Effects of *Rhamnus cathartica* (common buckthorn) stand age on decomposition

by

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Abstract

Many studies have investigated the effects of invasive species on biodiversity, but invasive plants can also directly alter ecosystem processes such as decomposition. Ecosystem changes resulting from invasion may be attributed to distinct physical traits introduced by the invasive. Plant traits can influence litter inputs and soil properties, both of which are important factors affecting biogeochemical processes. Common buckthorn (*Rhamnus cathartica*) is an exotic invasive shrub that can alter decomposition via inputs of high quality leaf litter and possible modifications to the soil environment (e.g. increased moisture and pH). These effects of buckthorn on decomposition may vary with age due to changes in litter chemistry since plants shift allocation of resources to different plant structures as they age. In addition, species modifications to the soil environment may be magnified as a stand ages. In this study, chemistry of leaf litter from mature and juvenile individuals was analyzed, and respiration rates and soil properties were measured under juvenile and mature buckthorn individuals in the field. It was expected that leaf litter from juvenile and mature shrubs would be chemically distinct and that these differences, along with soil modifications, would be translated into variations in respiration rates between the two age groups. As expected, leaf litter chemistry did differ between juvenile and mature individuals. Mature leaf litter had significantly ($p < 0.05$) higher concentrations of calcium, copper, sulfur, and zinc. Juvenile leaf litter had significantly ($p < 0.05$) higher concentrations of boron, iron, magnesium, and manganese. Notably, many of these chemicals are cations and metals, which are often linked to changes in soil properties. However, there were no significant differences in field respiration rates or soil properties (moisture, organic matter, pH). Though there are notable differences in the leaf litter chemistry of juvenile and mature buckthorn shrubs, it is unclear how this may impact decomposition. Understanding how decomposition changes with *Rhamnus cathartica* age will be crucial for predicting effects on forest communities and for improving management.

Keywords: decomposition; invasive species; respiration; *Rhamnus cathartica*; stand age

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Introduction

Over 50,000 species have been introduced to the United States, and although the majority of these species remain harmless, others become problematic and are considered "invasive" (Pimentel et al. 2005). Invasive species threaten biodiversity by replacing native species and creating monospecific patches. Decreasing biodiversity can inhibit ecosystem services, the natural ecosystem processes required to maintain human life such as production of goods, air and water purification, and decomposition (Daily et al. 1997). In addition to indirectly impacting ecosystem processes by decreasing biodiversity, invasive species can directly affect these processes by altering abiotic conditions such as soil nutrients. These direct changes can further

affect biodiversity since plant and animal communities are dependent on the abiotic environment.

An important ecosystem process is decomposition, which refers to the process of breaking down dead organic matter or detritus into smaller components through physical and chemical means (Chapin et al. 2002). Soil microorganisms such as bacteria and fungi are primarily responsible for decomposition. As the detritus breaks down, the soil microbes convert some of the nutrients into energy through respiration, resulting in the release of CO_2 as a byproduct. Therefore, the final result of decomposition is CO_2 released as a byproduct of microbial respiration and inorganic nutrients leftover from the microbes, which are then available to plants. These leftover nutrients continue moving through the ecosystem in a process called nutrient cycling. Given that relatively low inputs of nutrients enter an ecosystem, this recycling of nutrients is an essential ecosystem process (Aber and Melillo 1991). Since the rate of decomposition determines how fast nutrients are made available, decomposition is the primary control on nutrient cycling. The rate of decomposition is controlled by the physical environment as well as litter characteristics, both of which can vary from species to species. Plant species generally possess characteristics that reinforce the nutrient environment that is already present (Hobbie 1992). However, invasive plants often introduce characteristics that are atypical to that of the native plants, which can lead to unexpected changes in decomposition dynamics and other ecosystem processes.

One invasive species that has the ability to alter decomposition is *Rhamnus cathartica* (common buckthorn), which was introduced to the United States from Eurasia in the 1800s as an ornamental shrub. Buckthorn may impact decomposition through changes in litter characteristics and the soil environment. For example, buckthorn leaves have a higher concentration of nitrogen (N) than the leaves of many native species and subsequently decompose more rapidly (Heneghan et al. 2002, Heneghan et al. 2006). Also, buckthorn has been found to modify soil properties such as soil moisture and pH, which could also affect decomposition rates. Although buckthorn exhibits rapid decomposition, it contains secondary compounds, specifically emodin, which may influence decomposition rates in multiple ways. Generally, secondary compounds are thought to inhibit soil animals and microorganisms, which could decrease decomposition rates (Chapin et al. 2002). However, in some cases, secondary compounds may stimulate microbial activity (Swift et al. 1979, Hattenschwiler and Vitousek 2000). Due to these opposing effects, the

relationship between secondary compounds and decomposition is complex. Furthermore, chemical concentrations of secondary compounds and other nutrients can change as plants mature, which could potentially influence decomposition.

Age-related changes of invasive plants that impact critical processes such as decomposition may indicate how ecosystems will be transformed as a result of invasion. Understanding this will be crucial for predicting the effects of invasive species over time and for improving management strategies. Therefore, the objectives of this study are to 1) determine how litter quality and soil properties differ from juvenile buckthorn shrubs compared to mature shrubs and 2) evaluate the impact of these differences on decomposition. It is expected that as buckthorn stands age, decomposition rates will increase due to differences in leaf litter chemistry (e.g. lower secondary compounds) and buckthorn-induced modifications to the soil environment including increased soil moisture and elevated soil pH, which are magnified over time.

