1.5**[b]	**1.7**[c]	**1.6**[abc]	**1.7**[ac]	**1.6**[ab]
a17	2.1	2.1	2	1.9	2.1	2
10Me16	**4.6**[ab]	**5.2**[bc]	**7.1**[cd]	**5.4**[ab]	**7.8**[d]	**7.5**[d]
10Me18	1.7	1.8	1.8	1.9	1.7	1.9
18:2ω6	2.4	2	2.7	2.9	2.5	2
18:1ω9c	**6.8**[ac]	**6.4**[abcde]	**5.7**[bde]	**6.3**[cde]	**6.2**[de]	**5.8**[e]

Table 7, Cont.

	% mole abundance					
	Ash		Hickory		Oak	
PLFA	Tree	HS	Tree	HS	Tree	HS
18:00	2.1	1.9	2	2.1	1.8	1.7
i17:1ω7	2.8	2.8	2.8	2.9	2.9	2.9

Note: Samples were taken from the soils beneath native savanna trees (n = 3 species, n = 15 trees per species) in two locations (under an *L. maackii* shrub in the understory of a native tree species or in a spot in the tree understory that was free of *L. maackii*). All values are means ± 1 SE. Significant differences are indicated with boldface type and different superscript letters indicate significant differences ($p < 0.05$) in mean % mole abundance of the PLFA across tree species, sampling location, or the interaction between the two. For statistics, see Appendix 9.

Figure 5. Microsite and soil physicochemical properties differences among native tree species and associated *L. maackii* shrubs. Error bars show ± 1 SE.

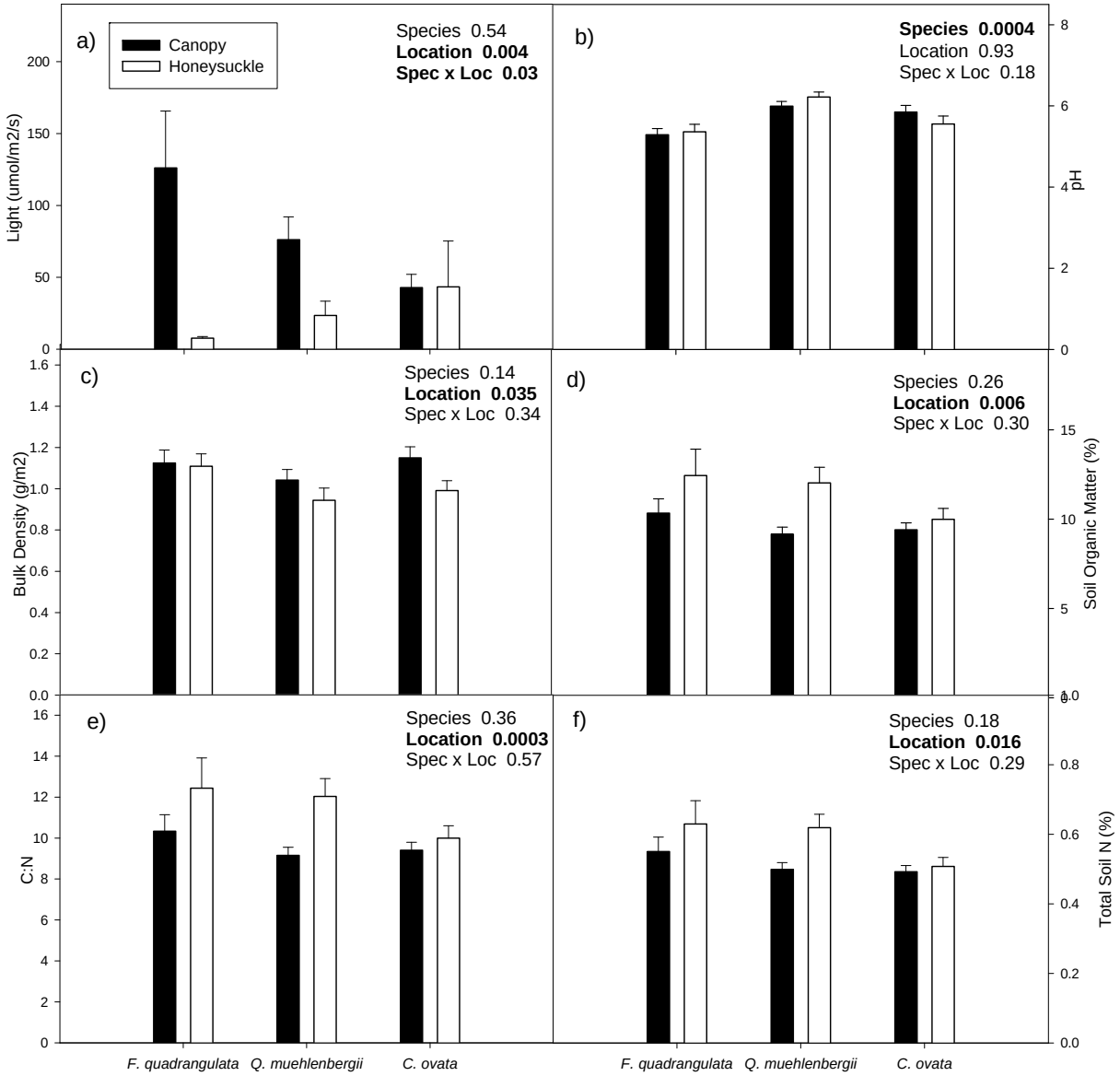


Figure 6. The total biomass (A), bacterial biomass (B), fungal biomass (C), and fungal:bacterial ratio(D) in the soil beneath the canopy of the native tree species and the *L. maackii* shrub in their understory. Error bars show ± 1 SE.

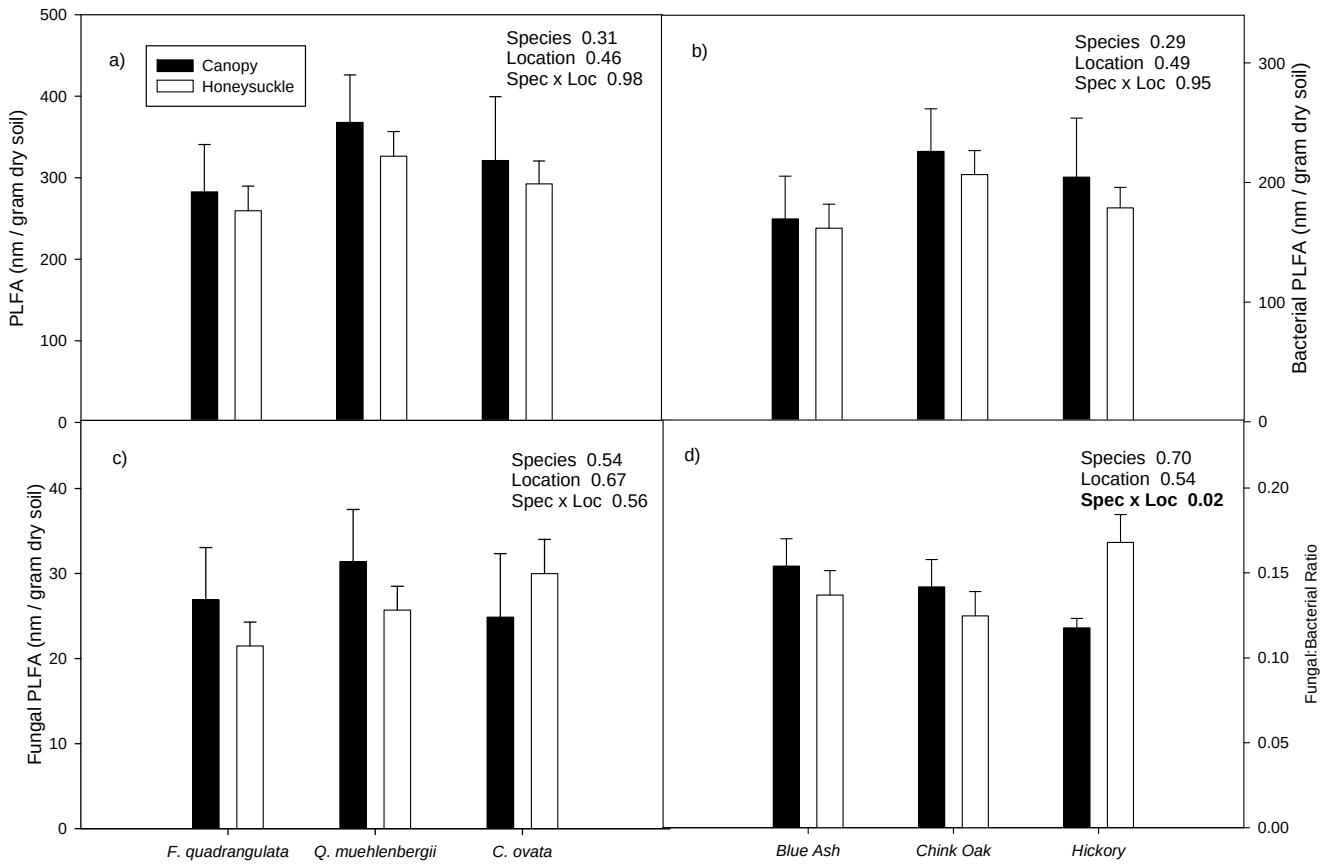


Figure 7. The % mole abundance of PLFAs pooled into the following taxonomic groups: gram-positive bacteria (A), gram-negative bacteria (B), non-specific bacteria (C), fungus (D), actinomycetes (E), and SRB and anaerobic bacteria in the soil beneath the canopy of the native tree species and the *L. maackii* shrub in their understory. Error bars show ± 1 SE.

