Ecosystem consequences of selective feeding of an insect herbivore: palatability-decomposability relationship revisited

HIDEKI KAGATA and TAKAYUKI OHGUSHI

Center for Ecological Research, Kyoto University, Japan

Correspondence: Hideki Kagata

Center for Ecological Research, Kyoto University,

Hirano 2-chome, Otsu, Shiga, 520-2113, Japan.

E-mail: kagata@ecology.kyoto-u.ac.jp

Tel: +81-77-549-8214

Fax: +81-77-549-8201

Running title: Palatability and decomposability

Abstract.

1. Relationship between leaf palatability and litter decomposability is critical to understanding the effects of selective feeding by herbivores on decomposition processes, and several studies have reported that there is a positive relationship between them.

2. However, palatability is not always positively correlated with decomposability, because of species-specific feeding adaptation of herbivores to host plants. Moreover, the effects of selective feeding by herbivores on soil decomposition processes should be understood in terms of the inputs of leaf litter and excrement.

3. The present study examined the relationships between leaf palatability and the decomposability of litter and frass, using *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae) and 15 temperate deciduous tree species.

4. Larvae of *L. dispar* exhibited a clear feeding preference, and subsequently the excreted frass mass differed among tree species. Litter and frass decomposability also differed among tree species, and frass was more rapidly decomposed than litter. There were no positive or negative correlations between palatability and decomposability of litter and frass.

5. These results indicate that *L. dispar* larvae may accelerate the decomposition process in temperate deciduous forests through selective feeding on plants with relatively low litter decomposability and the production of frass with higher decomposability than the litter.

Keywords: Decomposition, frass, host plant selection, *Lymantria dispar*, plant-insect interaction.

Introduction

Insect herbivores can influence soil decomposition processes through various pathways (Wardle and Bardgett 2004): changes in the quality and quantity of litter due to selective feeding on preferred plants and herbivore-induced plant responses (Chapman et al. 2003; Schmitz 2009) and return of the waste products and carcass to soil (Christenson et al. 2002; Yang 2006; Frost & Hunter 2007). The relationship between leaf palatability and litter decomposability is one of the factors determining the direction of effects of selective feeding by herbivores on decomposition processes (Bardgett and Wardle 2010). Several studies have shown that plants with foliage more palatable to generalist herbivores produce faster-decomposing litter (Grime et al. 1996; Schädler et al. 2003; Pálková and Leps 2008; but see Kurokawa and Nakashizuka 2008). Therefore, it is hypothesized that selective feeding by herbivores results in slow decomposition of leaf litter, according to such a positive relationship between palatability and decomposability (Hartley and Jones 2004; Bardgett and Wardle 2010), i.e., highly palatable (= highly decomposable) plants are selectively consumed by herbivores in a plant community and, as a result, poorly palatable (= poorly decomposable)

plants remain, and therefore, overall litter decomposition will be slow. The positive correlation between leaf palatability and litter decomposability results from the fact that plant defense traits against generalist herbivores, e.g., low nitrogen (N) and phosphorus, and high tannins and lignin, also reduce decomposer activity (Bardgett and Wardle 2010).

In most of the previous studies investigating the relationship between leaf palatability and litter decomposability, leaf palatability was determined by feeding experiments using slugs or crickets (Grime et al. 1996; Schädler et al. 2003; Pálková and Leps 2008; Kurokawa et al. 2010), which are suitable test organisms as generalist herbivores in determining leaf palatability. However, the leaf palatability derived from those herbivores would not always be applicable to other insect herbivores, because leaf palatability would depend on the identity of insect species due to species-specific feeding adaptation to host plants (Keathley and Potter 2008). Hence, a positive correlation between leaf palatability and litter decomposability may not always be expected in all plant-insect systems (Kurokawa and Nakashizuka 2008). To understand the effects of insect herbivores on the decomposition process through selective feeding, it is important to evaluate the leaf palatability using insect herbivores which are likely to have a significant impact on the decomposition process.

Moreover, insect herbivores can alter the energy and/or nutrient inputs to soil through return of the waste products, e.g., frass (Christenson et al. 2002; Frost & Hunter 2007). Insect frass contains larger amounts of N and labile carbon (C) than does leaf litter (Lovett and Ruesink 1995; Madritch et al. 2007). It can stimulate microbial activity (Frost and Hunter 2004), and in turn increase the decomposition rate (Zimmer and Topp 2002), N mineralization, and N immobilization (Lovett and Ruesink 1995; Frost and Hunter 2007). In addition, insect frass quality is strongly influenced by host leaf quality, such as nitrogen and tannins (Madritch et al. 2007; Kagata and Ohgushi 2011) which are known to be chemicals determining the decomposition efficiency of leaf litter (Enríquez 1993; Kraus et al. 2003). While selective feeding decreases the leaf litter produced by plants with high palatability, it would also lead to an increase in frass excreted by herbivores that fed on those plants. Therefore, the effects of selective feeding of insect herbivores on soil decomposition processes should be understood with respect to the inputs of both leaf litter and frass. However, the relationships between leaf palatability, litter decomposability and frass decomposability in herbivorous insects remain poorly known.