Literature Review

Invasive Species

Over 50,000 species have been introduced to the United States, and although the majority of these species remain harmless, others become problematic and are considered "invasive" (Pimentel et al. 2005). Although the exact definition is ambiguous, an invasive species can generally be defined as any species that has the potential to change natural community structures and alter ecosystem processes. Often, invasive species are also categorized as "non-native" or "exotic;" however, there are examples of native species that pose the same concerns as their foreign counterparts. Both native and exotic invasive species usually possess a number of characteristics that give them a competitive advantage over the desirable native species such as broad habitat tolerance, rapid growth, and prolific reproduction (Czarapata 2005). These traits enable invasive species to spread quickly across wide regions. Humans also have played a significant role in the introduction and spread of invasives. Numerous species have been introduced, intentionally for ornamental and agricultural purposes as well as accidentally in ship ballasts (Czarapata 2005). Following introduction, human activity further accelerates the spread of invasives by aiding in transport and creating disturbed areas, which invasive species are prone to colonize. As a result of rapid spread and distinct competitive advantages, invasive species

tend to dominate communities and replace native species posing a variety of ecological problems that subsequently lead to economic losses.

In the United States, approximately $120 billion is lost annually due to the damages inflicted by introduced species and the costs of control efforts to deter these problematic species (Pimentel et al. 2005). This estimated total cost does not take into account all invasive species in the country nor does it account for the many ecological damages that are imposed by these species but do not hold a monetary value. Though there is not a direct monetary cost associated with many ecological impacts of invasive species, the societal costs are substantial. For example, invasive species may inhibit ecosystem services, the natural ecosystem processes required to maintain human life. Examples of ecosystem services include production of goods, air and water purification, and decomposition (Daily et al. 1997). Preserving biodiversity is essential for maintaining these vital ecosystem services. For instance, decomposition is carried out by a diverse set of organisms, each responsible for breaking down different chemical components of waste. Given the tremendous amount of waste produced by humans, decomposition is a critical process required to break down these wastes and to release nutrients back into the ecosystem to be reused by plants, animals, and humans (Daily et al. 1997). Though direct, short-term economic benefits are not always apparent, the long-term importance of ecosystem services is obvious, which is why it is crucial to preserve these services (Daily et al. 1997). The greatest threat to biodiversity and ecosystem services is habitat destruction followed closely by invasive species (Daily et al. 1997). In order to protect these fundamental ecosystem services, it is imperative to have a comprehensive understanding of the ecological impacts of invasive species.

Past research has focused on the factors influencing invasibility of individual species and the direct effects of invasive species on community composition via competition. In addition to competitive interactions, it is apparent that invasive species have the potential to alter ecosystem processes (e.g. decomposition) that are, in part, subjected to individual species controls. Though frequently overlooked until recently, these invasive species impacts on ecosystem processes may be more important than the commonly studied direct consequences of competition given that ecosystem processes are one major determinant of the existing plant and animal communities (Ehrenfeld 2003).
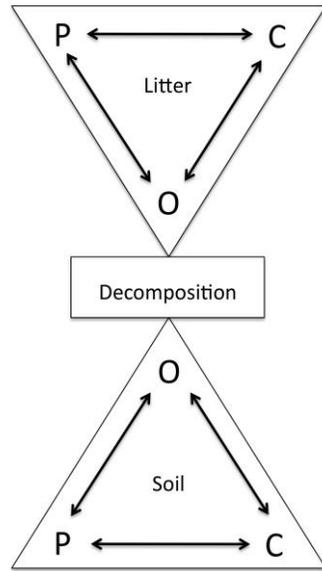
Decomposition and Nutrient Cycling

One of the most important ecosystem processes is decomposition, which refers to the process of breaking down dead organic matter, or detritus, into smaller components through physical and chemical means (Chapin et al. 2002). The entire process can be broken down into two stages: fragmentation and chemical alteration (Aerts 1997, Chapin et al. 2002). Fragmentation occurs when larger detritivores, such as earthworms, consume the detritus, breaking it down into smaller pieces (Aerts 1997, Chapin et al. 2002). These smaller pieces can then be broken down further by microorganisms, primarily bacteria and fungi, which produce extracellular enzymes that chemically break down the organic material (Chapin et al. 2002). As the detritus breaks down, the soil microbes convert some of the nutrients into energy through respiration, resulting in the release of CO_2 as a byproduct (Equation 1). Frequently, respiration (the amount of CO_2 released) is measured as a proxy for decomposition. In addition to releasing CO_2, microbes leave behind inorganic nutrients, which are then available to other organisms such as plants (Chapin et al. 2002).

Respiration Equation: $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + Energy$ (Equation 1)

These inorganic nutrients released by the microbes continue moving through the ecosystem in a process called nutrient cycling. Given that nutrient inputs into an ecosystem are relatively low, this recycling is an essential ecosystem process to make nutrients available to organisms (Aber and Melillo 1991). Since the rate of decomposition determines how fast nutrients are made available, decomposition is the primary control on nutrient cycling. The rate of decomposition is affected by three key factors in the soil environment and litter environment including microorganisms, physical attributes, and chemical composition (Chapin et al. 2002). Although these three factors interact within each environment, the soil and litter environments further interact to determine the rate of decomposition (Figure 1). The microbial community has a direct impact on decomposition because the microorganisms actually break down most of the detritus, but microbial community composition is determined by physical characteristics as well as chemical features. Therefore, the microbial community will be discussed in terms of how it responds to the physical and chemical environments. At a global scale, physical characteristics (including soil/litter temperature and moisture) play the most influential role in determining decomposition rates followed by litter characteristics, mainly litter quality (Aerts 1997).

Figure 1. Interacting factors in litter and soil that affect decomposition. Modified from Swift et al. (1979).