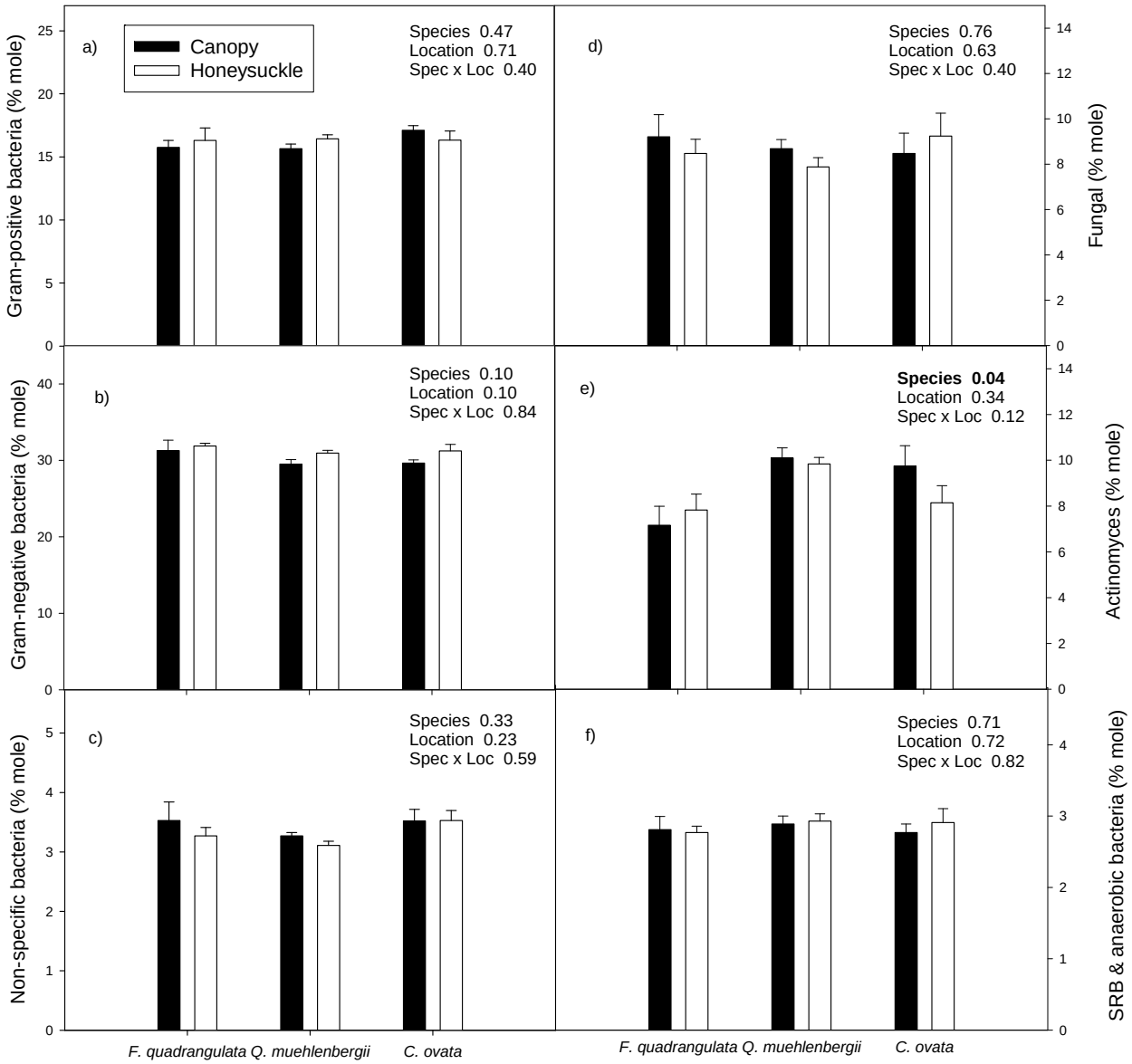
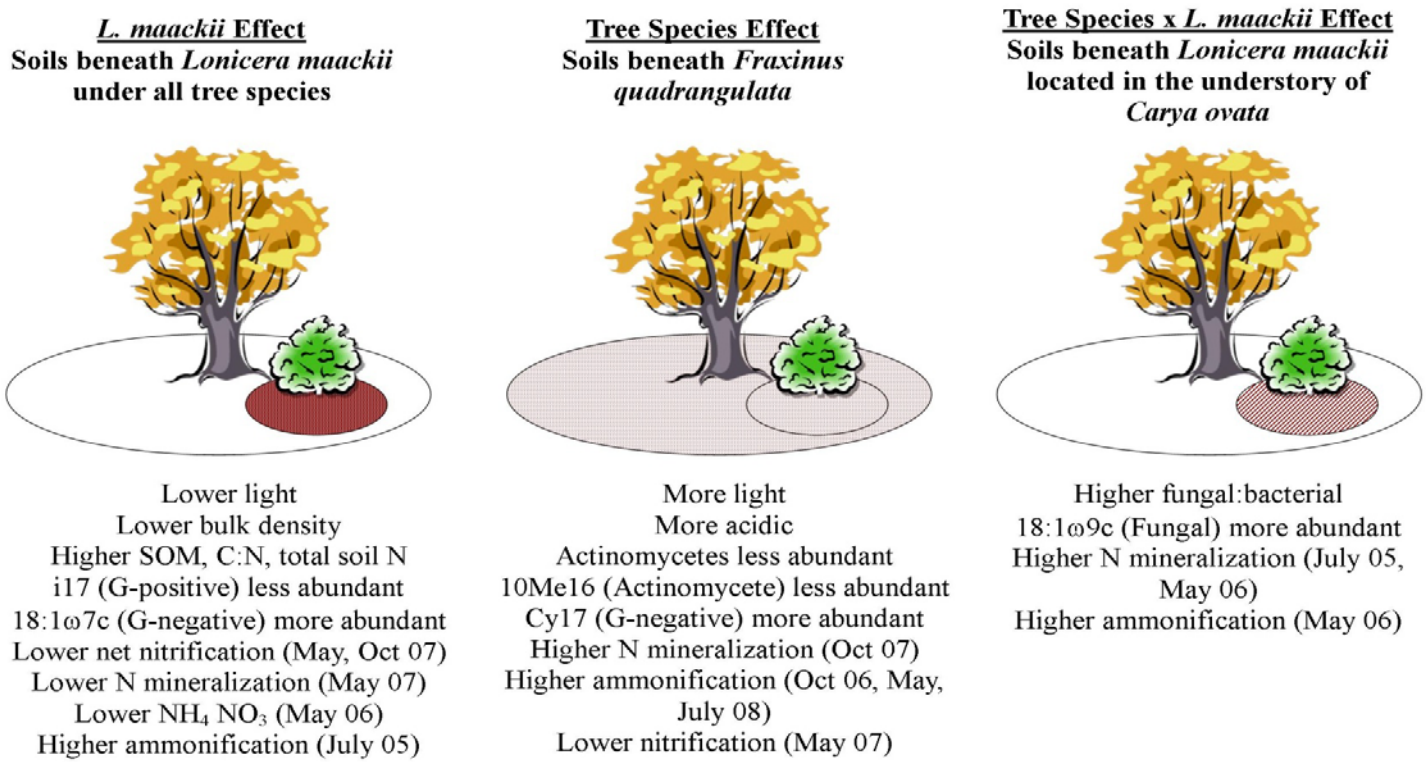


Figure 8. Summary of comparison between soil physicochemical conditions, N cycling, and the composition of the soil microbial community in the soils beneath *L. maackii*, *F. quadrangulata*, and *L.m aackii* shrubs located in the understory of *C. ovata*.



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Chapter 4

Throughfall nitrogen deposition is influenced by overstory native tree species, the invasive shrub *Lonicera maackii*, and season

Introduction

Throughfall is a critical component of the biogeochemical cycles of forest ecosystems (Levia and Frost 2006) conducting significant amounts of nutrients to forest soils. Throughfall is the portion of incident gross precipitation that passes through the canopy of a plant (Hewlett 1982) and can initially be intercepted by a vegetative surface and drip from the plant (release throughfall) or pass directly through the canopy (free throughfall) (Levia and Frost 2006). Terrestrial ecosystems production is often constrained by nitrogen (N) (Vitousek and Howarth 1991). Inorganic nitrogen deposited via throughfall can be taken up immediately by plants and such inputs may constitute an important pool of plant available nitrogen. Inorganic N inputs via throughfall vary, originating from three sources. (1) Wet deposition channels atmospheric compounds attached to precipitation droplets to the soil (Krupa 2002). Particulate forms of nitrogen including compounds of NH_4^+ and NO_3^- dissolve readily in water, leading to the wet deposition of inorganic N via rain or snowfall (Lovett and Lindberg 1993). (2) Dry substances entrained in the atmosphere can be deposited on leaf surfaces and washed off during precipitation events, transferring mineral matter like inorganic nitrogen from the surfaces of plants to the forest floor (Lindberg et al. 1986, Parker 1983). (3) Both uptake and the release of N occur at the leaf surface. Dry deposited compounds, including N, may be partially assimilated by the foliage directly (Lovett and Lindberg 1993, Horvath

2004), but inorganic N can also leach from leaves (Draaijers et al. 1994, Andre et al. 2008) and wash off with throughfall. Consequently, tree canopies can act as a net source or sink for inorganic nitrogen (Potter et al. 1991).

Numerous meterological factors like the magnitude and duration of precipitation events and site factors like the polluting environment can have an effect on throughfall chemistry (see review in Levia and Frost 2006, Andre et al. 2008). Effects of stand and tree species-specific characteristics on throughfall nutrient concentrations have been well documented (Henderson et al. 1977, Lovett and Lindberg 1986, Fenn and Bytnerowicz 1997, Andre et al. 2008, Alexander and Arthur 2012). Individual tree species differ in the physiology and permeability of foliar tissues, crown architecture, foliage distribution, the surface properties of aerial tissues, and seasonal changes in leaf area and physiological activity (Andre et al. 2008). These traits affect the interception of precipitation and can determine whether the canopy acts as a net sink or source for deposited chemical compounds (Talkner et al. 2010), thereby influencing throughfall fluxes. Previous work addressing tree species-specific influences on throughfall chemistry has largely been conducted under monoculture stands. Effects stemming from the heterogeneity of canopy structure are less well understood; it may be more difficult to predict throughfall nutrient fluxes in mixed stands with a complex, layered canopy structure and varied timing of phenological phases. Further, while inputs to the forest floor may be controlled by overstory tree species, understory vegetation can contribute to canopy heterogeneity, and therefore can alter throughfall before it reaches the soil. Little work to date has examined throughfall under shrub species, particularly in temperate systems. No studies that we are aware of have considered the interaction between

overstory trees and shrub species in the understory and their combined effects on throughfall chemistry.

Woody shrub invaders are beginning to profoundly alter the structure and function of forest ecosystems in the eastern United States (Webster et al. 2006). At least 129 exotic shrub species have invaded the eastern U.S. (Fridley 2008), and shrubs like *Lonicera maackii* (L) (Amur honeysuckle), *Berberis thunbergii* DC. (Japanese barberry), *Rhamnus cathartica* L. (common buckthorn), *Ligustrum sinense* Lour. (Chinese privet), and *Elaeagnus umbellata* Thunb. (autumn olive) have proven especially aggressive. Although the movement of these species into eastern deciduous forests has been gradual thus far, once established, they tend to form dense monotypic stands in the forest understory. These invasive shrubs may provide an extensive surface for dry deposition (McEwan et al. 2012) and the interception of throughfall that has passed through the canopy of overstory tree species. This new canopy layer in the understory and the leaves therein will likely differ from those in the overstory, resulting in heterogeneity that may alter throughfall deposition of limiting nutrients like nitrogen. Invaded ecosystems often have elevated soil N, higher rates of N mineralization, and increases in soil NH_4^+ and NO_3^- relative to native systems (Liao et al. 2008). Invasive shrubs like *L. maackii, R. cathartica,* and *B. thunbergii* have N-rich litter that decomposes rapidly, highlighting one possible pulse of N in invaded systems (Ehrenfeld et al. 2001, Heneghan et al. 2002, Poulette and Arthur 2012). Throughfall inorganic N deposition represents another potentially important source of soil nitrogen, and exotic species-mediated alterations to throughfall chemistry may represent another important feature of invaded systems.

The objective of this research was to examine the effects of a heterogeneous native-plus-invasive canopy layer on throughfall inorganic N deposition. To examine these native-invasive species effects, we utilized a savanna ecosystem in Kentucky that has been invaded by the invasive shrub *Lonicera maackii*. This system provides a novel opportunity to ask questions about throughfall chemistry and canopy heterogeneity. In mixed forest stands, it is difficult to parse out interactive effects of individual species. In our savanna system, invading shrubs often occupy only half of the understory of the native tree species. This allows us to examine throughfall deposition first under the overstory tree's canopy (tree canopy) and secondly beneath a shrub canopy in the understory (tree canopy + shrub canopy), providing unique insight into the throughfall deposition process in a heterogeneous system (Figure 9). This savanna-like ecosystem further allowed to examine the effects of an invasive species on throughfall inorganic nitrogen deposition and the species-specific nature of interactions between native trees and an invasive species in the understory.

The savanna system is dominated by *Fraxinus quadrangulata* (Mill.) K. Koch (blue ash), *Quercus muehlenbergii* E. (chinkapin oak), and *Carya ovata* M. (shagbark hickory). Savanna tree canopies are non-overlapping, so we were able to explore tree-species effects on throughfall N fluxes, using three tree species with varying crown architecture, foliage distribution, and leaf area (Wharton and Barbour 1973). Given these structural differences, we hypothesized that (1) inorganic N composition of throughfall would vary among the different native tree species. We were also able to measure the alteration of these throughfall inorganic N fluxes by the presence of an invasive, exotic shrub in the understory of the native trees and evaluate the uniform or variable nature of

this effect. We hypothesized that (2) the presence of the invasive *L. maackii* would alter inorganic N deposition to the soils beneath the native trees and (3) may do so in a variable manner depending on the identity of the overstory native tree species. Passive throughfall collectors were used to examine N (specifically NO_3^- and NH_4^+) (Fenn et al. 2002, Simkin et al. 2004). Throughfall chemistry is typically assessed on a precipitation event-based schedule (Fenn et al. 2002) resulting in sampling issues that arise due to the highly spatially and temporally variable nature of individual rainfall events and the resultant solute inputs. Throughfall collector systems have been developed that use a mixed bed ion exchange resin column; as precipitation moves through the column, anions and cations are captured on the positively and negatively charged exchange sites on the resin (Simkin et al. 2004). This allows for long-term, seasonal throughfall sampling (Simkin et al. 2004), enabling us to fully explore seasonal differences in throughfall N fluxes beneath the different native species and native-invasive species combinations.

Methods

Study Site

Research was conducted at Griffith Woods, a savanna woodland in Harrison County, Kentucky. See McEwan and McCarthy (2008) for a detailed description of oak savannas in central Kentucky. The regional climate of the Inner Bluegrass region of Kentucky is continental and characterized as temperate and humid (Wharton and Barbour 1991). The mean annual temperature at the nearby Cynthiana, KY weather station (located at 38.4456 latitude, -84.1615 longitude) is 12.2°C and mean precipitation is 108 cm (http://www.sercc.com). The savanna encompasses approximately 32.4 ha with 150-

to 300-year old trees widely scattered in a matrix of grasses and forbs. The dominant, native trees at the site were identified as *Fraxinus quadrangulata, Quercus muehlenbergii,* and *Carya ovata.* *L. maackii* has invaded the site and shrubs of varying sizes are located primarily in the understory of the savanna trees and in the adjacent woodland. Although *L. maackii* only grows beneath the trees in the savanna, its establishment in the understory of the savanna trees appears to follow no discernable pattern, occurring regardless of cardinal direction, overstory branching pattern, or any other apparent attribute.