Here we examined the relationship between leaf palatability and decomposability of

litter and frass, using the gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae), and 15 temperate deciduous tree species. *Lymantria dispar* is a suitable herbivorous insect for examining the relationship between palatability and decomposability in temperate deciduous forests for the following reasons: (1) *L. dispar* is an important pest for temperate deciduous trees and sometimes occurs at extremely high density (Kamata 2002), (2) *L. dispar* larvae are highly polyphagous but have a clear hierarchical feeding preference regarding tree species (Liebhold et al. 1995; Shields et al. 2003), and (3) defoliation by *L. dispar* larvae can have significant impacts on decomposition and soil nutrient availability in a forest ecosystem (Lovett et al. 2002; Frost and Hunter 2004). Therefore, selective feeding and subsequent frass excretion by *L. dispar* larvae would have a potentially large impact on soil processes in temperate deciduous forests. We investigated the leaf palatability to *L. dispar* larvae by feeding tests, and conducted incubation experiments of leaf litter and larval frass in a laboratory microcosm to determine their decomposability.

Materials and Methods

Collection of plant leaves and litter

Plant materials used for the present study were collected in and around an experimental field of the Center for Ecological Research (Forest of CER; 35˚ N, 136˚ E), Kyoto University in Shiga prefecture, central Japan. The secondary forest is dominated by *Quercus serrata* Murray (Fagaceae) and *Pinus densiflora* Siebold et Zucc. (Pinaceae), and includes more than 50 tree species occurring naturally or artificially. We selected 15 tree species in 11 families for the experiment (Table 1), all of which were deciduous broad-leaved trees that are common in temperate forests. For each tree species, we collected fully expanded leaves from 4-6 tree individuals in late May to early June 2009 for a feeding experiment and frass collection. We also collected litter of each tree species underneath 4-6 tree individuals in late November 2009 for a litter incubation experiment. The litter samples were air-dried for one month and stored at –20 ˚C until the incubation experiment and chemical analysis.

Collection of L. dispar larvae and frass

Lymantria dispar is univoltine and overwinters as eggs. Larvae hatch in April and the larval

period lasts two months through molting five or six times in central Japan (Furuno 1964). Larvae of *L. dispar* are commonly observed on several trees in the forest of CER. Third- and fourth-instar larvae were collected from several tree species in and around the forest of CER in late April to early May 2009. The collected larvae (more than 400 individuals) were placed with leaves of *Q. serrata* in rearing containers (3000 ml each) with a maximum of 20 individuals per container. The containers were kept in an environmental chamber at 25 ˚C with a 16L8D light cycle. Leaves of *Q. serrata* were replaced with new ones every day. When the larvae reached sixth-instar (the last instar for most larvae, but some pass through seventh-instar until pupation, Furuno 1964), they were used for the feeding experiment and frass collection. It is known that approximately 70 % of the leaf consumption and frass excretion of immature stages occurs during the sixth-instar period in *L. dispar* (Furuno 1964). Hence, leaf consumption and frass excretion during this stage are critical for assessing the effects of selective feeding of *L. dispar* larvae on the decomposition process.

Frass of *L. dispar* for the incubation experiment was collected from 10-20 larvae each of tree species. Prior to the frass collection, the larvae were kept for 24 h without diet to allow them to excrete the frass in their guts. The larvae were placed together with leaves of

each tree species in a rearing container (3000 ml each) with a maximum of six individuals per container. They were kept in an environmental chamber at 25 ˚C with a 16L8D light cycle. Leaves of each tree species were replaced daily with new ones. The larval frass was collected every day until pupation, and was stored at –20 ˚C until the incubation experiment, after it was oven-dried at 60 ˚C for one week.

Feeding experiment

The relative leaf palatability of 15 deciduous tree species for *L. dispar* was determined by a non-choice feeding trial, using the sixth-instar larvae obtained from laboratory rearing as described above. Prior to the feeding trial, the larvae were kept for 24 h without diet to allow them to excrete the frass in their guts. One larva was placed in a 250-ml plastic cup with a few leaves, about 2 g fresh weight equivalent, for each tree species in an environmental chamber at 25 ˚C with a 16L8D light cycle. The larvae and leaves were weighed before the feeding trial. After 24 h the leaves were removed and the larvae were kept for 24 h without diet to allow them to excrete the frass in the gut. Thereafter, the larval frass was collected. Leaves,

larvae, and frass were oven-dried at 60 ˚C for one week to determine dry weight. Leaves and frass were stored at –20 ˚C until C and N analyses. Nine to 11 replicates were conducted for each tree species (Table 1).

Consumed leaf mass, as an index of leaf palatability, was determined as the difference in leaf dry mass between the start and the end of the feeding trial. Leaf dry mass of each tree species at the start of the feeding trial was estimated from the leaf water concentration, which was measured using additional samples (n = 4-6 for each tree species). The water concentration was determined from the difference between the fresh and dry mass, which was measured after oven-drying at 60 ˚C for one week. In addition, larval growth mass was also determined as the difference in larval dry mass between the start and the end of the feeding trial. Similarly, larval dry mass at the start of the feeding trial was estimated from their water concentration, which was measured using additional samples (n = 10). Palatability was also evaluated by excreted frass mass, because there was a strong positive correlation between consumed leaf mass and excreted frass mass (see Results). We noted that the palatability of *L. dispar* larvae observed in the present study may, to some degree, be affected by previous food experience prior to the feeding trials (Mattson and Scriber 1987), i.e., all

larvae were reared on leaves of *Q. serrata* until the feeding trials.

Incubation experiment

Decomposability of leaf litter and insect frass was examined by incubation experiments in a laboratory microcosm. Prior to the experiment, leaf litter and insect frass were roughly ground, and mixed well for each treatment to obtain homogeneous quality. Litter or frass (750 mg) was placed in a 50-ml glass vial with 750 ml of soil and 2 ml of distilled water, which brought the samples to 60-70 % of the water capacity of the substrates. Soil was added as a soil microbe source, and soil-alone treatment was set as a control. The soil was collected underneath (< 5 cm in depth) several *Q. serrata* trees in the forest of CER in late November 2009. It was air-dried for one month and passed through a 2-mm sieve, and was stored at 5 ˚C until use for the experiment. Fifteen replicates were established for each leaf litter and frass, except for the frass originated from five tree species which were not examined due to insufficient mass of frass for the experiment (Table 1). The test samples were incubated in the dark at 25 ˚C in an environmental chamber for four weeks. After incubation, the samples were

oven-dried at 60 ˚C for two weeks to measure dry weight. The decomposability of litter and frass was determined by the reduction in dry mass during the incubation.