P = Physical
C = Chemical
O = Organisms

Physical characteristics, most importantly temperature and moisture, are largely driven by climate (Aerts 1997). Generally, increased temperature is associated with increased microbial respiration and thus faster rates of decomposition (Aber and Melillo 1991, Chapin et al. 2002). Increased moisture will also increase microbial activity and decomposition up to a certain point (Aber and Melillo 1991, Chapin et al. 2002). However, extreme high or low conditions that exceed the moisture threshold of most microbes will be associated with slower decomposition rates (Chapin et al. 2002). Overall, microbes favor warm, moist environments; therefore, faster rates of decomposition will occur under these conditions (Figure 2, Chapin et al. 2002). Although moisture and temperature conditions are independent between soil and litter environments, these physical characteristics are most commonly measured in the soil because it is difficult to accurately monitor temperature and moisture within plant litter.
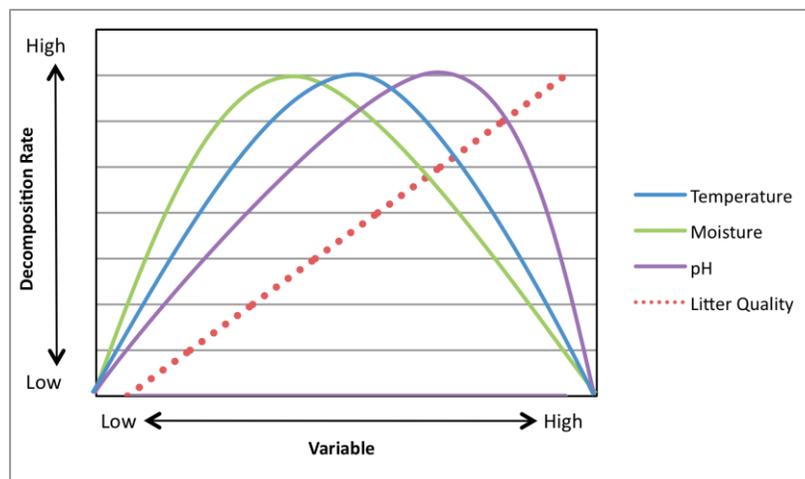
Figure 2: Theoretical decomposition trends based on major controlling factors including temperature, moisture, pH, and litter quality.

In addition to physical properties, chemical attributes also play an influential role in determining decomposition rates. In the soil environment, pH is an important characteristic that affects the microbial community and decomposition rates. Kittle et al. (1995) found that decomposition in wetlands was slower under low pH (2.5-4.5) conditions compared to higher pH

conditions (6.5). Although alkaline conditions were not tested in this particular study, it is documented that decomposition is generally faster under neutral pH (Figure 2, Chapin et al. 2002). In addition to pH, chemical composition is also a key factor influencing microbial activity and thus decomposition. Soil nutrients interact with leaf litter increasing the complexity of decomposition dynamics; however, to simplify the process, chemical composition will be discussed in terms of litter with the understanding that soil nutrients may complicate generalized relationships.

The chemical composition of litter, referred to as litter quality, is determined by factors such as nutrient concentration, chemical structure, and toxicity (Chapin et al. 2002). The litter quality is categorized as high (decomposes rapidly) or low (decomposes slowly) based on palatability to microbes (Figure 2). In order to explain general trends in decomposition dynamics, many studies focus on examining nutrient concentrations in litter and the ratio of those concentrations. Although many different nutrients influence decomposition, nitrogen (N) is one of the most important nutrients, and concentrations are frequently reported. Commonly, the carbon to nitrogen ratio (C:N) and lignin to nitrogen ratio (lignin:N) are considered to be some of the main determinants of decomposition rates. High N concentrations generally correlate to low C:N and low lignin:N, though not always. Since N is required to support microbial growth and activity, litter with high concentrations of N, low C:N, and low lignin:N decomposes more rapidly (Aber and Melillo 1991). The other important factor is the chemical structure of the litter. For example, cellulose has a regular repeating structure that is easily broken down by enzymes, while lignin possesses a highly irregular complex structure that is difficult to break apart. Thus, litter with high lignin concentrations (high lignin:N) tends to decompose more slowly (Chapin et al. 2002).

In addition to nutrient concentrations and chemical structure, secondary compounds can also influence decomposition rates. Generally, secondary compounds are thought to decrease decomposition rates because they are toxic and reduce microbial activity (Chapin et al. 2002). However, opposite effects on microbial activity have also been reported. For example, polyphenols, a specific class of secondary compounds, can either stimulate or inhibit microbial activity depending on the microbes and the types of secondary compounds present (Swift et al. 1979, Hattenschwiler and Vitousek 2000). These opposing effects further complicate the understanding of plant species effects on decomposition.

Invasive Species and Decomposition

Litter characteristics vary between species, resulting in species effects on decomposition (Hobbie 1992). In general, plants from low-nutrient environments produce low quality litter that contains more secondary compounds and decomposes slowly, while high-nutrient environments have plants that produce high quality litter with fewer secondary compounds that decomposes rapidly (Hobbie 1992). Therefore, there is a positive feedback loop between the nutrient environment and the plant community in which plant species reinforce the original nutrient conditions (Hobbie 1992). However, invasive plants often possess characteristics that are atypical to that of the native plants, which can lead to unexpected changes in decomposition dynamics and other ecosystem processes. For example, out of 12 studies reviewed by Ehrenfeld (2003), total litter mass of invaded areas compared to non-invaded areas increased in six cases, decreased in five, and remained constant in one case. These differences in litter mass can be attributed to unique plant traits introduced by the invasives such as varying plant size, biomass allocation, and litter chemistry (Ehrenfeld 2003). Furthermore, all of these traits interact. For instance, the litter of many invasive species contains high N concentrations and decomposes rapidly (Heneghan et al. 2002, Ehrenfeld 2003, Allison and Vitousek 2004, Ashton et al. 2005); however, this pattern is not universal (Ehrenfeld 2003). There are cases where invasive plant litter decomposes slower than that of natives (Ehrenfeld 2003), possibly due to secondary compounds, which are frequently found in exotic plants (Cappuccino and Arnason 2006). The overall impacts of a given species may depend on the relative difference between the characteristics of the invasive compared to that of the native species (Ehrenfeld 2003, D'Antonio and Hobbie 2005).