Passive Throughfall Collectors

Passive throughfall collectors were designed to sit at a height of approximately 61 cm above the ground so as to fit under *L. maackii* shrubs. In the field, a PVC pipe section approximately 20 cm long was pounded several cm into the ground. A hole was drilled just above ground level to allow for drainage from the pipe. Fiberglass stakes were pounded in and lashed to the pipes for stability. In the lab, resins were prepared by rinsing 1 L batches of an equal mix of a Lewatit Lanxess MonoPlus S100 resin (H^+ form) (Siemens) and an Ionac Sybron ASB-1P resin (OH^- form) (Siemens) with 3L of double de-ionized water. Resins were poured into a disposable polypropylene BioRad Econo-Pac column (20 mL capacity) (Simkin et al. 2004). Filled resin columns were affixed to a plastic, unpigmented HDPE funnel (20.32 cm outer diameter) with a piece of rubber tubing and placed in the PVC pipes in the field, with the funnel resting on the top of the pipe mouth and the resin column concealed within. Glass wool was placed in the neck of each funnel to prevent entry of debris. Five trees of each of the dominant savanna

species (n = 5 per species, n = 3 species) were randomly selected from a pool of 45 savanna trees for inclusion in this study. Four collectors were placed under each tree: two under each *L. maackii* shrub and two under the tree canopy in a location free of *L. maackii* for a total of 60 collectors. All collectors were located approximately 1 m from the bole of the tree. Two additional collectors were placed in the open savanna at a distance of 30 meters from the dripline of any savanna tree to establish bulk deposition of inorganic N for comparison to throughfall samples. Throughfall collectors were occasionally torn apart by animals (probably raccoons) or colonized by ants; as a result, there was some variation in the number of experimental units during each sampling period. This variation was accounted for in subsequent statistical analyses of the data.

Throughfall N Inputs

Resin columns were placed in the field on March 20, 2009 and monitored and collected at the end of three months to assess spring inputs of N via throughfall. The collected resin columns were replaced with fresh columns on June 20, September 20, and December 20 of 2009 to sample summer, fall, and winter throughfall inputs for a total of four collections. In the lab, resins were removed from each column and extracted two times with 50 ml of 1N KCl. Samples were then filtered through No. 1 Whatman paper. The resultant 100 ml of extractant was analyzed colorimetrically for nitrate and ammonium on an automated continuous-flow analyzer (Bran-Luebbe Autoanalyzer 3, Bran+Luebbe, Chicago, Illinois, USA). Background levels of nitrate and ammonium in the resin columns were determined using the same extraction procedure and were subtracted from the sample totals. Ten percent of all the samples were analyzed as

replicates. Seasonal throughfall N deposition (kg/ha) was calculated as content (extract concentration x extractant volume) divided by the collection area of the funnel. Net throughfall inorganic N deposition was then calculated by subtracting the bulk deposition of inorganic N (NO_3-N or NH_4-N) measured by the collector in the open savanna from the total throughfall deposition of N (TF – BD) under each tree and tree + *L. maackii* shrub.

Statistical Analysis

Differences in net throughfall N deposition were analyzed within a repeated measures (tree = repeated unit) mixed model ANOVA with time, tree species (n = 3 species), and location (under tree, under tree + *L. maackii*) as fixed main effects and tree number (replication) as a random effect. Throughfall N deposition data were summed across seasons to calculate annual deposition for each sampling location and analyzed in the above manner. For significant main-effects differences ($p < 0.05$), least squares means were compared using the LSMEANS procedure.

Results

Annual inorganic nitrogen deposition in the collectors placed in the open savanna away from the drip-line of any savanna trees (bulk deposition) was 1.26 kg ha^{-1}. Bulk deposition (BD) was dominated by NH_4-N, with NO_3-N contributing approximately 23% of total inorganic N when annual bulk NH_4-N and NO_3-N were summed (Table 8). Season had a significant effect on throughfall N deposition ($p < 0.001$). Deposition of total inorganic N and NH_4-N in throughfall was higher during the spring than the other

seasons and this effect persisted under all three tree species (Figure 10a,c). Throughfall deposition of NO_3-N was significantly lower during the summer compared to deposition in all other seasons (Figure 10b). Contributions to total throughfall inorganic N also varied by season. NO_3-N contributed approximately 25% of total inorganic N concentrations in the fall and winter (averaged across all tree species) and approximately 12-14% of total inorganic N concentrations in the spring and summer, respectively (averaged across all tree species, $p < 0.0001$).

Annual throughfall (TF) inorganic N (NH_4-N and NO_3-N) and NH_4-N fluxes were higher than those of bulk deposition (TF > BD), and the annual enrichment ratio (TF:BD) for both exceeded 1.0 under all three tree species (Table 8). This effect largely persisted across seasons with one exception: NH_4-N deposition under *F. quadrangulata* in the fall decreased relative to bulk deposition (Figure 1). Throughfall NO_3-N deposition was mixed, increasing (TF > BD) and decreasing (TF < BD) across seasons and species (Table 8 and Figure 10).

Tree species had a significant effect on total annual net inorganic N deposition (net throughfall deposition = TF - BD). Annual net deposition of throughfall inorgaic N was positive under all three tree species (TF > BD), but was significantly less under *F. quadrangulata* than the other tree species ($p = 0.02$, Figure 11). Net throughfall NO_3^-N deposition was often negative (TF < BD), especially in the understory of *F. quadrangulata* (Figure 12). There was a tree species effect on net throughfall NO_3-N deposition in the winter ($p = 0.006$) and summer ($p = 0.005$). In the summer (June 20-September 20), net inorganic N deposition was negative (BD > TF) under all three species, but this effect was most pronounced under *F. quadrangulata,* with significantly

less net throughfall NO_3-N deposition beneath its canopy compared to the other tree species ($p = 0.005$, Figure 4f). In the winter, net throughfall NO_3-N deposition was negative (BD > TF) under *F. quadrangulata* and positive (BD < TF) under the other two tree species (Figure 12j). This effect persisted when net throughfall NO_3-N deposition was summed across the seasons, with less NO_3-N penetrating the canopy of *F. quadrangulata* annually ($p = 0.006$, Figure 12b). Annual net NO_3-N deposition was negative (BD > TF) under the canopy of *F. quadrangulata* and positive (BD < TF) under the canopy of the other two tree species. Annual net NH_4-N deposition also tended to be lower beneath the canopy of *F. quadrangulata*, although the effect was not significant (Figure 12).

The presence of the invasive shrub *L.maackii* in the understory did not significantly affect annual net throughfall deposition of inorganic N, NO_3-N, or NH_4-N. However, *L. maackii* did have a seasonal effect on throughfall NO_3-N. In the spring, *L. maackii* significantly increased net throughfall NO_3^--N deposition (BD < TF) under all three tree species ($p = 0.02$, Figure 11d). While net NO_3-N deposition under *L. maackii* (tree + HS) was positive in the spring (BD < TF), deposition beneath all three tree species (tree only) was negligible and negative (BD > TF) under *F. quadrangulata* and *Q. muehlenbergii* (Figure 11d). The presence of *L. maackii* (tree + HS) significantly decreased net throughfall NH_4^+-N deposition ($p = 0.02$, Figure 12c). In this case, net NH_4-N deposition was positive beneath both the native trees (tree) and *L. maackii* (tree + HS).

Discussion

Annual inorganic N in bulk deposition was 1.26 kg ha^{-1} which is low compared to rates of deposition found across North America and in Central Europe (Mustajarvi et al. 2008, see review in Pelster et al. 2009). Fluxes are similar to those measured in two recent event-based throughfall studies conducted within 100 miles of our site (Alexander and Arthur 2010, McEwan et al. 2012) and are similar to levels found in a low-pollution stand in California (Fen and Bytnerowicz 1997). This indicates that our site receives little atmospheric deposition relative to forests in the Northeastern United States and Central Europe. The site is in a relatively isolated rural area; the nearest urban areas are Lexington KY (population 295,803) and Louisville KY (population 597,337) located approximately 30 and 90 miles respectively from the savanna-woodland (U.S. Census Bureau 2010). Our site is also situated within a lower zone of annual NO_3 and NH_4 deposition as recorded by the National Atmospheric Deposition Program (NADP).

Tree species effects

The identity of the native tree species had a significant effect on annual throughfall inorganic N and NO_3-N deposition, supporting our first hypothesis. The native tree *F. quadrangulata* appeared to drive most of these differences and it is important to note that these differences are identified as those that occurred in the understory of both the native tree (tree) and the understory of *L. maackii* shrubs beneath the native tree (tree + HS). Significantly less annual net throughfall N and NO_3-N were deposited beneath the canopy of *F. quadrangulata* compared to the other two tree

species, and *F. quadrangulata* acted as a sink for NO_3-N across the sampling year (Figure 11 and 12b).

Tree canopies retain N in three ways: absorption onto the surface of the leaf, foliar uptake, or assimilation by microorganisms (Wilson 1992, Lovett 1994, Krupa 2003, Pelster et al. 2009). The branching structure of *F. quadrangulata* may partially account for these differences. Compared to the other two native tree species at our site, *F. quadrangulata* trees tended to have a tall, narrow, and highly irregular crown structure. The interception of precipitation as it passes through the irregular crown may be greater, trapping more inorganic nitrogen on leaf surfaces. In the winter, *F. quadrangulata* acted as a sink for NO_3-N (TF < BD) while the other two tree species acted as a source (TF > BD). This suggests that the branching structure of *F. quadrangulata* may be an important feature in the interception of precipitation. The distribution of branches can significantly influence spatial patterns of throughfall nutrient deposition (including NH_4 and NO_3) during the growing season and non-foliated periods (Staelens et al. 2006). The water storage capacity of branches is larger than that of leaves (Llorens and Gallart 2000) and the interception of rain and snowfall precipitation and the nutrients stored within during the winter may have been higher in the canopy of *F. quadrangulata.*

In addition to structural properties of the canopy, interception, deposition and leaching are dependent on species specific leaf attributes like leaf area and the physical and chemical properties of the leaf surface (Talkner et al. 2010). In the summer, all three tree species acted as a sink for NO_3-N (BD > TF), but net throughfall NO_3-N deposition was significantly lower under *F. quadrangulata*. On the whole, throughfall NO_3-N

deposition was lower in the summer than any of the other seasons ($p < 0.0001$), so very little NO_3-N was deposited via wet deposition or dry deposition. Attributes of *F. quadrangulata* leaves may have helped the trees trap inorganic N more efficiently than the other species, making NO_3-N from wet and dry deposition more available for uptake at the leaf surface.

Total inorganic N and NH_4-N throughfall fluxes were generally higher than fluxes in bulk deposition (Figures 11 and12a). Native tree canopies acted largely as a source of NH_4-N over the course of the year. Throughfall studies are inconsistent, with some showing enrichment of inorganic nitrogen in throughfall (Henderson et al. 1977, Herrmann et al. 2006, Terauda and Nikodemus 2007) and others show assimilation of inorganic nitrogen in the canopy (Potter et al. 1991, Pryor and Barthelmie 2005, Duchesne and Houle 2006, Pelster et al. 2009). Positive net throughfall values (TF > BD) in our study suggests either leaching from the canopy or the wash-off of dry deposited inorganic nitrogen particles (Balestrini et al. 2007, Pelster et al. 2009). Net throughfall NH_4-N peaked in the spring (March 20-June 20); tree species leaf emergence occurred at the tail end of this spring sampling period.

This significant seasonal difference in net deposition ($p < 0.0001$) may be due in part to leaching from the newly emerging canopy. Our site is located in close proximity to agricultural operations (crops and livestock) so atmospheric deposition of inorganic nitrogen compounds may also have been higher at this time of year. The influence of agricultural activities and ammonia emissions from livestock farming has been associated with higher concentration of NH_4-N in bulk deposition and throughfall in other studies (Draaijers et al. 1989, Asman et al. 1998). Gaseous ammonia in a forest in Germany

accounted for throughfall deposition rates of NH4-N that were 4 times higher than bulk deposition rates (Herrmann et al. 2006); dry deposition of NH_4^+ and gaseous ammonia accounted for 66% of the total NH_4^+ throughfall deposition at that site (Marques et al. 2001).

Invasive species effects

In partial support of our second hypothesis, the invasive shrub *L. maackii* had little effect on net throughfall inorganic N deposition, with one notable exception. In the spring, soils beneath the tree canopies (tree) and *L. maackii* canopies (tree + HS) received approximately the same amount of net inorganic N deposition. However, net throughfall inorganic N under the tree canopies (tree) was almost entirely comprised of NH_4-N while soils beneath *L. maackii* (tree + HS) received a mixture of NH_4-N and NO_3-N. Soils beneath *L. maackii* received less net throughfall NH_4^+-N deposition and more net throughfall NO_3^-N relative to soils beneath the trees where *L. maackii* was absent (Figure 4c, d). This effect was uniform and did not vary based on associations with native tree species, refuting our third hypothesis.

Like many other woody invasive shrubs, *L. maackii* has a longer leaf phenology than comparable native species, leafing out earlier in the spring and retaining its leaves late into the fall and early winter (McEwan et al. 2009). In central Kentucky, *L. maackii* leaves emerge in early March and can be fully expanded by late March (McEwan et al. 2009), a full four weeks prior to the emergence and expansion of the native tree leaves at our study site (personal observation). This emergent leaf area is available for dryfall

interception of inorganic nitrogen compounds and may also be more prone to leaching at this stage of development.

One possible explanation may lie in the differential colonization of the emerging phyllosphere by litter microbial communities. Both the dry deposition and leaching of NH_4 may occur at the leaf surface of both trees and *L. maackii* making more inorganic N available. However, the more mature *L. maackii* leaves present later in the spring when the tree leaves are just beginning to emerge may support a more robust nitrifying bacteria community that may convert some of that NH_4^+ into NO_3^-. This would explain the similar amounts of total net inorganic N but variable contributions of NH_4-N and NO_3-N to the throughfall pool beneath and away from the invasive shrub.