Carbon and nitrogen analyses

Prior to the analysis, all samples (fresh leaves, leaf litter, and insect frass) were ground to fine powder. Total C and N contents were determined using an elemental analyzer (JM 1000CN, J-Science Co., Ltd, Kyoto, Japan). Carbon and N contents of the frass originated from three tree species were not measured due to insufficient mass of the frass for the analyses (Table 1).

Statistical analyses

Leaf consumption, frass excretion, and litter and frass decomposition were compared among plant species by one-way ANOVAs. Differences in C:N ratio among leaf, litter, and frass were tested by paired t tests. The difference between decomposition of litter and frass was also tested by paired t test. Relationships among litter and frass C:N ratio, palatability and

decomposability of litter and frass were evaluated using correlation coefficients (i.e., species-level analysis in which plant phylogeny was not considered). These relationships were also analyzed using phylogenetically independent contrasts (PICs) (Garland et al. 1992). A phylogenetic hypothesis for the studied plants was constructed using a recently inferred phylogenetic tree based on the Angiosperm Phylogeny Group classification (APG III 2009). This phylogenetic tree was resolved at the family level. Hence, we placed the genera as branches within families and species as branches within genera, where we had multiple species within a family (Fig. 1). We calculated PICs for each measured parameter, assuming that all branch lengths were equal. Because the statistical results in PICs were similar to the results in species-level analysis (see Table 2), the results were described on the basis of PICs in the Results section. All analyses were conducted using JMP version 6 (SAS Institute Japan, Tokyo, Japan), except for the PICs which were analyzed using the package *ape* in R (R Development Core Team 2010).

Results

Feeding experiment

Larvae of *L. dispar* showed strong preferences for certain tree species: consumed leaf mass differed significantly among tree species (ANOVA: $df = 14,139$, $F = 17.39$, $P < 0.0001$, Fig. 2a). Excreted frass mass also differed significantly among tree species (ANOVA: $df = 14,139$, $F = 22.48$, $P < 0.0001$, Fig. 2b). A strong positive correlation was detected between consumed leaf mass and excreted frass mass ($r = 0.88$, $P < 0.0001$, Table 2). Larvae converted approximately 60 % of consumed leaf mass to frass. Larval growth was significantly and positively correlated with consumed leaf mass ($r = 0.89$, $P < 0.0001$).

There was no significant correlation between leaf C:N ratio and consumed leaf mass or between leaf C:N ratio and excreted frass mass (Table 2). Frass C:N ratio did not differ from leaf C:N ratio (Paired t test, $df = 11$, $t = 2.00$, $P = 0.07$, Fig. 3a), and it was strongly, positively correlated with leaf C:N ratio ($r = 0.91$, $P = 0.0002$, Table 2).

Incubation experiment

Litter decomposability, expressed by dry weight loss during litter incubation, significantly differed among tree species (ANOVA: df = 12,210, F = 254.92, P < 0.0001, Fig. 2c). While soil-alone treatment lost only 10.1 mg of substrate mass, litter treatments lost 40.7-179.1 mg of mass during the four-week incubation. Frass decomposability also differed significantly among tree species (ANOVA: df = 9,139, F = 876.9, P < 0.0001, Fig. 2d), with loss of substrate mass ranging from 111.6 to 311.6 mg during the incubation. A significant correlation between litter and frass decomposability was not detected (r = 0.55, P = 0.09; Table 2).

Frass C:N ratio was significantly lower than litter C:N ratio (Paired t test: df = 11, t = – 4.55, P = 0.0008, Fig. 3a). Frass decomposability was significantly greater than litter decomposability (Paired t test: df = 9, t = 4.84, P < 0.0009, Fig. 3b). The reduction of mass in the frass incubation was approximately double compared to that in the litter incubation. Litter and frass decomposability were not correlated with litter or frass C:N ratio, respectively (Table 2).

Relationships between palatability and decomposability

Palatability, expressed by consumed leaf mass, was not correlated significantly with litter or frass decomposability, although correlation coefficients showed negative values for each relationship (Table 2, Fig. 4a and c). When excreted frass mass was also regarded as an index of palatability because there was a strong positive correlation between consumed leaf mass and excreted frass mass (Table 2), the excreted frass mass was significantly, negatively correlated with frass decomposability (Table 2 and Fig. 4d). On the other hand, it was not correlated significantly with litter decomposability, although correlation coefficient was negative (Table 2 and Fig. 4c).

Discussion

Palatability to L. dispar

Lymantria dispar larvae are generalist herbivores, which can feed on over 500 plant species, and exhibit a clear hierarchical feeding preference (Liebhold et al. 1995; Shields et al. 2003). Several studies have examined factors involved in determining the host plant selection of *L.*

dispar larvae, and demonstrated that the larvae preferred plant species that have no or low levels of alkaloids in leaves (Barbosa and Krischik 1987; Shields et al. 2003), but the larval preference was not affected by foliar tannins (Barbosa and Krischik 1987; Shields et al. 2003), lignin (Brodeur-Campbell et al. 2006), or other C-based secondary metabolites (Barbosa and Krischik 1987).