Rhamnus cathartica

One example of an invasive species that possesses the ability to alter decomposition is *Rhamnus cathartica*, often referred to as common buckthorn or European buckthorn. Buckthorn is an invasive plant found throughout the northeastern and midwestern United States as well as parts of southern Canada (Kurylo et al. 2007). Originating in Europe and Asia, buckthorn was introduced to North America in the 1800s as an ornamental shrub used in hedgerows because of its ability to grow densely. Buckthorn usually grows as a shrub with multiple stems but can

sometimes form a small tree up to nine meters in height (Godwin 1943, Czarapata 2005, Knight et al. 2007). Young buckthorn shrubs have gray to brown bark, which becomes rough with age and turns to a dark gray or black color. The bark of both young and mature individuals is speckled with prominent light-colored lenticels. Buckthorn is recognizable by its simple dark green leaves that grow nearly opposite each other. The leaves, which flush in early May and senesce in late October to November, are ovate and sometimes pointed at the tip with distinct converging veins (Godwin 1943, Czarapata 2005). Between May and June the plant forms clusters of small light green to white fragrant flowers (Czarapata 2005). Later, female shrubs develop copious amounts of small black fruits that ripen in September and October (Godwin 1943) and are commonly dispersed by birds (Knight et al. 2007). Since its introduction to the United States, buckthorn has spread rapidly and become a major concern in many Midwestern states including Wisconsin, Minnesota, Illinois, Iowa, and Michigan (Czarapata 2005, Kurylo et al. 2007) due to its broad habitat tolerance, rapid growth, and resistance to management.

Buckthorn is tolerant of a wide range of moisture and light conditions (Knight et al. 2007), which allows it to grow in a variety of habitats including interior woods, forest edges, prairies, old fields, and roadsides (Czarapata 2005). In addition, buckthorn exhibits rapid growth (Knight et al. 2007) and the ability to grow clonally, often resulting in dense monospecific thickets. Although it is commonly thought to be a particular problem in forest understories, Mascaro and Schnitzer (2007) found that buckthorn was the dominant woody species in forest sites of southern Wisconsin up to a size class of 25 cm dbh making it competitive with larger trees as well as understory plants. Compared to native plants, buckthorn leafs out early and senesces late, increasing time for photosynthesis and giving the shrub a competitive advantage (Knight et al. 2007), which may allow it to dominate forest sites. Buckthorn is known to be associated with arbuscular mycorrhizae (Godwin 1943), which may aid its aggressive growth pattern and ability to become a forest dominant. It has also been reported that buckthorn is highly resistant to imposed management such as cutting, burning, and grazing (Godwin 1943), which may be attributed to its mutualism with arbuscular mycorrhizae since it seems to maintain its mycorrhizal associations even after the stems have been cut down (Petrovic 2007). Buckthorn's ability to grow rapidly, become dominant, and resist management make it a successful invader with the potential to impact ecosystems in multiple ways.

One crucial ecosystem impact is the influence of buckthorn on decomposition and nutrient cycling (Knight et al. 2007) primarily via changes in the litter characteristics and the physical environment. It has been noted that high quality buckthorn leaves have a higher concentration of N than the leaves of many native species (Heneghan et al. 2002, Heneghan et al. 2006). As expected for high N concentrations, buckthorn leaf litter decomposes rapidly in relation to native species (Heneghan et al. 2002). Furthermore, when buckthorn-invaded sites were compared to non-invaded sites, lower litter mass was observed in the invaded sites supporting the idea that buckthorn increases decomposition rates (Heneghan et al. 2004).

Although it is apparent that litter characteristics of buckthorn can affect decomposition, it is unknown how these litter characteristics change with age. As plants mature, they allocate nutrients to different structures. For example, young plants expend the majority of their energy on growth, allocating resources to stem development. However, as plants age, growth begins to slow and biomass does not increase as quickly. After the plant reaches reproductive maturity, more nutrients are allocated to reproductive structures such as fruits. In addition to changing nutrient concentrations, levels of secondary compounds used for defense may also vary according to age. In a study of *Populus tremuloides* (trembling aspen) clones, Donaldson et al. (2006) found that phenolic glycosides in leaves decreased between juvenile and mature ramets aged 1-10 years, suggesting that defense compounds in leaves may be more important in juveniles whose growth and development is more vulnerable to herbivory. Buckthorn also contains concentrations of secondary compounds, specifically the polyphenol emodin, which may vary with age. Buckthorn contains other secondary compounds, but emodin is the most prominent (Cappuccino and Arnason 2006) and has been reported to be found in the bark, leaves, stems, flowers, and seeds of 23 different *Rhamnus* species (Izhaki 2002). Based on the findings of Donaldson et al. (2006), it is possible that emodin and other secondary compounds in buckthorn leaves decrease as the plant ages. Concentrations of secondary compounds in leaf litter have not been reported for buckthorn nor have the potential impacts on decomposition been studied.

Additionally, buckthorn has been found to modify soil properties such as moisture, pH, and soil nutrients, which may contribute to the rapid decomposition associated with invaded sites. Buckthorn has been associated with increased soil moisture and higher pH. Heneghan et al. (2004) found that gravimetric water content was 59% higher in invaded sites compared to

non-invaded sites, a finding that is likely attributed to the shade differences between the growth form of shrubs compared to trees. Furthermore, Heneghan et al. (2004) found that soil pH was also significantly increased in the soil of buckthorn-invaded sites compared to non-invaded sites. In addition, Heneghan et al. (2004) found that invaded sites had lower soil C:N compared to non-invaded sites. Although the overall soil C:N was lower, percentage of soil N was twice as high in the invaded sites, and percentage of soil C was 80% higher in the invaded areas suggesting that buckthorn increases soil nutrients (Heneghan et al. 2006), which could affect decomposition rates. It is likely that these alterations will be magnified as the buckthorn stand ages.

Together, these previous studies show that buckthorn has the potential to alter decomposition dynamics, yet it remains unclear how these effects vary as the plant matures and as the entire stand ages. Age-related changes of invasive plants that impact critical processes such as decomposition may indicate how ecosystems will be transformed as a result of invasion. Understanding these changes will be crucial for predicting the effects of invasive species and for improving management strategies. Therefore, the objectives of the following study were to 1) determine how litter quality and soil properties differ from juvenile buckthorn shrubs compared to mature shrubs and 2) evaluate the impact of these differences on decomposition. It was expected that as buckthorn stands age, decomposition rates would increase due to variation in leaf litter chemistry (e.g. lower secondary compounds) of mature shrubs and soil modifications including increased soil moisture and elevated soil pH.