For the remainder of the year, the invasive shrub had no effect on throughfall inorganic N deposition. It is possible that once the overstory native tree canopy fully emerged, *L. maackii* was shielded from atmospheric dry deposition of nitrogen and did not act as a novel deposition surface in the understory, limiting shrub contributions to net throughfall inorganic nitrogen. Alternatively, it may suggest that while dry deposition still occurred, *L. maackii* and/or the microorganisms living on the leaf surface were highly efficient at assimilating inorganic nitrogen. It further suggests that any leaching of inorganic nitrogen from the leaf surface of *L. maackii*, is limited to leaf emergence in the spring and does not occur during the remainder of the growing season or during leaf senescence in the fall.

Regardless of the mechanism, spring throughfall inorganic N fluxes to the soil beneath *L. maackii* contained more NO_3-N than fluxes beneath the native tree species. Plant preferences for different forms of inorganic N in the soil can vary (Templer and

Dawson 2004) and *L. maackii* preferences are unknown. Nitrate is highly soluble in soil water and diffuses more rapidly to plant roots (Lambers et al. 1998, Templer and Dawson 2004). Early leaf emergence may be of critical importance to *L. maackii* as it maximizes photosynthetic capacity prior to upper canopy closure by the native tree species. Pulses of throughfall NO_3-N in the spring may help *L. maackii* rapidly assimilate nitrogen to build up leaf mass and photosynthetic enzymes prior to canopy closure, facilitating its growth.

Forests in the northeastern United States have come under increased pressure from similar invasive shrubs like *B. thungergii*. These systems tend to receive much higher atmospheric N deposition and are also showing signs of N saturation (Lovett et al. 2000, Lawrence et al. 2004). Throughfall fluxes were relatively low at our site. If spring throughfall flux patterns are similar under invasive shrubs in areas with significantly higher throughfall N deposition, pulses of NO_3 may prove highly problematic. These larger pulses may not only facilitate the growth of invasive species in these systems but could also increase leaching of NO_3 into groundwater and surface waters. Future examinations of heterogenous native tree-invasive shrub throughfall should break up early season throughfall sampling periods to account for different leaf emergence times. This would allow for a further exploration of invasive shrub impacts on throughfall inorganic N deposition during early season leaf emergence and potential feedbacks in the soil.

Another examination of *L. maackii* effects on throughfall inorganic N deposition was conducted in three forests in central Kentucky, including woodland adjacent to our savanna site (McEwan et al. 2012). This event based-precipitation study used a

conventional throughfall collector and examined throughfall deposition along a forest transect in collectors located both under and away from *L. maackii* shrubs. The sampling of a precipitation event in May revealed no effect of *L. maackii* on NH_4-N and NO_3-N throughfall deposition. However, NH_4-N throughfall deposition was significantly lower under *L. maackii* following an August and November precipitation event (McEwan et al. 2012). While event based sampling is informative, it can be highly temporally variable due to the varying magnitude, duration, and intensity of the precipitation event and differences in wind conditions at the time of sample collection (see review in Levia and Frost 2006). The use of passive collectors provides an opportunity to examine long-term, seasonal throughfall inorganic N inputs and may ultimately provide a better understanding of invasive species effects.

The use of conventional throughfall collectors in McEwan et al.'s study (2012) did reveal significantly lower throughfall volume beneath *L. maackii* shrubs which we were unable to assess using the long-term resin collectors. While the expansive, dense canopy of *L. maackii* may intercept a significant amount of rainwater/throughfall from overstory trees, it is unclear if this water is retained by the canopy or funneled to the base of the shrub via stemflow. Stemflow conducts precipitation down plant branches and stems depositing water and nutrients directly at the plant stem (Levia and Frost 2003). It is often volumetrically insignificant and low in nutrients in comparison to throughfall and as such is not often addressed in the literature (Levia and Frost 2003). Stemflow contributions by volume are highly variable among shrub species and appear to be related to the aerial structure of the shrub species (Llorens and Domingo 2007). Stemflow – derived nutrients represent another unexplored avenue of N enrichment in the soils

beneath invasive shrubs like *L. maackii*. Studies in desert ecosystems demonstrate that shrubs can redirect intercepted rainfall via stemflow to deep storage among root channels (Martinez-Meza and Whitford 1996) facilitating the creation of 'fertile islands' beneath desert shrubs (Whitford et al. 1997). The multi-stemmed growth form characteristic of *L. maackii* may intercept significant amounts of precipitation and nutrients and funnel them to its base. Although challenging to measure on shrubs with multiple stems, future studies could incorporate stemflow measurements into examinations of throughfall beneath invasive shrubs like *L. maackii*.

Passive throughfall collection under shrubs

Two features of the passive throughfall collection system may have biased our results. First, the height of the collectors was low relative to other studies. To measure deposition under *L. maackii* shrubs, collectors were elevated approximately 24 cm off of the ground. To stay consistent, collectors in all three locations (under tree, under tree+HS, in the open) were constructed in the same manner. In other studies, open collectors (bulk deposition) are commonly elevated much higher off the ground, sometimes over the top of the forest canopy. Dry deposition may have been lower in our short collectors relative to a taller collector, resulting in an underestimation of bulk deposition. Second, the use of ion exchange resins in throughfall sampling is a promising but relatively new technique. Field tests of the system have found that throughfall inorganic N deposition measurements in a conventional throughfall solution collector and an ion exchange resin column were statistically equal (Fenn et al. 2002). There are some indications that background levels of NH_4^+ may become slightly elevated over time due

to a slow release of quaternary amine groups from the anion exchange resin beads (Fenn and Poth 2004). This is likely to be more of an issue in collectors left in the field for longer periods of time (ie 12 months). The replacement of resin columns every three months to capture seasonal throughfall is likely to have lessened the risk of any such bias.

Ultimately, the use of passive throughfall collectors offered insight into throughfall inorganic nitrogen deposition fluxes beneath a heterogenous tree-invasive shrub canopy. Consistent with the literature, we observed species-specific tree effects on throughfall inorganic N deposition, with *F. quadrangulata* acting as a sink for NO_3-N while *Q. muehlenbergii* and *C. ovata* acted as a source. All three species acted as a source of annual throughfall inorganic nitrogen, although fluxes were significantly lower beneath *F. quadrangulata* relative to the other tree species. The presence of the invasive species altered throughfall deposition of inorganic nitrogen in the spring, with increased net NO_3-N throughfall deposition relative to the native tree species. Future examinations of dry deposition, leaching, and canopy uptake at the leaf surface, microbial community identity and development at the phyllosphere, and stemflow deposition throughout the growing season may shed light on invasive species effects and the potential implications of increasing forest invasion by exotic woody species. Finally, dissolved organic nitrogen is an important component of throughfall N deposition and canopy exchange that we did not explore, despite its potentially important role in N cycling (Neff et al. 2002). Collection of dissolved organic nitrogen is not possible using ion exchange resin collectors, so examinations of this pool of throughfall nitrogen would require move conventional throughfall collectors. Throughfall deposition is a major pathway in

nutrient recycling in forest systems and its potential alteration by the presence of an invasive species bears further investigation.

Table 8. Annual bulk deposition (BD), throughfall deposition (TF), and the enrichment ratio (TF:BD deposition ratio) of inorganic N under the three native tree species. Annual deposition rates in kg ha^{-1}. N_I – total inorganic N deposition (NO_3-N + NH_4-N).

		F. quadrangulata		*Q. muehlenbergii*		*C. ovata*	
	BD	TF	TF:BD	TF	TF:BD	TF	TF:BD
NO_3-N	0.3	0.23	0.68	0.46	1.43	0.3	1.06
NH_4-N	0.97	1.8	1.68	2.28	2.27	2.23	2.26
N_I	1.26	2.03	1.47	2.74	2.09	2.53	1.98

Figure 9. Partitioning of throughfall in the savanna (P_g) is incident gross precipitation, TF_T is throughfall deposition under the tree only, TF_{T+S} is throughfall deposition under the tree and the shrub. Adapted from Levia and Frost (2006).

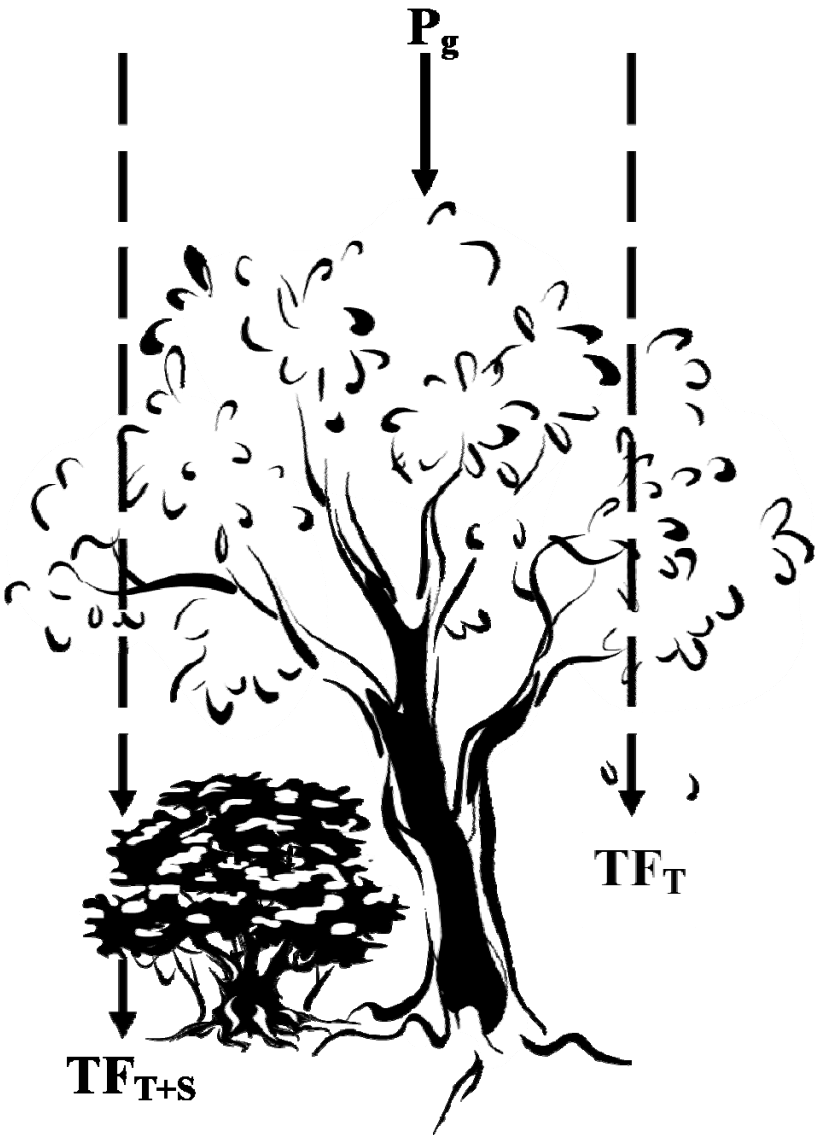


Figure 10. Annual and seasonal inorganic N deposition (NO_3-N + NH_4-N) (a), NO_3-N deposition (b), and NH_4-N deposition in bulk deposition and throughfall collected in the understory of the native tree species. Note the change in scale between panels. Different letters above bars indicate significant differences within sampling periods ($p < 0.05$). Error bars show ± 1 SE.