Tannins are common as anti-herbivore defensive substrates in a diverse group of woody plants (Feeny 1970; Barbosa and Krischik 1987). However, there is increasing evidence that tannins are not always a feeding deterrent against insect herbivores (Ayres et al. 1997; Forkner et al. 2004; Keathley and Potter 2008). In particular, they have little or no effect on host selection of generalist insect herbivores, such as *L. dispar* and *Popillia japonica* (Barbosa and Krischik 1987; Keathley and Potter 2008). In contrast, alkaloids have strong feeding deterrence for the host plant selection of *L. dispar* larvae (e.g. Barbosa and Krischik 1987). It is known that alkaloids are rare or absent in Betulaceae, Fagaceae, and Salicaceae, and are present in Magnoliaceae, Araliaceae, and *Prunus* (Rosaceae) (Barbosa and Krischik 1987). In fact, our results showed that the former plants were relatively preferred, while the latter plants were rejected by *L. dispar* larvae. Although it is not clear whether other plant

species used in the present study contain alkaloids, the leaf palatability to *L. dispar* larvae shown in the present study is most likely determined by foliar alkaloids, rather than by C-based secondary metabolites, such as tannins and lignin.

Decomposability of litter and frass

It is well known that the decomposability of litter is largely dependent on the quality of the litter, i.e., the concentrations of N, phosphorus, tannins, and lignin, and their relative ratios across various plant species (Gallardo and Merino 1993; Aerts 1997; Kraus et al. 2003, Osono and Takeda 2005; Kurokawa and Nakashizuka 2008). In particular, tannins and lignin are important chemicals which suppress the litter decomposition rate (Kraus et al. 2003). Several studies demonstrated that litter with higher levels of tannins and/or lignin was decomposed more slowly for temperate deciduous trees (Gallard and Merino 1993; Osono and Takeda 2005). In contrast, the effects of N-based secondary metabolites, such as alkaloids, on litter decomposition have not been well explored. Siegrist et al. (2010) showed that alkaloids were not detected in the litter of a grass, *Schedonorus arundinaceus*, in spite of high levels of

alkaloids in leaves, and that alkaloids had little effect on the decomposition process in a litter bag experiment with alkaloid addition. Therefore, it is most likely that tannins and/or lignin, but not alkaloids, were the important determinants of the decomposability shown in the present study.

Our results also showed that frass decomposability differed among tree species, and there was no strong correlation between litter and frass decomposability. Although litter and frass traits, such as C:N ratio and tannins, tend to be positively correlated with fresh leaf traits (Madritch et al. 2007; Kurokawa and Nakashizuka 2008), we did not find a significant relationship between litter and frass C:N ratio. Hence, the chemical characteristics of the litter and frass may be determined in independent manners, resulting in the lack of a strong relationship between litter and frass decomposability. On the other hand, our results showed that frass was more rapidly decomposed than leaf litter. In general, insect frass contains larger amounts of N and labile C than does leaf litter (Lovett and Ruesink 1995; Madritch et al. 2007), which causes acceleration of the decomposition rate of frass (Zimmer and Topp 2002). Although the frass C:N ratio was lower than the litter C:N ratio, frass and litter decomposability was not explained by the C:N ratio (see Table 2). Specific compounds, such

as condensed tannins, rather than C:N ratio, may be important in determining frass and litter decomposability (Hättenschwiler and Jørgensen 2010).

315

Relationships between palatability and decomposability

Several studies have shown that the palatability of leaves to generalist herbivores is positively correlated with litter decomposability (Grime et al. 1996; Schädler et al. 2003; Pálková and

320 Leps 2008), which suggests that the factors determining leaf palatability might also determine litter decomposability (Bardgett and Wardle 2010). However, we found no positive correlations between palatability and decomposability in the system of gypsy moth and temperate deciduous trees. Similarly, Kurokawa and Nakashizuka (2008) found that while litter decomposability was largely determined by condensed tannins and lignin:N ratio, leaf

325 palatability was not determined by such simple factors, resulting in the lack of a relationship between leaf palatability and litter decomposability in a tropical rain forest. The lack of a positive relationship between palatability and decomposability in the present study was probably also due to some difference between the key factors determining palatability and

decomposability: leaf palatability to *L. dispar* larvae is likely to be determined by alkaloids, whereas litter and frass decomposability are likely to be determined by tannins and lignin.

Moreover, our results showed a negative correlation between excreted frass mass and frass decomposability. The correlation coefficient was also negative between leaf palatability and litter decomposability, although it was not statistically significant at $P = 0.05$. These negative relationships indicate that the factors determining palatability and decomposability may be negatively associated in our experimental system. As mentioned above, the palatability in this study was probably determined by alkaloids, while decomposability was probably determined by C-based secondary metabolites such as tannins and lignin. Several researchers have hypothesized that there is a negative association between C-based and N-based anti-herbivore defenses in plants (Bryant et al. 1983; Coley et al. 1985). Actually, Stevens et al. (1995) showed that alkaloids were negatively correlated with tannin content in Crassulaceae plants. On the other hand, there is increasing evidence that no clear trade-offs are found between alkaloid defense and other C-based defenses (Steward and Keeler 1988; Koricheva 2002). Further understanding of the associations among multiple anti-herbivore defenses in plants would contribute to clarifying the mechanisms responsible

for the palatability-decomposability relationships.