Methods

Site Description

The field component of this study was conducted at the River Bend Nature Center located in Racine, Wisconsin (42º45' N, 87º49' W) along the Root River. The River Bend Nature Center was first established as a nature sanctuary in the 1950s and currently consists of 80 acres of natural areas including young oak-hickory forests, wetlands, and open fields. The dominant overstory trees consist of *Quercus alba* (white oak), *Quercus rubra* (red oak), *Carya ovata* (shagbark hickory), and *Prunus serotina* (black cherry), while the understory is primarily dominated by the exotic shrubs *Rhamnus cathartica* (common buckthorn) and *Lonicera tatarica* (Tartarian honeysuckle). Invasive species within the site have not been managed in recent years, and buckthorn stands of varying ages are prevalent throughout the site.

Litter Collection

Size was used as an estimation of general age, and two categories (juvenile and mature) were determined based on the general size of buckthorn individuals containing berries, a sign of reproductive maturity. Juveniles were defined as any plant with a dbh less than 2 cm and a height less than 2 m, while adults had a dbh greater than 6 cm and a height greater than 2 m. Given that buckthorn commonly grows in clumps with multiple stems, dbh was measured at a height of 1.4 m for only the largest stem in each clump. Leaves from juvenile and mature buckthorn shrubs were harvested at River Bend Nature Center prior to leaf fall on October 22, 2009 and brought back to the laboratory at Carthage College where they were air-dried and stored. Six subsamples of air-dried leaves (three juvenile and three mature) were sent to the Louisiana State University AgCenter Soil Testing and Plant Analysis Laboratory (LSU AgCenter 2010) in Baton Rouge, Louisiana to be analyzed for nutrients (C, N, P, K, Ca, Mg, S, B, Zn, Cu, Fe, Mn, Mo, Al, Na). Six additional samples (three of each age group) were sent to the University of Wisconsin-Madison Soil and Forage Analysis Laboratory (Schulte et al. 1987) in Marshfield, Wisconsin to be analyzed for total dry matter, lignin, and acid detergent fiber (ADF).

Field Experiment

A total of 20 buckthorn individuals were selected (ten juveniles and ten adults). In order to evaluate effects of stand age, individuals were selected from buckthorn patches containing plants of approximately the same size. Under each selected individual, soil properties (temperature, pH, moisture, organic matter) and respiration rates were measured immediately following buckthorn litterfall on November 19, 2009.

In the field, soil temperature was measured at a depth of 10 cm under each selected individual, then three soil samples were collected using a soil corer (2 cm diameter, 15 cm depth) and pooled, resulting in a total of 20 samples transported back to the laboratory. Soil pH was measured by creating two duplicate slurries (5 mL soil to 25 mL of distilled water) per soil sample. The pH was measured after the slurries settled for 24 hours, and the mean pH of the duplicates was used to estimate the overall pH of each soil sample. In addition, subsamples (approximately 5 g) were taken from each soil sample to calculate soil moisture using standard gravimetric techniques. After calculating soil moisture, about 2 g of each subsample was placed

in a muffle furnace for three hours at 300°C and then reweighed to determine soil organic matter (SOM) content.

Soil respiration was measured under each individual shrub using a modified version of the soda-lime method (Edwards 1982). Small containers (29.6 mL) were filled with approximately 10 g of soda lime granules and oven-dried at 105°C to a constant mass. While attempting to minimize disturbance, one soda-lime container was carefully placed on the soil/litter surface under each selected shrub and covered with a respiration chamber (437 mL) that was sealed to the ground. Additionally, six "blanks," each consisting of a soda-lime container placed in an empty sealed chamber, were randomly assigned and placed under three juvenile and three mature individuals. The respiration chambers were left in the field to incubate for 24 hours before they were retrieved and brought back to the laboratory where the soda-lime containers were oven-dried at 105°C to a constant mass. The amount of CO_2 (mg) absorbed was calculated according to the protocol in Edwards (1982); however, a revised correction factor of 1.69 was used to account for water formation (Equation 2, Grogan 1998).

Calculation: $(\text{Mass}_{final} - \text{Mass}_{initial} - \text{Mean } \Delta \text{ Mass}_{blank})*1000*1.69 = CO_2 \text{ in mg}$ (Equation 2)

Results

The results from the chemistry analyses indicate that there are significant differences in the chemistry of leaf litter from juvenile buckthorn individuals and mature buckthorn individuals. Mature leaf litter had significantly ($p < 0.05$) higher concentrations of calcium, copper, sulfur, and zinc (Table 1). There was a marginally significant ($p = 0.055$) difference in sodium concentrations with mature leaf litter having more sodium. Juvenile leaf litter had significantly ($p < 0.05$) higher concentrations of boron, iron, magnesium, and manganese (Table 1). Juvenile leaf litter also had a significantly ($p = 0.005$) higher percent of acid detergent fiber (ADF). There were no significant differences in carbon, nitrogen, or lignin concentrations (Table 1).

Overall, there were minimal differences between juvenile individuals and mature individuals in terms of respiration and soil properties. The mean respiration rates were not significantly different ($p = 0.86$) under mature buckthorn shrubs compared to juvenile shrubs (Figure 3). The mean respiration rate under mature shrubs was 150.8 ± 7.1 mg CO_2 and $149.2 \pm$

5.6 mg CO_2 under juveniles. Differences in soil moisture were slightly more pronounced, though still not significant (p = 0.21). The mean percent of soil moisture was marginally higher under juvenile buckthorn shrubs at 27.7 ± 1.3% compared to mature buckthorn shrubs at 24.7 ± 1.9% (Figure 4). Soil organic matter (SOM) also differed only slightly between the two groups (p = 0.38). The mean percent of soil organic matter was slightly higher under juvenile buckthorn shrubs at 8.5 ± 0.9% compared to mature buckthorn shrubs at 7.2 ± 1.1% (Figure 5). There was also no significant difference in the soil pH between the two groups (p = 0.64). The mean soil pH was 6.921 ± 0.027 under mature buckthorn individuals compared to 6.901 ± 0.033 under juvenile buckthorn individuals (Figure 6).