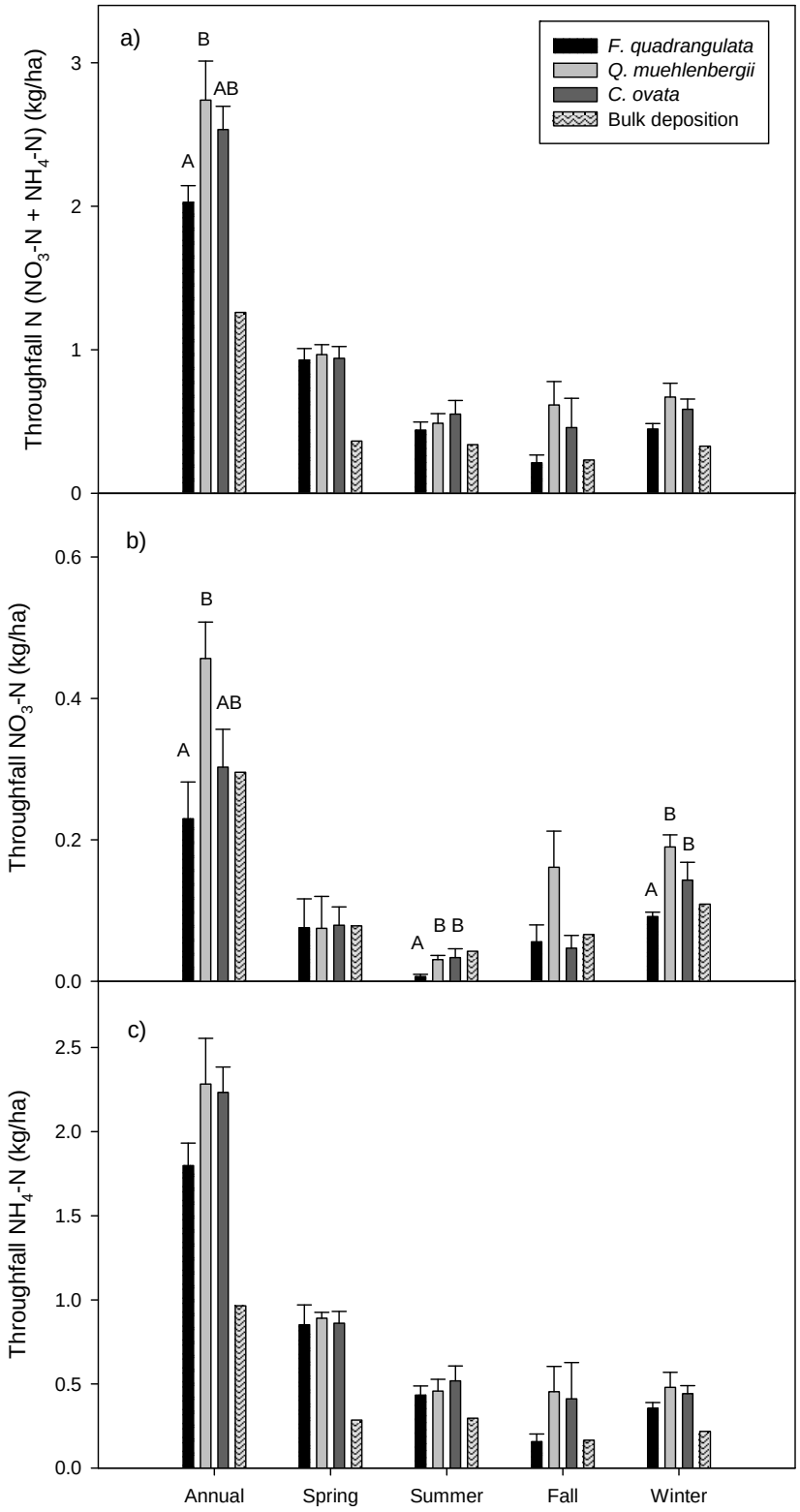

a)
b)
c)
F. quadrangulata
Q. muehlenbergii
C. ovata
Bulk deposition
Throughfall N (NO3-N + NH4-N) (kg/ha)
Throughfall NO3-N (kg/ha)
Throughfall NH4-N (kg/ha)
A
B
AB
A
B
AB
A
B B
A
B
B
Annual
Spring
Summer
Fall
Winter

Figure 11. Annual net throughfall (throughfall – bulk deposition) inorganic N deposition ($NH_4^+ + NO_3^-$) in the understory of the native tree species (n = 3) and *L. maackii* shrubs located under the trees. Error bars show ± 1 SE.

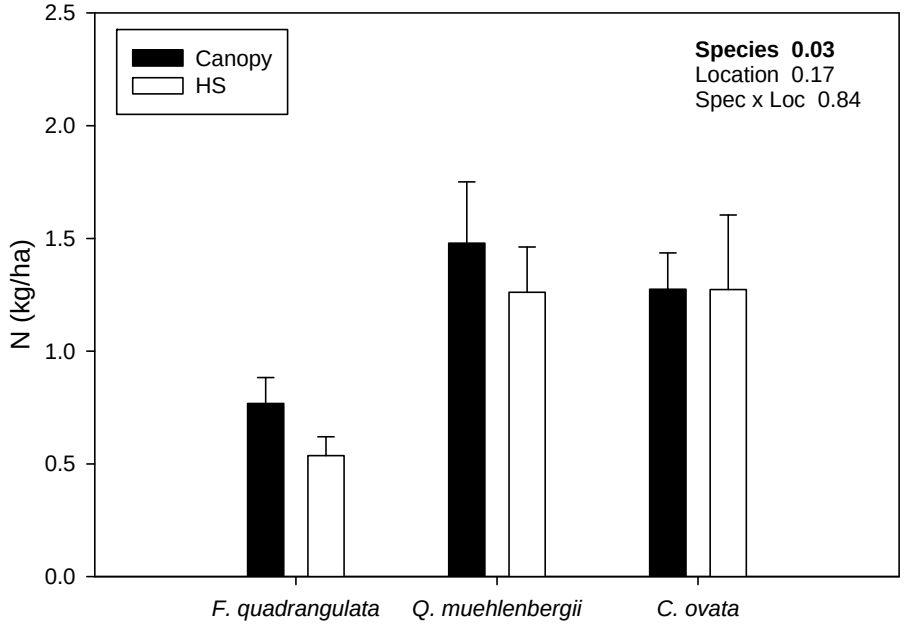
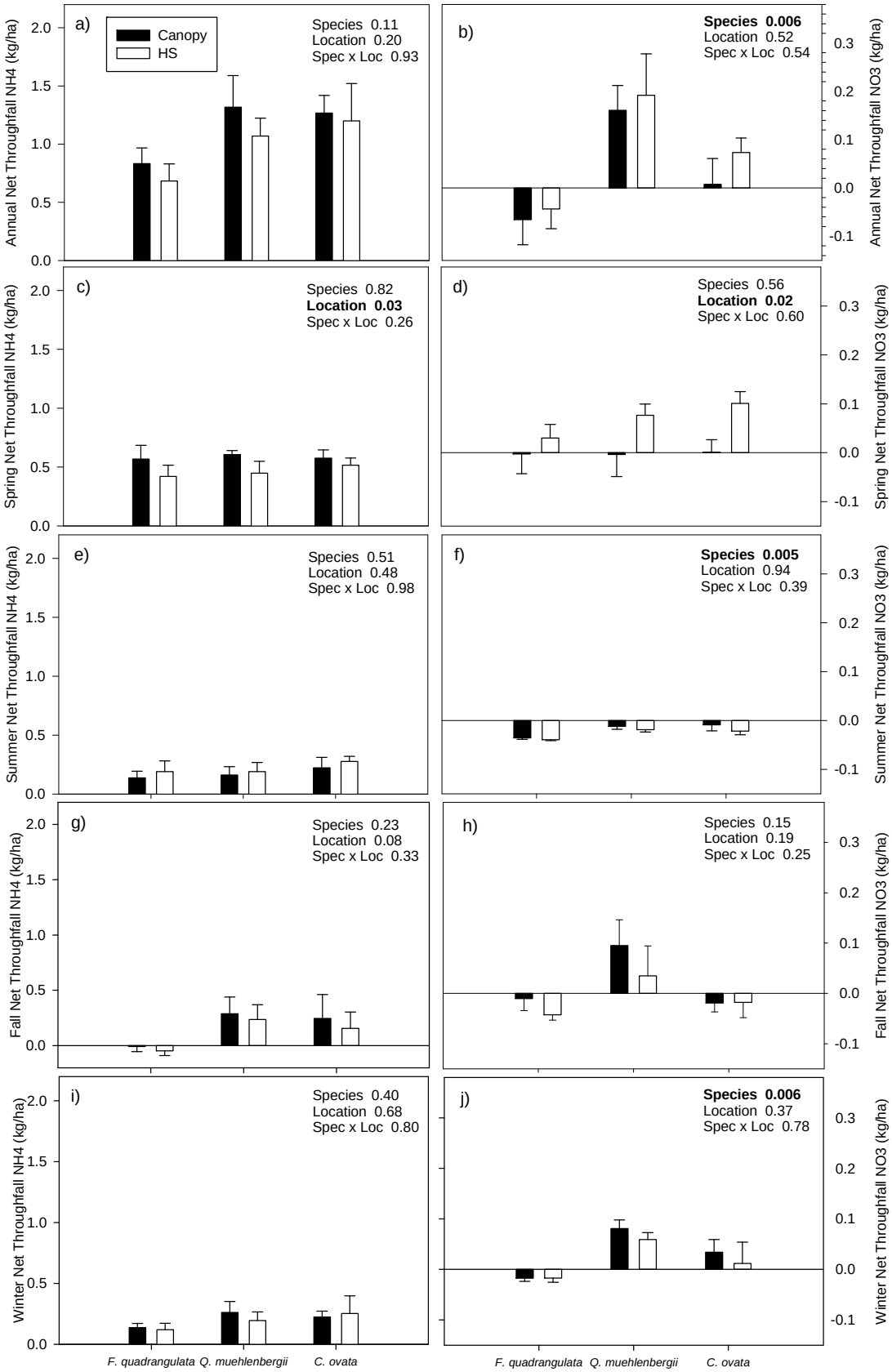


Figure 12. Net seasonal throughfall N deposition (throughfall – bulk deposition) in the understory of the native tree species (n = 3) and *L. maackii* shrubs located under the trees. Error bars show ± 1 SE.


a)
Canopy
HS
Species 0.11
Location 0.20
Spec x Loc 0.93
Annual Net Throughfall NH4 (kg/ha)
b)
Species 0.006
Location 0.52
Spec x Loc 0.54
Annual Net Throughfall NO3 (kg/ha)
c)
Species 0.82
Location 0.03
Spec x Loc 0.26
Spring Net Throughfall NH4 (kg/ha)
d)
Species 0.56
Location 0.02
Spec x Loc 0.60
Spring Net Throughfall NO3 (kg/ha)
e)
Species 0.51
Location 0.48
Spec x Loc 0.98
Summer Net Throughfall NH4 (kg/ha)
f)
Species 0.005
Location 0.94
Spec x Loc 0.39
Summer Net Throughfall NO3 (kg/ha)
g)
Species 0.23
Location 0.08
Spec x Loc 0.33
Fall Net Throughfall NH4 (kg/ha)
h)
Species 0.15
Location 0.19
Spec x Loc 0.25
Fall Net Throughfall NO3 (kg/ha)
i)
Species 0.40
Location 0.68
Spec x Loc 0.80
Winter Net Throughfall NH4 (kg/ha)
j)
Species 0.006
Location 0.37
Spec x Loc 0.78
Winter Net Throughfall NO3 (kg/ha)
F. quadrangulata
Q. muehlenbergii
C. ovata

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CHAPTER 5: CONCLUSIONS AND FUTURE DIRECTIONS

A growing body of literature has served to document the spread and impacts of woody invasive shrubs in eastern US forests. Studies on one especially pervasive woody invasive shrub, Amur honeysuckle (*Lonicera maackii)*, have proliferated in recent years. Through a suite of physiological and reproductive traits, this aggressive, woody invasive shrub is poised to significantly alter community and ecosystem dynamics in eastern forests in the US. Amur honeysuckle shrubs have a multi-stemmed growth form with a high degree of plasticity in branch architecture, a long growing season, and highly successful reproductive strategies. These characteristics, in part, allow for this invasive shrub to successfully establish in the forest understory. There is evidence that the resulting formation of dense, monotypic stands significantly reduces the plant and animal biodiversity in these systems. Animal population dynamics may be affected by restricted movement through forests, changes in predator-prey dynamics, a reduction in palatable food for native herbivores, and alterations to the forest food web via negative effects on arthropods. The suppression of native plant communities, tree seedlings in particular, may have a significant influence on the productivity, successional dynamics, and disturbance regimes in these systems, although these long-term effects are poorly understood. While a clearer picture of the population and community level effects of Amur honeysuckle has begun to emerge, ecosystem impacts of this invasive shrub have not been addressed as fully in the literature.

In an effort to address current gaps in the literature, the research presented in this dissertation examined Amur honeysuckle's potential to alter litter decomposition dynamics, nutrient cycling, soil properties, and the soil microbial community in invaded

systems. Five years of field data reveal that Amur honeysuckle may have significant effects on ecosystem processes. Like other invasive shrubs, it has an N-rich litter that rapidly decomposes and loses N, especially in comparison to the litter of native tree species. Honeysuckle's litter synergistically accelerated N loss of native tree litter at particular times during a decomposition experiment. New work by Arthur et al. (in review) examining Amur honeysuckle litter decomposition has confirmed the findings presented herein that honeysuckle litter breaks down more rapidly than native litter. However, they also discovered that the decomposition of both Amur honeysuckle litter and native tree litter is slower under honeysuckle shrubs. Further examination of decomposition dynamics both in the understory of honeysuckle and in invaded plots is clearly needed to clarify these findings. Measurements of the species composition and depth of the litter layer beneath Amur honeysuckle throughout the fall, winter, and spring may help to understand the timing of decomposition and resulting nutrient pulses. Lysimeters or ion exchange resin bags installed beneath honeysuckle shrubs could be used to link aboveground decomposition dynamics and belowground pulses of nutrients at critical times during the growing season.

The presence of Amur honeysuckle altered spring throughfall deposition of inorganic nitrogen beneath native tree species. An examination of dry deposition, leaching, and canopy uptake at the leaf surface may shed light on the mechanisms behind this effect. Honeysuckle's branch architecture and leaf surface area may also allow for a more successfull capture of stemflow, potentially altering the ecohydrology of invaded habitats. In particular, stemflow may alter the soil moisture beneath Amur honeysuckle shrubs which can in turn affect litter decomposition, nutrient cycling, and the soil

microbial community. Water conducted to the invasive shrubs via stemflow may carry high concentrations of nutrients leached from overstory species. Stemflow nutrients may constitute a previously unexplored flux of nutrients to the soils beneath invasive shrubs like Amur honeysuckle.