Ecosystem consequences of herbivore selective feeding

A positive association between leaf palatability and litter decomposability implies slower litter decomposition due to selective feeding of generalist herbivores, because plants that produce more decomposable litter are preferentially consumed (Hartley and Jones 2004; Bardgett and Wardle 2010). However, the present study found no evidence of such a positive relationship, and even showed a negative relationship between palatability and decomposability. We also found that frass of *L. dispar* was decomposed faster than leaf litter. These findings indicate that *L. dispar* larvae may accelerate the decomposition process in temperate deciduous forests through selective feeding on plants with relatively low litter decomposability and producing frass with higher decomposability than the litter. In addition, selective feeding by insect herbivores could also accelerate litter decomposition via induction of plant regrowth, i.e., selective feeding on plants that tolerate defoliation by mounting a regrowth response, which would produce litter with high decomposability (Hunter 2001). The

effects of such herbivore-induced plant responses on litter decomposability were not examined in the present study. However, the plant responses to insect herbivory would be important factor determining the relationship between palatability and decomposability through changes in the litter decomposition process (Findlay et al. 1996; Uselman et al. 2011).

Although the relationship between palatability and decomposability is critical for understanding the effects of selective feeding by herbivores on the decomposition process, this relationship is still a controversial issue because it may depend on the plant-herbivore systems, mechanistic pathways by which herbivores influence decomposition processes, and temporal/spatial scale (Hunter 2001). Our results were derived from short-terms and small-scale laboratory experiment, which may have influenced the observed palatability and/or decomposability. Nevertheless, present study would raise an important issue that there was no positive or negative relationship between palatability and decomposability for *L. dispar*, in contrast to previous studies that showed a positive relationship for slugs and crickets (Grime et al. 1996; Schädler et al. 2003; Pálková and Leps 2008). Further studies examining which types of herbivores show positive, negative or neutral relationships between palatability and decomposability will be needed to clarify how herbivores influence

decomposition processes.

Acknowledgements

We thank I. Kojima and A. Matsumoto for identification of tree species, A. Kawakita for help in statistical analyses, and E. Nakajima for English proofreading of the manuscript. This study was supported by a Grant-in-Aid for Scientific Research (B-20370010), and Kyoto University Global COE Program (A06).

References

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

APG-III (2009) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society*, **161**, 105-121.

Ayres, M.P., Clausen, T.P., MacLean Jr., S.F., Redman, A.M., & Reichard, P.B. (1997) Diversity of structure and antiherbivore activity in condensed tannins. *Ecology*, **78**, 1696-1712.

Barbosa, P. & Krischik, V.A. (1987) Influence of alkaloids on feeding preference of eastern deciduous forest trees by the gypsy moth *Lymantria Dispar*. *The American Naturalist*, **130**, 53-69.

Bardgett, R.D. & Wardle, D.A. (2010) *Aboveground-Belowground Linkages* Oxford University Press, New York.

Brodeur-Campbell, S.E., Vucetich, J.A., Richter, D.L., Waite, T.A., Rosemier, J.N., & Tsai, C.

(2006) Insect herbivory on low-lignin transgenic aspen. *Plant-Insect Interactions*, **35**, 1696-1701.

Bryant, J.P., Chapin, F.S., & Klein, B. (1983) Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos*, **40**, 357-368.

Chapman, S.K., Hart, S.C., Cobb, N.S., Whitham, T.G., & Koch, G.W. (2003) Insect herbivory increases litter quality and decomposition: an extension of the acceleration hypothesis. *Ecology*, **84**, 2867-2876.

Christenson, L.M., Lovett, G.M., Mitchell, M.J., & Groffman, P.M. (2002) The fate of nitrogen in gypsy moth frass deposited to an oak forest floor. *Oecologia*, **131**, 444-452.

Coley, P.D., Bryant, J.P., & Chapin, F.S. (1985) Resource availability and plant antiherbivore defense. *Science*, **230**, 895-899.

Enríquez, S., Duarte, C.M., & Sand-Jensen, K. (1993) Patterns in decomposition rates among photosynthetic organisms: the importance of detritus C:N:P content. *Oecologia*, **94**, 457-471.

Feeny, P. (1970) Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. *Ecology*, **51**, 555-581.

Findlay, S., Carreio, M., Krischik, V., & Jones, C.G. (1996) Effects of damage to living plants on leaf litter quality. *Ecological Applications*, **6**, 269-275.

Forkner, P.E., Marquis, R.J., & Lill, J.T. (2004) Feeny revisited: condensed tannins as anti-herbivore defenses in leaf-chewing herbivore communities of *Quercus*. *Ecological Entomology*, **29**, 174-187.

Frost, C.J. & Hunter, M.D. (2004) Insect canopy herbivory and frass deposition affect soil nutrient dynamics and export in oak mesocosms. *Ecology*, **85**, 3335-3347.

Frost, C.J. & Hunter, M.D. (2007) Recycling of nitrogen in herbivore feces: plant recovery, herbivore assimilation, soil retention, and leaching losses. *Oecologia*, **151**, 42-53.

Furuno, T. (1964) On the feeding quantity of the gypsy moth (*Lymantria dispar* Linne) and the camphor silk moth (*Dictyoploca japonica* Bulter). *Journal of the Japanese Forest Society*, **46**, 14-19.

Gallardo, A. & Merino, J. (1993) Leaf decomposition in two mediterranean ecosystems of southwest Spain: influence of substrate quality. *Ecology*, **74**, 152-161.

Garland, T., Harvey, P.H., & Ives, A.R. (1992) Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Systematics Biology*, **41**, 18-32.

Grime, J.P., Cornelissen, J.H., & Hodgson, J.G. (1996) Evidence of a causal connection between anti-herbivore defense and the decomposition rate of leaves. *Oikos*, **77**, 489-494.

Hartley, S.E. & Jones, T.H. (2004). Insect herbivores, nutrient cycling and plant productivity. *Insects and Ecosystem Function* (ed. by W.W. Weisser and E. Siemann), pp. 27-52. Springer-Verlag, Berlin.