Table 1: Mean concentrations of chemical components in leaf litter from juvenile and mature buckthorn individuals

	Juvenile	Mature	
	Mean ± Stdev	Mean ± Stdev	p-value
Carbon %	44.80 ± 1.04	43.70 ± 2.08	0.474
Nitrogen %	2.17 ± 0.07	2.23 ± 0.03	0.311
C:N	20.62 ± 0.65	19.63 ± 1.13	0.275
Aluminum (ppm)	74.29 ± 13.02	53.52 ± 0.89	0.109
Boron (ppm)	56.49 ± 2.96	42.98 ± 1.24	**0.008**
Calcium %	3.36 ± 0.10	3.91 ± 0.16	**0.011**
Copper (ppm)	6.26 ± 0.14	7.39 ± 0.26	**0.007**
Iron (ppm)	149.11 ± 2.72	122.53 ± 0.95	**0.001**
Magnesium %	0.47 ± 0.02	0.41 ± 0.01	**0.019**
Manganese (ppm)	52.35 ± 1.96	40.87 ± 4.37	**0.030**
Phosphorus %	0.15 ± 0.01	0.18 ± 0.02	0.104
Potassium %	2.46 ± 0.02	2.90 ± 0.25	0.090
Sodium (ppm)	18.91 ± 1.30	21.91 ± 0.16	0.055
Sulfur %	0.20 ± 0.01	0.23 ± 0.01	**0.025**
Zinc (ppm)	12.16 ± 0.33	16.66 ± 0.49	**0.0004**
Dry Matter %	94.02 ± 0.23	93.83 ± 0.08	0.297
ADF % of DM	18.33 ± 0.43	15.99 ± 0.18	**0.005**
Lignin % of DM	3.79 ± 0.07	3.48 ± 0.25	0.152
Lignin:N	1.75 ± 0.09	1.56 ± 0.12	0.107

* Bold values indicate significant differences using two-tailed t-tests assuming unequal variance.
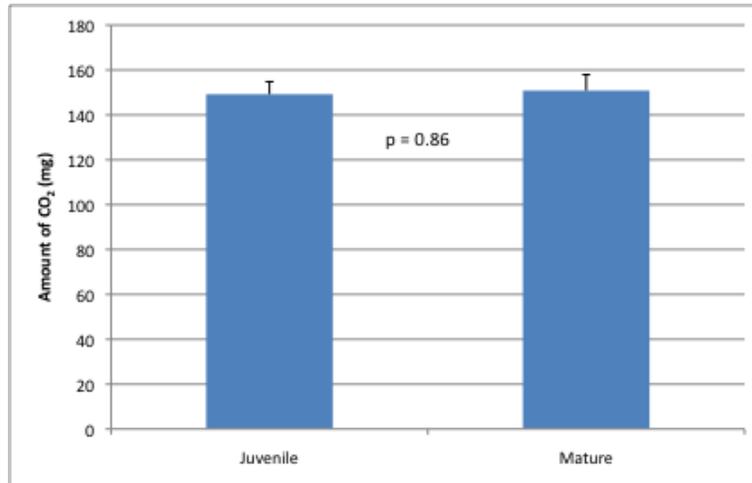
Figure 3: Mean respiration (mg of CO_2) after a 24-hour incubation under juvenile and mature buckthorn individuals.
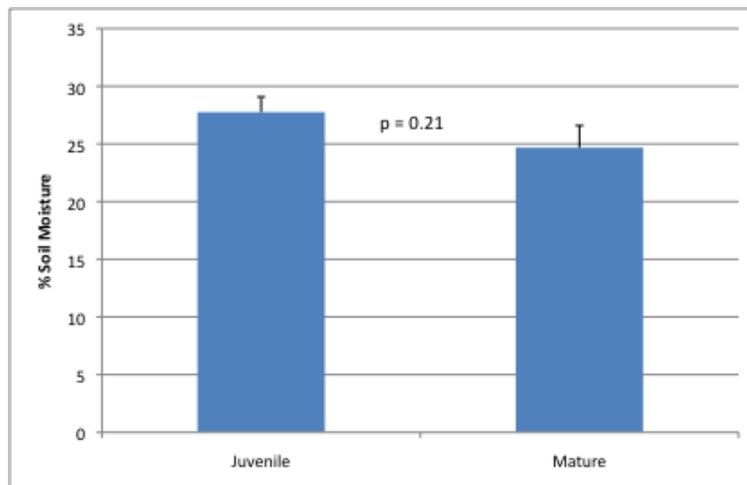

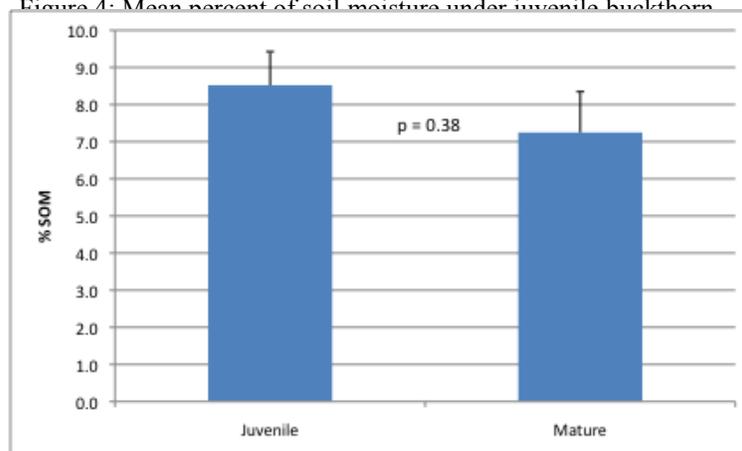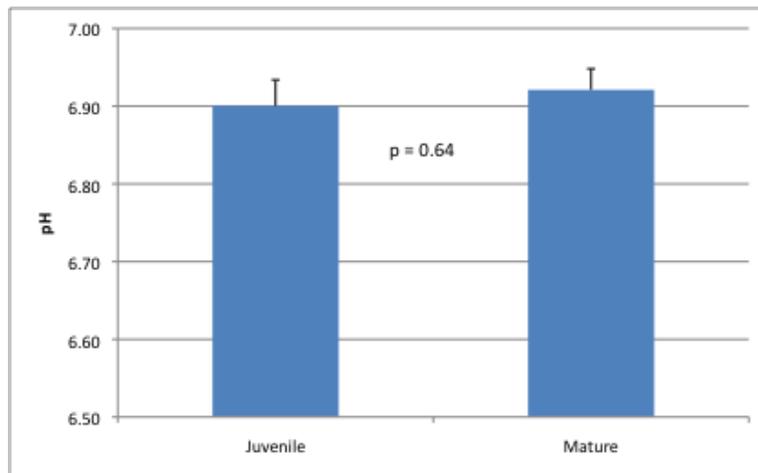Figure 4: Mean percent of soil moisture under juvenile buckthorn