Soils beneath Amur honeysuckle were different from soils beneath native tree species, suggesting that the invasive shrub can alter soil physicochemical conditions. Soils under honeysuckle had a lower bulk density, elevated soil organic matter, C:N, and total soil N and an altered soil microbial community. At times, the presence of honeysuckle was associated with changes to the soil N cycle, with seasonal increases in net N mineralization and ammonification observed throughout the experiment. Further work is needed to elucidate the mechanisms behind these effects. Invasive plants often assimilate more C via photosynthesis into both plants and soils, enhancing C accumulation in invaded ecosystems (Liao et al. 2008). Certain ecophysiological traits like a higher net photosynthetic activity, longer growing season, and a rapidly decomposing, N-rich litter may give *L. maackii* the ability to assimilate more N into the soils beneath its canopy. However, the relationship between increased pools of soil C and N in the soils beneath *L. maackii,* N cycling, and resultant feedbacks are difficult to tease out. It would be helpful to examine the partitioning of SOM among active, slow, and passive pools in the soils beneath *L. maackii* coupled with examinations of the activity of extracellular enzymes involved in the degradation of SOM. Further, relating these pools and enzyme activities to the rooting depth of the different trees and the invasive species would be useful. Root exudates and root turnover also provide an

important source of organic carbon to the soil microbial community and it would be useful to partition out litter and root contributions to the SOM pool.

The composition of the microbial community in the soils beneath Amur honeysuckle also differed from soils beneath the native tree species. Continued assessments of the soil microbial community coupled with measurements of enzyme activity may provide further insight into this effect. Future work should include examinations of both the rhizosphere and bulk soils to \assess both short- and long-term effects of honeysuckle invasion. Arthur et al.'s (in review) decomposition study revealed distinct microbial communities on Amur honeysuckle litter that likely originated prior to leaf senescence. An examination of the development of the phyllosphere microbial community throughout the growing season could potentially inform decomposition and throughfall dynamics.

The use of a savanna site allowed an examination of interactions between Amur honeysuckle and several native tree species. Invasive plants may have variable effects within a given environment, depending on their interactions with the dominant native species, yet little research has examined such species-species interactions within a site. Data presented in this dissertation characterize distinct species-species interactions. The rate at which honeysuckle litter decomposed depended on the identity of the associated tree, with litter from the invasive decomposing and losing N more rapidly under shagbark hickory trees than under the other two native tree species. Seasonal increases in net N mineralization and ammonification and differences in the composition of the microbial community were observed in the soils beneath honeysuckle under hickory trees. If invasive species affect some ecosystem processes in a variable manner, as influenced by

associated native species, this suggests the need for a more nuanced approach to examinations of invaded communities. Future work could examine the growth and performance of Amur honeysuckle shrubs in the understory of native trees to further examine these community interactions.

In future work, it will be important to determine how alterations to soil, decomposition, and nutrient cycling processes by invading shrubs effect the health and productivity of canopy trees and to identify the mechanisms involved. Passing reference to the shallow, abundant root systems of invasive shrubs is made throughout the literature with little effort to quantify their extent. If invading shrubs are able to aggressively compete with mature trees for water and nutrients, the success of this effort will likely vary with different tree species. The use of an ^{15}N tracer to assess nutrient uptake by individual trees and shrubs may help to shed light on this potential interaction. In coordination with management efforts, a better understanding of legacy effects of treated patches of exotic shrubs is also needed to predict the ability of forest systems to recover from invasions. The establishment of long-term monitoring projects will also be necessary to assess the effect of invasive shrubs on productivity and succession. A number of other forces (climate change, anthropogenic disturbance, exotic pathogens and pests) acting on forest succession and productivity will make it difficult to tease out individual effects. Modeling may prove useful in this regard, and species-specific assessments of tree seeding-shrub competition in invaded sites could allow for accurate predictions of future regeneration and succession patterns. A more thorough understanding of these long-term and unseen effects of invaders can contribute to

advancements in our understanding of invasion biology in general, and our ability to anticipate problem species in the future.

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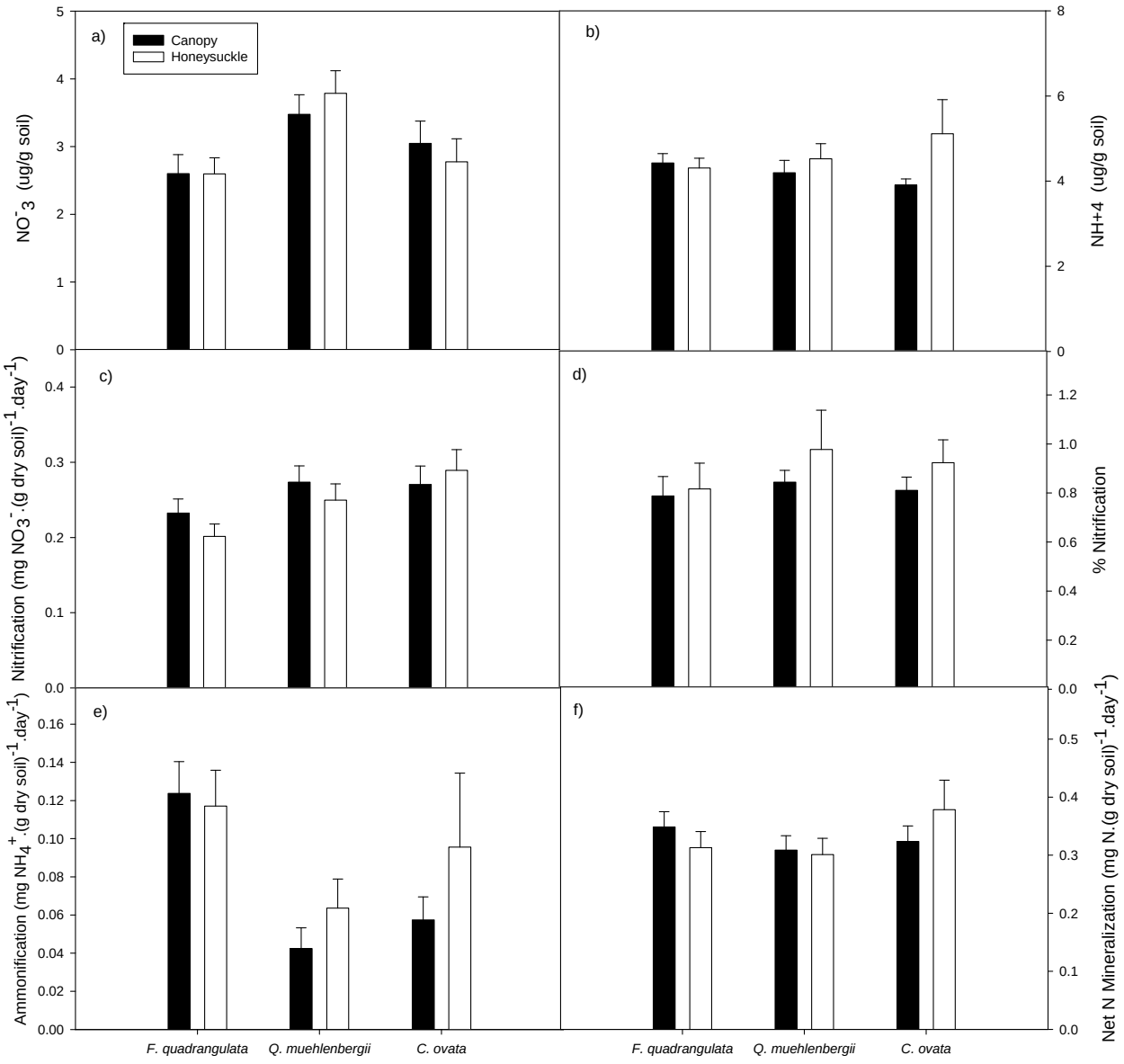
Appendix 1.Microsite and soil physicochemical properties associated with native tree species and the invasive *L. maackii*.

Characteristics	*F. quadrangulata*		*Q. muehlenbergii*		*C. ovata*	
	Canopy	*L. maackii*	Canopy	*L. maackii*	Canopy	*L. maackii*
Light (µmol/m2/s)	126 ± 40^{a}	7.7 ± 1.0	76.2 ± 16	23.5 ± 9.9	42.8 ± 9.2	43.3 ± 32
Bulk density (g/m^2)	1.1 ± 0.06^{a}	1.1 ± 0.06^{ac}	1.0 ± 0.05^{ab}	0.9 ± 0.06^{bc}	1.2 ± 0.05^{a}	1.0 ± 0.05^{c}
pH	5.3 ± 0.15^{a}	5.4 ± 0.19^{a}	6.0 ± 0.12^{bc}	6.2 ± 0.12^{b}	5.8 ± 0.16^{bc}	5.6 ± 0.19^{ac}
C (%)	6.0 ± 0.47^{acd}	7.2 ± 0.86^{bd}	5.3 ± 0.23^{c}	7.0 ± 0.51^{d}	5.5 ± 0.23^{ac}	5.8 ± 0.35^{abcd}
C:N	10.9 ± 0.17^{ac}	11.3 ± 0.19^{b}	10.7 ± 0.14^{c}	11.2 ± 0.16^{ab}	11.1 ± 0.15^{ab}	11.4 ± 0.23^{b}
TN (%)	0.55 ± 0.04^{abcd}	0.63 ± 0.07^{bd}	0.50 ± 0.02^{c}	0.62 ± 0.04^{bd}	0.49 ± 0.02^{ac}	0.51 ± 0.03^{abcd}
Soil N Content (g/m^2)	898 ± 62^{ab}	1000 ± 93^{b}	776 ± 35^{a}	855 ± 52^{ab}	841 ± 36^{ab}	766 ± 51^{a}

Notes: Soil samples were taken to a depth of 12 cm at a distance of 1m from the bole of the tree. Canopy light measurements were taken under the tree in a spot free of *L. maackii* while *L. maackii* light measurements were taken beneath the invasive shrubs located in the understory of the native tree. All values are means ±1 SE.

Appendix 2. Average extractable soil NO_3- N, extractable NH_4-N, net N mineralization rates, net ammonification rates, net nitrification rates, and % nitrification. Data have been averaged across 10 sampling periods (seasonal collections from 2005-2008).



Appendix 3. ANOVA results for NO_3-N, NH_4-N, N mineralization, nitrification, and ammonification. The analysis is broken into two specific time components: differences across seasons or differences within years. Anova results are first given for seasons (May, July, and October) then years (2005, 2006, 2007, 2008)

May

	Species		Location		Species x Location		Year		Species x Year		Location x Year		Species x Location x Year	
	F	p	F	p	F	p	F	p	F	p	F	P	F	p
NO3-N	2.19	0.12	1.67	0.2	0.37	0.69	**12.82**	**<0.0001**	0.75	0.56	1.53	0.22	0.85	0.5
NH4-N	1.81	0.17	2.52	0.12	0.46	0.63	**25.61**	**<0.0001**	0.76	0.55	1.89	0.16	0.72	0.58
N Mineralization	1.18	0.31	0.26	0.61	**3.21**	**0.05**	2.04	0.14	1.87	0.12	**3.72**	**0.03**	0.82	0.52
Nitrification	2.38	0.1	2.53	0.12	1.04	0.36	1.61	0.2	0.62	0.65	**3.11**	**0.05**	0.2	0.9
Ammonification	2.39	0.09	1.95	0.17	0.07	0.93	0.2	0.82	**2.61**	**0.04**	**3.6**	**0.03**	**4.03**	**0.005**
% Nitrification	**3.89**	**0.03**	3.01	0.09	0.72	0.49	1.52	0.23	1.53	0.2	0.99	0.38	1.32	0.27

Appendix 3, Cont.

July

	Species		Location		Species x Location		Year		Species x Year		Location x Year		Species x Location x Year	
	F	p	F	p	F	p	F	p	F	P	F	P	F	p
NO3-N	**6**	**0.004**	0	0.95	2.2	0.12	**39.2**	**<0.0001**	1	0.4	0	1	0.6	0.74
NH4-N	0.9	0.41	2.4	0.12	0.8	0.47	**75.4**	**<0.0001**	**2.2**	**0**	0.2	0.91	0.7	0.65
N Mineralization	**4.4**	**0.02**	3.6	0.06	1.6	0.21	**45.1**	**<0.0001**	0.9	0.5	0.6	0.59	1.2	0.32
Nitrification	**4.7**	**0.01**	1.6	0.22	1.3	0.27	**43.2**	**<0.0001**	1.2	0.3	0.5	0.69	0.3	0.95
Ammonification	**7.2**	**0.002**	0.1	0.81	0.2	0.8	**11.4**	**<0.0001**	1.3	0.3	0.9	0.47	1	0.41
% Nitrification	**5.4**	**0.006**	0.1	0.77	0.6	0.53	**4.21**	**0.007**	1	0.4	0.8	0.52	1.3	0.25

Appendix 3, Cont.