Hättenschwiler, S. & Jørgensen, H.B. (2010) Carbon quality rather than stoichiometry controls litter decomposition in a tropical rain forest. *Journal of Ecology*, **98**, 754-763.

Hunter, M. D. (2001) Insect population dynamics meets ecosystem ecology: effects of herbivory on soil nutrient dynamics. *Agricultural and Forest Entomology*, **3**, 77-84

Kagata, H. & Ohgushi, T. (2011) Ingestion and excretion of nitrogen by larvae of a cabbage armyworm: the effects of fertilizer application. *Agricultural and Forest Entomology*, **13**, 143-148.

Kamata, N. (2002) Outbreaks of forest defoliating insects in Japan, 1950-2000. *Bulletin of Entomological Research*, **92**, 109-117.

Keathley, C.P. & Potter, D.A. (2008) Quantitative resistance traits and suitability of woody plant species for a polyphagous scarab, *Popillia japonica* Newman. *Environmental*

Entomology, **37**, 1548-1557.

Koricheva, J. (2002) The carbon-nutrient balance hypothesis is dead; long live the carbon-nutrient balance hypothesis ? *Oikos*, **98**, 537-539.

Kraus, T.E.C., Dahlgren, R.A., & Zasoski, R.J. (2003) Tannins in nutrient dynamics of forest ecosystems - a review. *Plant and Soil*, **256**, 41-66.

Kurokawa, H. & Nakashizuka, T. (2008) Leaf herbivory and decomposability in a Malaysian tropical rain forest. *Ecology*, **89**, 2645-2656.

Kurokawa, H., Peltzer, D.A., & Wardle, D.A. (2010) Plant traits, leaf palatability and litter decomposability for co-occurring woody species differing in invasion status and nitrogen fixation ability. *Functional Ecology*, **24**, 513-523.

Liebhold, A.M., Gottschalk, K.W., Muzika, R., Montgomery, M.E., Young, R., O'Day, K., & Kelley, B. (1995) *Suitability of North American tree species to the gypsy moth: a summary of field and laboratory tests* USDA Forest Service, Delaware, Ohio.

Lovett, G.M. & Ruesink, A.E. (1995) Carbon and nitrogen mineralization from decomposing gypsy moth frass. *Oecologia*, **104**, 133-138.

Lovett, G.M., Christenson, L.M., Groffman, P.M., Jones, C.G., Hart, J.E., & Mitchell, M.J.

(2002) Insect defoliation and nitrogen cycling in forests. *BioScience*, **52**, 335-341.

Madritch, M.D., Donaldson, J.R., & Lindroth, R.L. (2007) Canopy herbivory can mediate the influence of plant genotype on soil processes through frass deposition. *Soil Biology & Biochemistry*, **39**, 1192-1201.

Mattson, W.J. & Scriber, J.M. (1987). Nutritional ecology of insect folivores of woody plants: nitrogen, water, fiber, and mineral considerations. *Nutritional Ecology of Insects, Mites, Spiders, and Related Invertebrates* (ed. by F. Slansky & J.G. Rodrigues), pp. 105-146. John Wiley & Sons, New York.

Osono, T. & Takeda, H. (2005) Decomposition of organic chemical components in relation to nitrogen dynamics in leaf litter of 14 tree species in a cool temperate forest. *Ecological Research*, **20**, 41-49.

Pálková, K. & Leps, J. (2008) Positive relationship between plant palatability and litter decomposition in meadow plants. *Community Ecology*, **9**, 17-27.

Schädler, M., Jung, G., Auge, H., & Brandl, R. (2003) Palatability, decomposition and insect herbivory: patterns in a successional old-field plant community. *Oikos*, **103**, 121-132.

Schmitz, O.J. (2009) Effects of predator functional diversity on grassland ecosystem function.

Ecology, **90**, 2239-2345.

Shields, V.D.C., Broomell, B.P., & Salako, J.O.B. (2003) Host selection and acceptability of selected tree species by gypsy moth larvae, Lymantria dispar (L.). *Annals of the Entomological Society of America*, **96**, 920-926.

Siegrist, J.A., McCulley, R.L., Bush, L.P., & Phillips, T.D. (2010) Alkaloids may not be responsible for endophyte-associated reductions in tall fescue decomposition rates. *Functional Ecology*, **24**, 460-468.

Stevens, J.F., Hart, H.T., van Ham, R.C.H.J., Elema, E.T., van den Ent, M.M.V.X., Wildeboer, M., & Zwaving, J.H. (1995) Distribution of alkaloids and tannins in the Crassulaceae. *Biochemical Systematics and Ecology*, **23**, 157-165.

Steward, J.L. & Keeler, K.H. (1988) Are there trade-offs among antiherbivore defenses in *Ipomoea* (Convolvulaceae)? *Oikos*, **53**, 79-86.

Uselman, S.M., Snyder, K.A., & Blank, R.R. (2011) Insect biological control accelerates leaf litter decomposition and alters short-term nutrient dynamics in a *Tamarix*-invaded riparian ecosystem. *Oikos*, **120**, 409-417.

Wardle, D.A. & Bardgett, R.D. (2004). Indirect effects of Invertebrate herbivory on the

decomposition subsystem. *Insects and Ecosystem Function* (ed. by W.W. Weisser and E. Siemann), pp. 53-70. Springer-Verlag, Berlin.

Yang, L.H. (2006) Interactions between a detrial resource pulse and a detritivore community. *Oecologia*, **147**.

Zimmer, M. & Topp, W. (2002) The role of coprophagy in nutrient release from feces of phytophagous insects. *Soil Biology & Biochemistry*, **34**, 1093-1099.