Figure 5: Mean percent of soil organic matter (SOM) under juvenile buckthorn individuals and mature buckthorn individuals.

Figure 6: Mean soil pH under juvenile and mature buckthorn.

Discussion

      The purpose of this study was to determine how leaf litter chemistry varied between juvenile and mature buckthorn individuals and to compare soil properties in stands of juvenile and mature buckthorn in order to assess how these factors (litter chemistry and soil properties) may impact decomposition. Though significant differences were observed in leaf litter chemistry between the two age groups, there were no differences in soil properties or respiration rates, contrary to expectations.

      Leaf litter concentrations of C, N, and lignin did not significantly differ between juvenile and mature buckthorn (Table 1), indicating that these chemicals are not highly variable with age in this species. Similarly, Donaldson et al. (2006) found that *Populus tremuloides* (trembling aspen) leaf litter N concentrations did not exhibit high variability among six age classes of ramets. However, Donaldson et al. (2006) did find that phenolic compounds decreased with age, which suggests that trembling aspen may require less defense in leaves as it matures and becomes less vulnerable to herbivory. Though secondary compounds (e.g. polyphenols) were not directly analyzed in the buckthorn leaf litter, acid detergent fiber (ADF) was significantly

higher in juvenile litter (Table 1). This finding indirectly suggests the possibility of more secondary C compounds in juvenile leaves, since secondary compounds sometimes bind with cellulose that is incorporated into the ADF (Swift et al. 1979). Though concentrations of C, N, and lignin have been found to be correlated with decomposition rates and are frequently the focus of many studies (e.g. Melillo et al. 1982, Aerts 1997), secondary compounds may play an influential role in determining decomposition dynamics. Secondary compounds may be especially important when considering changes within a single species since these compounds may be subject to more developmental variability compared to the usual decomposition metrics such as nitrogen and lignin.

It is unclear what specific effects secondary compounds may have on decomposition in buckthorn stands, though these compounds are commonly thought to slow decomposition by inhibiting microbial activity (Chapin et al. 2002). In previous studies (Heneghan et al. 2002), decomposition rates have been found to be higher in buckthorn stands, so perhaps the microbial community has coevolved with buckthorn to be more resistant to secondary compounds specific to buckthorn (i.e. emodin). For example, it is known that buckthorn is associated with arbuscular mycorrhizae (though it is unclear whether or not this is a specialized relationship with a certain species of fungi), and arbuscular mycorrhizae may indirectly influence litter decomposition by altering soil nutrients. Some researchers have also suggested that arbuscular mycorrhizae may directly affect decomposition, though evidence of this has not been consistent (Smith and Read 2008).

Other chemical constituents of buckthorn leaf litter, mostly metals and cations, also differed between juvenile and mature shrubs (Table 1). One of the most notable differences was that calcium was significantly higher in leaf litter from mature individuals (Table 1). Calcium and other cations have been linked to changes in soil properties such as increased soil pH (Reich et al. 2005). Therefore, the increased calcium concentrations in mature leaf litter is likely correlated with the slightly higher soil pH that was found in mature buckthorn stands. Although soil pH was not significantly different between juvenile and mature stands, buckthorn patches have been correlated with increased soil pH when compared to sites relatively free of buckthorn (Heneghan et al. 2004). A positive feedback may exist between leaf litter calcium and soil pH such that soil with a higher pH may hold more calcium, and then increases in leaf litter calcium may reinforce that pattern by increasing inputs of calcium to the upper soil layers. This increase

of calcium (or other cations) and associated changes in pH may then affect decomposition in the organic layers by altering the microbial community. For instance, bacteria tend to prefer more alkaline pH conditions, while fungi have a higher tolerance for acidic environments (Brady and Weil 2000). Bacteria are often associated with faster decomposition rates compared to fungi (Brady and Weil 2000), resulting in increased rates of decomposition in calcium-rich, more alkaline environments.

Though changes in litter properties can happen relatively quickly as plants pull nutrients from deeper layers of the soils, significant changes in soil properties often occur over relatively long time periods, so it is perhaps not surprising that soil properties did differ significantly between young and mature buckthorn (Figures 4, 5, 6). For example, within a 30 year period Reich et al. (2005) found differences in soil properties correlated to leaf litter calcium, noting that this was a rapid change. The speed at which soil changes occur may be influenced by multiple factors such as plant growth rate and decomposition rate. Reich et al. (2005) examined soil changes in monospecific plots of tree species, which have a relatively slow growth rate compared to shrubs such as buckthorn. It is reasonable to believe that species-induced soil modifications may occur more rapidly beneath plants that grow faster since inputs (e.g. plant litter) to the soil environment would increase as the plant grows. Further, areas with faster rates of decomposition may exhibit more rapid changes in soil properties since leaf litter chemicals influencing soil properties are released into the soil environment more quickly. Buckthorn grows rapidly and produces high quality litter; therefore, it may be linked to faster rates of soil changes than tree species. In this study, individual ages, and subsequently exact age differences between mature and juvenile stands, were not determined. It is possible that more pronounced differences in soil properties might be observed if measured among stands with a larger, more defined age difference.