October

	Species		Location		Species x Location		Year		Species x Year		Location x Year		Species x Location x Year	
	F	P	F	p	F	p	F	p	F	P	F	P	F	p
NO3-N	**4**	**0.02**	0.5	0.48	0	1	**3.46**	**0.04**	1.5	0.2	2	0.14	0.8	0.51
NH4-N	0.4	0.64	2.3	0.14	1.8	0.18	1.26	0.29	1.8	0.1	1	0.37	1.1	0.35
N Mineralization	1.4	0.25	0	0.99	0	0.99	**5.92**	**0.004**	1.3	0.3	0.2	0.86	**3.7**	**0.01**
Nitrification	0.2	0.82	1.6	0.22	0.4	0.65	**3.08**	**0.05**	1.1	0.3	0.1	0.88	0.9	0.47
Ammonification	**7.5**	**0.001**	0.5	0.5	0.4	0.69	**16.2**	**<0.0001**	1.3	0.3	2.3	0.11	0.3	0.87
% Nitrification	0.9	0.43	0.1	0.78	0.8	0.46	0.49	0.61	0.4	0.8	**3.1**	**0.05**	0.7	0.6

Appendix 3, Cont.

2006

	Species		Location		Species x Location		Month		Species x Month		Location x Month		Species x Location x Month	
	F	P	F	p	F	p	F	p	F	P	F	P	F	p
NO3-N	**3.1**	**0.05**	1.3	0.26	1.6	0.22	**41.9**	**<0.0001**	**2.6**	**0.1**	2.8	0.06	0.2	0.93
NH4-N	1.6	0.21	2.4	0.13	0.3	0.74	**41.9**	**<0.0001**	2.4	0.1	**5.4**	**0.01**	0.1	0.98
N Mineralization	2	0.15	0.2	0.68	**5.3**	**0.01**	**9.19**	**2E-04**	0.4	0.8	0.5	0.63	1.2	0.34
Nitrification	**3.6**	**0.03**	0.3	0.59	1.4	0.26	**7.62**	**9E-04**	0.9	0.5	1	0.38	0.1	0.98
Ammonification	**4.8**	**0.01**	**7.6**	**0.01**	1.7	0.19	**28.6**	**<0.0001**	**3.2**	**0**	**3.1**	**0.05**	1	0.39
% Nitrification	0.8	0.48	0.6	0.43	0.6	0.56	0.94	0.39	2.4	0.1	0.5	0.62	0.8	0.5

Appendix 3, Cont.

2007

	Species		Location		Species x Location		Month		Species x Month		Location x Month		Species x Location x Month	
	F	p	F	p	F	p	F	p	F	P	F	P	F	p
NO3-N	**5.1**	**0.01**	0.1	0.8	0.1	0.87	**15.3**	**<0.0001**	0	1	0.1	0.94	0.3	0.91
NH4-N	1.4	0.27	1.3	0.25	1.1	0.34	**13**	**<0.0001**	0.7	0.6	0.6	0.56	2.2	0.08
N Mineralization	**3.9**	**0.03**	1	0.33	2	0.15	**17.8**	**<0.0001**	**2.9**	**0**	2	0.15	1.4	0.23
Nitrification	1.6	0.2	3.9	0.05	0.1	0.93	**9.68**	**2E-04**	3	0	2.4	0.1	1	0.44
Ammonification	**3.5**	**0.04**	0.3	0.58	1.2	0.31	**5.1**	**0.008**	0.9	0.5	0.7	0.48	1.6	0.19
% Nitrification	**3.2**	**0.05**	1.5	0.23	0.2	0.86	**3.42**	**0.04**	0.7	0.6	1.3	0.27	0.7	0.59

Appendix 3, Cont.

2008

	Species		Location		Species x Location		Month		Species x Month		Location x Month		Species x Location x Month	
	F	P	F	p	F	p	F	p	F	P	F	P	F	p
NO3-N	2.6	0.08	1	0.33	1	0.39	**28.1**	**<0.0001**	0.6	0.7	3	0.06	1.7	0.16
NH4-N	1	0.39	0.6	0.45	1.3	0.28	**12.7**	**<0.0001**	1.5	0.2	0.9	0.42	0.3	0.87
N Mineralization	0.6	0.55	1.6	0.21	0.9	0.4	**8.07**	**6E-04**	1.1	0.4	0.9	0.4	0.8	0.51
Nitrification	**3.7**	**0.03**	0	0.9	0.2	0.83	**14.6**	**<0.0001**	2.1	0.1	2.1	0.13	0.4	0.78
Ammonification	**9.2**	**3E-04**	0	0.93	0.5	0.6	1.38	0.26	0.9	0.5	0.9	0.39	0.4	0.83
% Nitrification	2.6	0.08	0	0.88	1	0.37	2.15	0.12	0.3	0.9	1.5	0.22	2.2	0.08

Note: Samples were taken from the soils beneath native savanna trees (n = 3 species, n = 15 trees per species) in two locations (under an *L. maackii* shrub in the understory of a native tree species or in a spot in the tree understory that was free of *L. maackii*). Boldface type indicates significant differences ($p < 0.05$).

Appendix 4. Comparison of seasonal and yearly N-cycling in the soils under *L. maackii* (honeysuckle) and the native tree species. Statistical analysis was broken into two specific time components: differences across seasons and differences within years. For each N-cycling response variable, soil N values for tree species and *L. maackii* combinations are given first across seasons (e.g. May averages data from May of 2006, 2007, and 2008) and then years (e.g. 2006 averages data from May, July, and October of 2006).

	F. quadrangulata		*Q. muehlenbergii*		*C. ovata*	
	Canopy	Honeysuckle	Canopy	Honeysuckle	Canopy	Honeysuckle
Initial NO3-N (ug/g soil)						
May	2.08 ± 0.31	2.25 ± 0.36	2.67 ± 0.34	2.40 ± 0.22	1.96 ± 0.22	2.31 ± 0.54
July	1.61 ± 0.31	1.47 ± 0.24	2.02 ± 0.22	2.50 ± 0.30	2.07 ± 0.31	1.42 ± 0.25
October	3.91 ± 0.66	4.00 ± 0.62	5.61 ± 0.51	5.75 ± 0.68	4.59 ± 0.68	3.52 ± 0.73
2006	2.50 ± 0.37	2.66 ± 0.42	3.74 ± 0.46	3.55 ± 0.51	3.21 ± 0.53	1.95 ± 0.48
2007	2.38 ± 0.43	2.49 ± 0.43	3.21 ± 0.41	3.53 ± 0.50	2.81 ± 0.39	2.88 ± 0.64
2008	3.71 ± 0.71	3.44 ± 0.46	4.31 ± 0.70	4.95 ± 0.78	3.74 ± 0.84	3.72 ± 0.64
All	2.60 ± 0.28	2.60 ± 0.24	3.48 ± 0.29	3.79 ± 0.33	3.05 ± 0.33	2.78 ± 0.34

Appendix 4, Cont.

	F. quadrangulata		*Q. muehlenbergii*		*C. ovata*	
	Canopy	Honeysuckle	Canopy	Honeysuckle	Canopy	Honeysuckle
Initial NH4-N (ug/g soil)						
May	4.61 ± 0.53	3.84 ± 0.27	3.88 ± 0.45	3.43 ± 0.22	3.95 ± 0.23	6.33 ± 2.45
July	4.18 ± 0.27	4.47 ± 0.39	3.75 ± 0.28	4.60 ± 0.44	3.87 ± 0.27	4.16 ± 0.31
October	3.86 ± 0.25	3.66 ± 0.25	5.11 ± 1.02	5.73 ± 1.41	4.04 ± 0.33	4.93 ± 0.63
2006	3.37 ± 0.20	2.87 ± 0.18	3.07 ± 0.21	2.87 ± 0.25	3.29 ± 0.22	3.15 ± 0.28
2007	3.74 ± 0.27	3.62 ± 0.27	4.63 ± 0.80	5.13 ± 0.95	3.74 ± 0.25	4.35 ± 0.43
2008	6.02 ± 0.56	5.70 ± 0.59	5.01 ± 0.47	5.42 ± 0.54	4.67 ± 0.31	7.18 ± 2.09
All	4.43 ± 0.22	4.31 ± 0.23	4.20 ± 0.29	4.53 ± 0.35	3.91 ± 0.15	5.11 ± 0.80
Net N Mineralization (ugN/g soil/day)						
May	0.40 ± 0.05	0.32 ± 0.05	0.39 ± 0.04	0.33 ± 0.04	0.41 ± 0.05	0.36 ± 0.11
July	0.38 ± 0.05	0.37 ± 0.05	0.32 ± 0.04	0.32 ± 0.04	0.38 ± 0.05	0.55 ± 0.08
October	0.24 ± 0.04	0.18 ± 0.04	0.12 ± 0.05	0.08 ± 0.05	0.14 ± 0.03	0.13 ± 0.07
2006	0.25 ± 0.04	0.22 ± 0.03	0.19 ± 0.03	0.14 ± 0.04	0.23 ± 0.04	0.39± 0.07
2007	0.32 ± 0.05	0.21 ± 0.06	0.16 ± 0.05	0.20 ± 0.07	0.27 ± 0.05	0.17 ± 0.04
2008	0.33 ± 0.05	0.33 ± 0.05	0.40 ± 0.05	0.29 ± 0.06	0.40 ± 0.06	0.39 ± 0.10
All	0.35 ± 0.03	0.30 ± 0.03	0.28 ± 0.03	0.27 ± 0.03	0.32 ± 0.03	0.37 ± 0.05

Appendix 4, Cont.

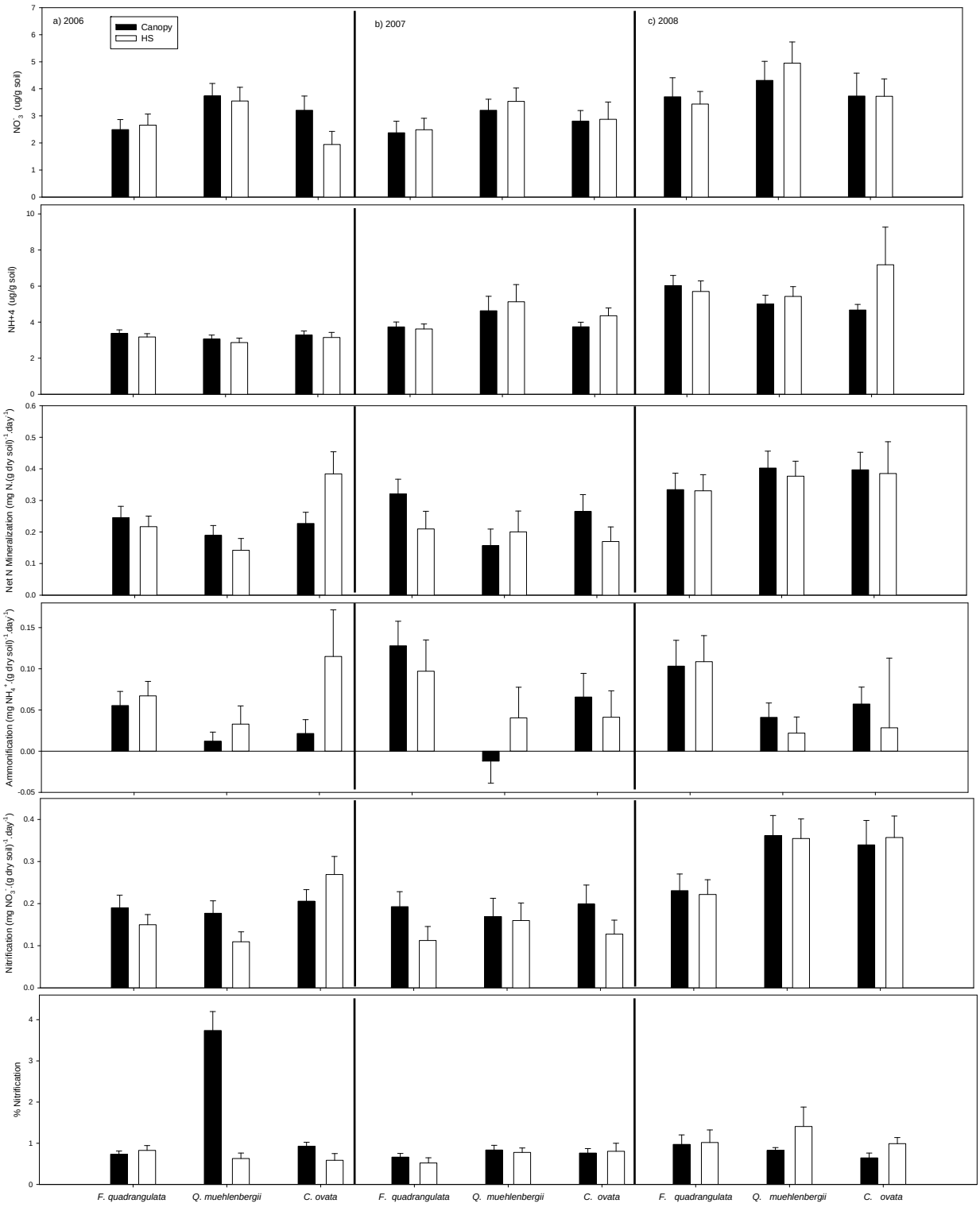
	F. quadrangulata		*Q. muehlenbergii*		*C. ovata*	
	Canopy	Honeysuckle	Canopy	Honeysuckle	Canopy	Honeysuckle
Nitrification (ugN/g soil/day)						
May	0.28 ± 0.03	0.18 ± 0.02	0.35 ± 0.04	0.31 ± 0.04	0.34 ± 0.04	0.30 ± 0.04
July	0.22 ± 0.03	0.22 ± 0.03	0.26 ± 0.04	0.26 ± 0.04	0.31 ± 0.04	0.39 ± 0.05
October	0.17 ± 0.03	0.13 ± 0.03	0.15 ± 0.04	0.10 ± 0.04	0.13 ± 0.03	0.12 ± 0.04
2006	0.19 ± 0.03	0.15 ± 0.02	0.18 ± 0.03	0.11 ± 0.02	0.21 ± 0.03	0.27 ± 0.04
2007	0.19 ± 0.04	0.11 ± 0.03	0.17 ± 0.04	0.16 ± 0.04	0.20 ± 0.04	0.13 ± 0.03
2008	0.23 ± 0.04	0.22 ± 0.04	0.36 ± 0.05	0.35 ± 0.05	0.34 ± 0.06	0.36 ± 0.05
All	0.22 ± 0.02	0.19 ± 0.02	0.26 ± 0.02	0.23 ± 0.02	0.26 ± 0.03	0.28 ± 0.03
Ammonification (ugN/g soil/day)						
May	0.12 ± 0.03	0.14 ± 0.04	0.034 ± 0.02	0.087 ± 0.03	0.074 ± 0.03	0.065 ± 0.001
July	0.16 ± 0.03	0.14 ± 0.03	0.063 ± 0.01	0.059 ± 0.02	0.068 ± 0.02	0.16 ± 0.05
October	0.067 ± 0.02	0.050 ± 0.02	-0.038 ± 0.03	-0.022 ± 0.03	0.014 ± 0.02	0.014 ± 0.005
2006	0.055 ± 0.02	0.067 ± 0.02	0.012 ± 0.01	0.033 ± 0.02	0.022 ± 0.02	0.12 ± 0.06
2007	0.13 ± 0.03	0.10 ± 0.04	-0.012 ± 0.03	0.04 ± 0.04	0.066 ± 0.03	0.041 ± 0.03
2008	0.10 ± 0.03	0.11 ± 0.04	0.041 ± 0.02	0.022 ± 0.02	0.057 ± 0.02	0.028 ± 0.08
All	0.12 ± 0.02	0.11 ± 0.02	0.024 ± 0.01	0.045 ± 0.02	0.053 ± 0.01	0.085 ± 0.04