Table 1: Tree species used in the present study. Sample size for leaf and litter collection, and number of replications in the feeding trial, litter and frass incubation experiments are also presented. Blanks indicate no data. Tree = number of tree individuals for which leaves and litter were collected. Feeding = number of replications in the feeding trial. L inc = number of replications of the litter incubation. F inc = number of replications of frass incubation. L CN = sample size in C and N analyses for leaves and litter. F CN = sample size in C and N analyses for frass.

Family	Species	Sample size or replication					
		Tree	Feeding	L inc	F inc	L CN	F CN
Magnoliaceae	*Magnolia obovata* Thunb.	5	11	15		5	11
Betulaceae	*Alnus sieboldiana* Matsumura	6	11	15	15	6	11
Fagaceae	*Castanea crenata* Siebold & Zucc.	5	10	15	15	5	10
	Quercus acutissima Carruth.	6	9	15	15	6	9
	Quercus serrata Murray	6	10	15	15	6	10
Ulmaceae	*Zelkova serrata* Makino	6	10	15	15	6	10
Rosaceae	*Prunus grayana* Maxim.	6	10	15		6	10
	Prunus jamasakura Nakai	6	11	15		6	
Fabaceae	*Wisteria floribunda* (Willd.)	4	10	15	15	4	10
Euphorbiaceae	*Mallotus japonicus* (Thunb.)	6	10	15	15	6	10
Salicaceae	*Salix eriocarpa* Franch. & Sav.	5	10	15	15	5	10
	Populus tremula L.	6	9	15	15	6	9
Anacardiaceae	*Rhus javanica* L.	6	11	15	15	6	11
Clethraceae	*Clethra barbinervis* Siebold & Zucc.	6	11	15		6	
Araliaceae	*Gamblea innovans* (Siebold & Zucc.)	6	11	15		6	

Table 2: Correlation coefficients in species-level analyses (above the diagonal) and in phylogenetically independent contrasts (below the diagonal and shown by bold) between measured parameters, i.e., leaf C:N ratio, litter C:N ratio, frass C:N ratio, leaf consumption, frass excretion, litter decomposition, and frass decomposition. They were explained on the basis of the analyses in phylogenetically independent contrasts in the text. ***: P < 0.0001, **: P < 0.001, *: P < 0.05, (*): P < 0.1.

	Leaf CN	Litter CN	Frass CN	Consumption	Excretion	Litter dec	Frass dec
Leaf CN		0.23	0.90 ***	– 0.16	– 0.21	– 0.37	0.26
Litter CN	**0.17**		– 0.03	– 0.29	– 0.40	– 0.21	0.37
Frass CN	**0.91** **	**– 0.08**		0.13	– 0.01	– 0.34	0.38
Consumption	**– 0.13**	**– 0.16**	**0.06**		0.93 ***	– 0.48 (*)	– 0.59 (*)
Excretion	**– 0.18**	**– 0.33**	**0.01**	**0.88** ***		– 0.52 *	– 0.74 *
Litter dec	**– 0.39**	**– 0.30**	**– 0.36**	**– 0.40**	**– 0.46** (*)		0.46
Frass dec	**0.01**	**– 0.48**	**– 0.03**	**– 0.35**	**– 0.63** *	**0.55** (*)	

Figure legends

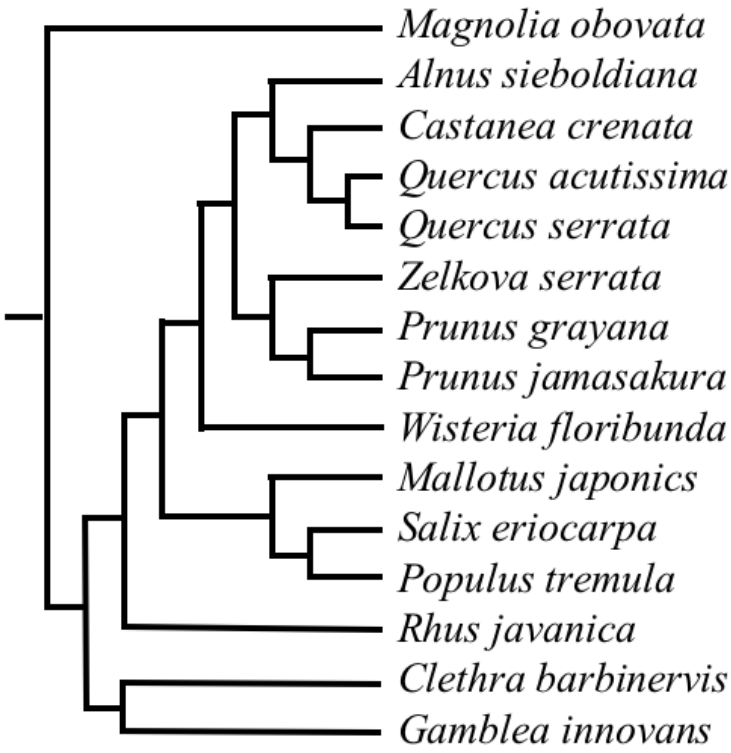
Fig. 1. Phylogenetic hypothesis used for calculation of phylogenetically independent contrasts, which is based on the Angiosperm Phylogeny Group classification (APG III 2009).

Fig. 2. (a) Consumed leaf mass, (b) frass mass excreted by *L. dispar* larvae in the feeding experiment (c) decrease of litter mass, and (d) decrease of frass mass in the incubation experiment. Means with SE are presented.