Though not measured in this study, another aspect that could be influenced by changes in leaf litter calcium, and subsequent changes in soil properties, is potential shifts in the soil biological community. For example, calcium has been found to be positively correlated to earthworm abundances (Reich et al. 2005). In a common garden study of monospecific tree plots, Reich et al. (2005) found that plots containing species with calcium-rich litter had higher abundances of earthworms, predominantly *Lumbricus terrestris* (Hobbie et al. 2006). At the same common garden site, Hobbie et al. (2006) found forest floor removal rates to be positively

related to litter calcium (and subsequently earthworm abundance), while decomposition in litter bags (excluding earthworms) was best predicted by lignin concentrations and unrelated to forest floor removal. It has been well established that earthworms increase decomposition rates leading to faster disappearance of the litter layer (e.g. Bohlen et al. 2004b, Hale et al. 2005, Heneghan et al. 2007, Hale et al. 2008), though studies of earthworm activity and litter decomposition have primarily focused on litter N concentrations as the key determinant of litter palatability (Hendriksen 1990). The novel link established by Hobbie et al. (2006) between litter calcium, earthworm activity, and forest floor dynamics may be important when considering effects of buckthorn on decomposition.

Buckthorn has previously been linked to high abundances of invasive Eurasian earthworms, and it has been proposed that one or both of these species may facilitate the spread of the other (Frelich et al. 2006, Heneghan et al. 2007). In a recent study of a Chicago woodland, Heneghan et al. (2007) sampled earthworm abundance and biomass in five forest areas (upland forest, two mesic woodlands, dry mesic forest, and buckthorn-dominated forest). Plots dominated by buckthorn were found to have significantly higher abundances of earthworms when compared to only two other sites (upland forest and one of the mesic woodlands), while earthworm biomass was significantly higher in buckthorn plots compared to all other plot types (Heneghan et al. 2007). The significantly higher earthworm biomass in buckthorn plots compared to all other plot types may be attributed to the species of earthworms present in each plot type, though species were not reported in the study. The higher biomass in buckthorn plots may suggest that larger species of earthworms were present, such as *Lumbricus terrestris* (common nightcrawler).

Lumbricus terrestris is an anecic species that forms deep burrows integrating leaf litter from the surface into the deeper mineral soils, thus reducing the thickness of the O-layer and increasing decomposition rates (Eisenhauer et al. 2007). *Lumbricus* species have complex calciferous glands that excrete calcium carbonate, and as a result, these species have a higher calcium requirement than other earthworm species (Piearce 1972). Hobbie et al. (2006) suggested that the calcium requirement of *Lumbricus terrestris* may explain why earthworm abundance and biomass were positively correlated with trees producing calcium-rich leaf litter. Leaf litter from mature buckthorn had significantly more calcium than litter from juveniles (Table 1), which may influence the relationship between buckthorn and earthworms. As

buckthorn stands age, increasing calcium in litter as well as soil changes resulting from this calcium (and other cations) may be linked to increasing earthworm populations.

Though many of the measured soil differences between juvenile and mature stands were subtle (such as only marginal shifts in soil moisture and SOM, Figures 4 and 5), overall decomposition may be affected by the combination of these modifications. In this study, respiration rates were measured under juvenile and mature buckthorn individuals and used as a proxy for decomposition. Contrary to expectations, respiration rates did not differ between juvenile and mature stands (Figure 3). However, respiration rates are not always positively correlated to decomposition rates (Salamanca et al. 1998), and measuring mass loss using litterbags would be a more representative assessment of overall decomposition rates since it could incorporate both physical and microbial decomposition.

Furthermore, this field study did not separate the effects of litter chemistry from the effects of the soil environment. Though litter and soil interact to influence decomposition, it would be useful to understand each individually. For example, understanding how litter chemistry affects decomposition separate from the soil environment may be important given that significant differences did exist between leaf litter from juvenile and mature buckthorn shrubs. Separating the effects of litter chemistry and the soil environment in controlled laboratory experiments will also help eliminate possible confounding factors that may have influenced field results. For instance, individual buckthorn shrubs were selected from within an 80-acre site and unapparent differences among these individuals may have influenced both soil properties and respiration.

Additionally, other changes not considered here may occur as buckthorn stands age that could affect decomposition. For example, as plants mature they produce larger quantities of litter, which may have an impact on decomposition rates. Further, reproductively mature female shrubs produce copious amounts of drupes that reportedly have high N concentrations and contain secondary compounds (Knight et al. 2007). Sometimes these high N drupes go uneaten and are left to fall to the forest floor, in which case, they may affect decomposition. However, few, if any, studies have investigated the effects of fallen fruits on decomposition dynamics.

Conclusion

Overall, some differences exist between juvenile and mature buckthorn stands (i.e. litter chemistry) though it is still unclear how these differences may impact decomposition. Even if buckthorn-induced changes to soil properties and decomposition are minimal, these seemingly small modifications may have larger, unforeseen impacts, such as shifts in the abundance of earthworms, which can dramatically change the soil structure and soil food web. More research is needed to completely understand how changes resulting from buckthorn stand age may influence decomposition in the long term. Future studies should use more discrete age classes, separate litter and soil effects, utilize litterbags to measure mass loss, and investigate the effects of buckthorn drupes on decomposition. Understanding the differences between juvenile and mature buckthorn shrubs and the possible long-term effects that these differences have on an ecosystem will be crucial for targeting management efforts and developing forest restoration techniques. For example, if soil properties and ecosystem processes are altered more by mature buckthorn shrubs compared to juvenile shrubs, then additional restoration work may be needed after mature buckthorn removal such as C additions to balance out the high N litter. This could be important since in many cases, large mature individuals are left intact because they are more difficult to remove than juvenile individuals.

Acknowledgements

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