Appendix 4, Cont.

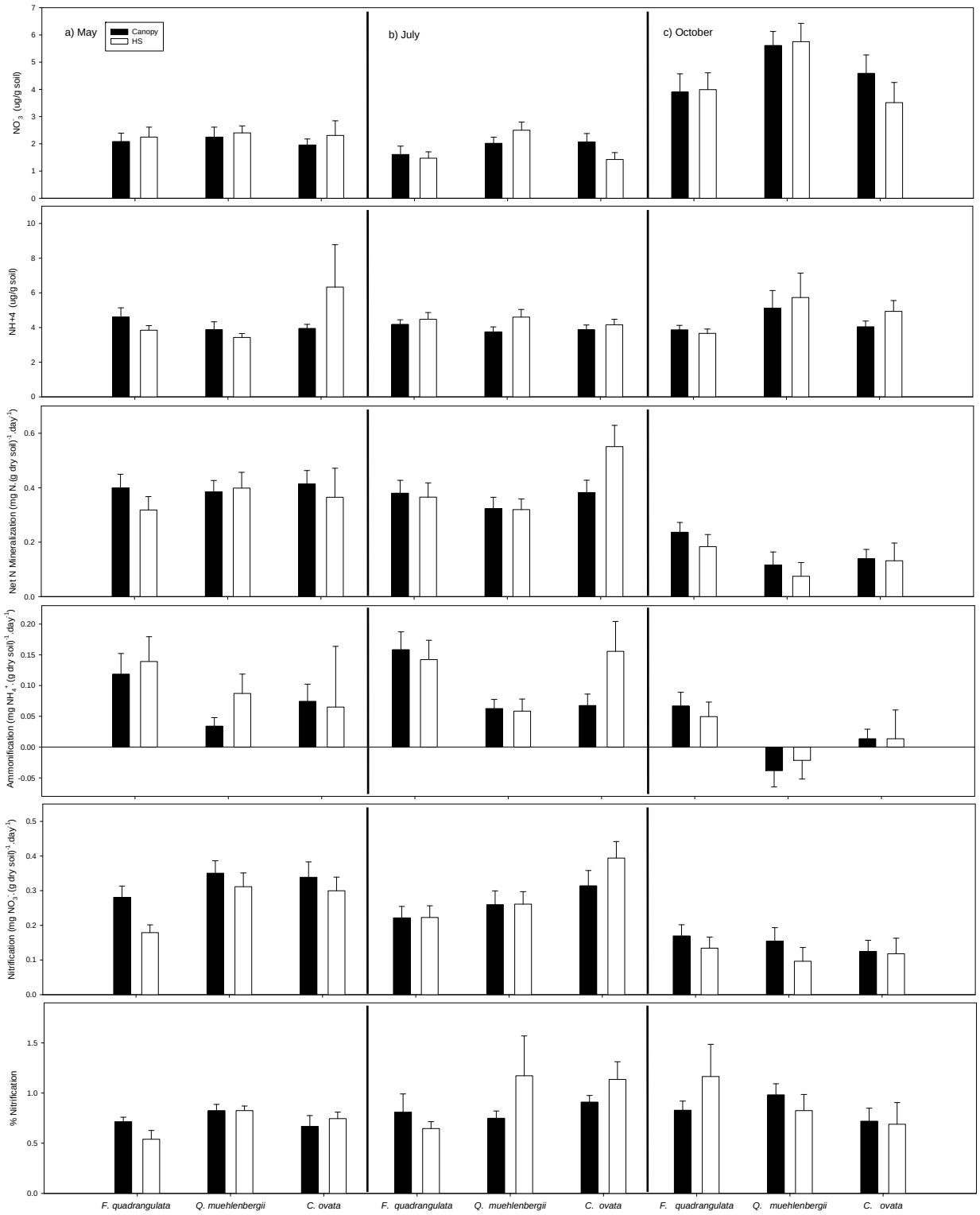
	F. quadrangulata		*Q. muehlenbergii*		*C. ovata*	
	Canopy	Honeysuckle	Canopy	Honeysuckle	Canopy	Honeysuckle
% Nitrification						
May	0.72 ± 0.04	0.54 ± 0.09	0.83± 0.06	0.83 ± 0.05	0.67 ± 0.1	0.75 ± 0.06
July	0.77 ± 0.2	0.65 ± 0.07	0.72 ± 0.08	1.11 ± 0.4	0.91 ± 0.07	0.94 ± 0.2
October	0.83 ± 0.09	1.17± 0.3	0.98 ± 0.1	0.83 ± 0.2	0.72 ± 0.1	0.69 ± 0.2
2006	0.73 ± 0.08	0.83 ± 0.12	0.83 ± 0.10	0.63 ± 0.13	0.93 ± 0.10	0.58 ± 0.16
2007	0.66 ± 0.09	0.52 ± 0.1	0.84 ± 0.1	0.78 ± 0.11	0.76 ± 0.11	0.80 ± 0.2
2008	0.97 ± 0.2	1.02 ± 0.3	0.82 ± 0.07	1.41 ± 0.47	0.64 ± 0.12	0.99 ± 0.15
All	0.77 ± 0.08	0.77 ± 0.1	0.83 ± 0.05	0.94 ± 0.16	0.78 ± 0.06	0.81 ± 0.09

Note: Samples were taken from the soils beneath native savanna trees (n = 3 species, n = 15 trees per species) in two locations (under an *L. maackii* shrub in the understory of a native tree species or in a spot in the tree understory that was free of *L. maackii*). N values are means ± 1 SE

Appendix 5. Annual extractable soil NO_3- N, extractable NH_4-N, net N mineralization rates, net ammonification rates, net nitrification rates, and % nitrification for 2006 (a), 2007 (b), and 2008 (c). In each year, data was averaged across seasons (e.g. % Nitrification for 2006 is the average of May, July, and Oct 2006 % nitrification). Error bars show ± 1 SE.


a) 2006
b) 2007
c) 2008
Canopy
HS
NO-3 (ug/g soil)
NH+4 (ug/g soil)
Net N Mineralization (mg N.(g dry soil)-1.day-1)
Ammonification (mg NH4+.(g dry soil)-1.day-1)
Nitrification (mg NO3-.(g dry soil)-1.day-1)
% Nitrification
F. quadrangulata
Q. muehlenbergii
C. ovata

Appendix 6. Annual extractable soil NO_3- N, extractable NH_4-N, net N mineralization rates, net ammonification rates, net nitrification rates, and % nitrification for May (a), July (b), and October (c). In each season, data was averaged across years (e.g. % Nitrification for May is the average of May 2007, July 2007, and Oct 2007 % nitrification). Error bars show ± 1 SE.


a) May
Canopy
HS
b) July
c) October
NO-3 (ug/g soil)
NH+4 (ug/g soil)
Net N Mineralization (mg N (g dry soil)-1 day-1)
Ammonification (mg NH+4 (g dry soil)-1 day-1)
Nitrification (mg NO-3 (g dry soil)-1 day-1)
% Nitrification
F. quadrangulata
Q. muehlenbergii
C. ovata
F. quadrangulata
Q. muehlenbergii
C. ovata
F. quadrangulata
Q. muehlenbergii
C. ovata

Appendix 7. Mixed model ANOVA results comparing mean relative abundance (% mole) of the different microbial taxa occupying the soils beneath the native tree species and the invasive *L. maackii* shrubs in their understory.

Taxa	Relative Abundance	Species		Location		Species x Location	
	(% mole ± SE)	F	p	F	p	F	p
Gram-negative bacteria	30.7 ± 0.3	3.05	0.1	2.89	0.1	0.18	0.84
Gram-positive bacteria	16.3 ± 0.2	0.82	0.47	0.15	0.71	1.03	0.4
Actinomycetes	8.8 ± 0.3	**4.49**	**0.04**	1	0.34	2.72	0.12
Fungi	8.7 ± 0.3	0.27	0.76	0.25	0.63	1.01	0.4
Non-specific bacteria	3.4 ± 0.07	1.25	0.33	1.63	0.23	0.56	0.59
SRB and anaerobes	2.8 ± 0.05	0.36	0.71	0.14	0.72	0.2	0.82
Protozoa	1.2 ± 0.09	1.48	0.28	2.29	0.16	0.9	0.44

Note: Samples were taken from the soils beneath native savanna trees (n = 3 species, n = 15 trees per species) in two locations (under an *L. maackii* shrub in the understory of a native tree species or in a spot in the tree understory that was free of *L. maackii*). Boldface type indicates significant differences ($p < 0.05$). Relative abundance values are means ± 1 SE. SRB = sulfate reducing bacteria.

Appendix 8. Mixed model ANOVA results comparing % mole abundance of the most common microbial phospholipid fatty acids (PLFAs) found in the soils beneath the native tree species and the invasive *L. maackii* shrubs in their understory.

PLFA	Taxa	Species		Location		Species x Location	
		F	p	F	p	F	P
16:1ω7c	Gram-negative bacteria	0.65	0.54	1.06	0.33	0.11	0.9
cy17	Gram-negative bacteria	**4.97**	**0.04**	1	0.34	0.03	0.97
18:1ω7c	Gram-negative bacteria	1.53	0.27	**5.49**	**0.04**	0.07	0.93
18:1ω5	Gram-negative bacteria	2.2	0.16	0.1	0.76	0.33	0.73
cy19	Gram-negative bacteria	2.44	0.14	0	0.95	0.93	0.43
i14	Gram-positive bacteria	3.04	0.1	1.93	0.2	2.01	0.19
i15	Gram-positive bacteria	1.66	0.24	0.01	0.93	0.63	0.56
i16	Gram-positive bacteria	1.93	0.2	3.37	0.1	0.14	0.87
i17	Gram-positive bacteria	2.48	0.14	**5.93**	**0.04**	0.1	0.99
a17	Gram-positive bacteria	1.69	0.24	1.48	0.26	0.89	0.44
10Me16	Actinomycetes	**5.11**	**0.03**	1.43	0.26	2.59	0.13
10Me18	Actinomycetes	0.15	0.86	2.6	0.14	0.01	0.99
18:2ω6	Fungi	0.66	0.54	0.24	0.63	0.19	0.83
18:1ω9c	Fungi	2.29	0.16	0.07	0.8	**4.5**	**0.04**
18:00	Non-specific bacteria	1.41	0.29	1.65	0.23	2.73	0.12
i17:1ω7	SRB and anaerobes	0.36	0.71	0.14	0.72	0.2	0.82

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Notes: Samples were taken from the soils beneath native savanna trees (n = 3 species, n = 15 trees per species) in two locations (under an *L. maackii* shrub in the understory of a native tree species or in a spot in the tree understory that was free of *L. maackii*). Boldface type indicates significant differences ($p < 0.05$).

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Publications

- **Poulette, M.M**. and M.A. Arthur. 2012. The impact of the invasive shrub *Lonicera maackii* on the decomposition dynamics of a native plant community. Ecological Applications 22(2):412-424.
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