Fig. 3. (a) C:N ratio in fresh leaves, litter, and frass, and (b) decomposition of litter and frass. Decomposition is shown as decrease of substrate mass (mg). Means with SE are presented.

Fig. 4. Relationship between palatability and decomposability as indicated by relationships between: (a) leaf consumption and litter decomposition, (b) frass excretion and litter decomposition, (c) leaf consumption and frass decomposition, and (d) frass excretion and frass decomposition. Statistical values were based on the analyses using phylogenetically independent contrasts (see also Table 2).

Fig. 1


Magnolia obovata
Alnus sieboldiana
Castanea crenata
Quercus acutissima
Quercus serrata
Zelkova serrata
Prunus grayana
Prunus jamasakura
Wisteria floribunda
Mallotus japonics
Salix eriocarpa
Populus tremula
Rhus javanica
Clethra barbinervis
Gamblea innovans

545 Fig.2

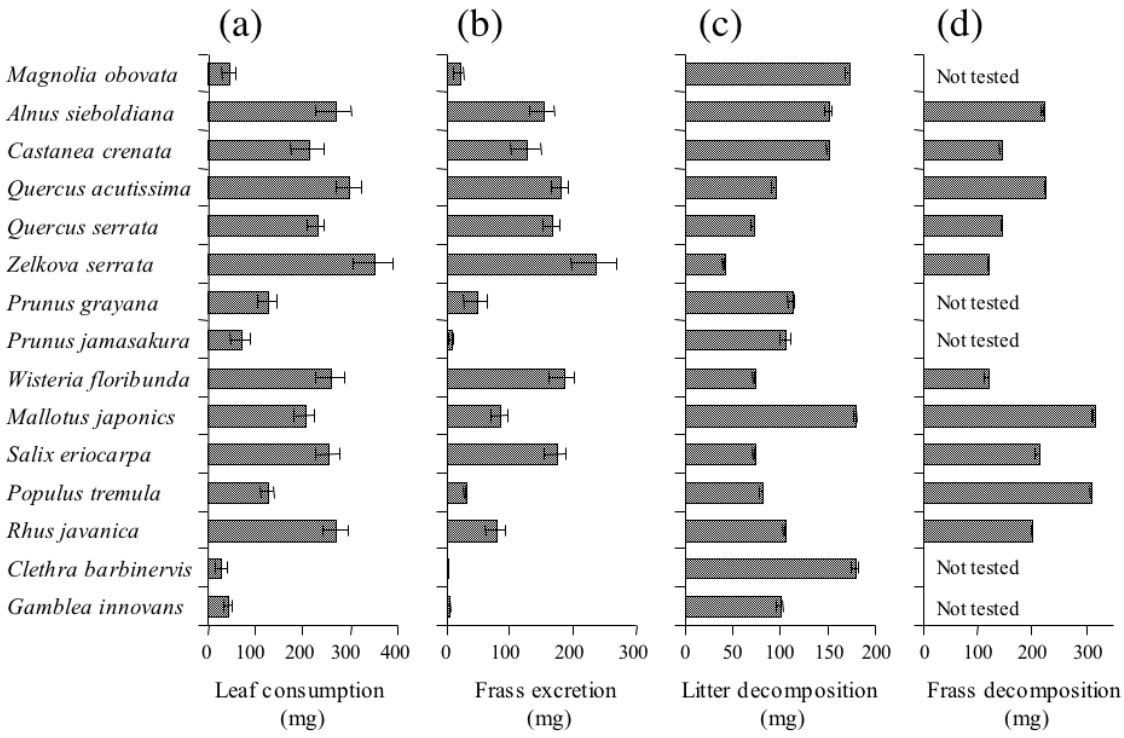
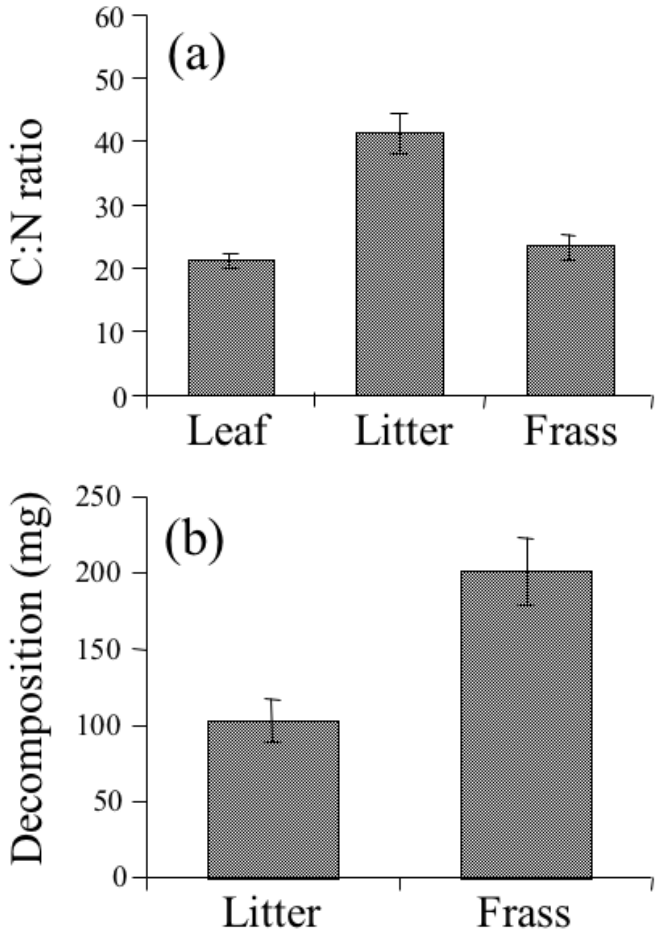
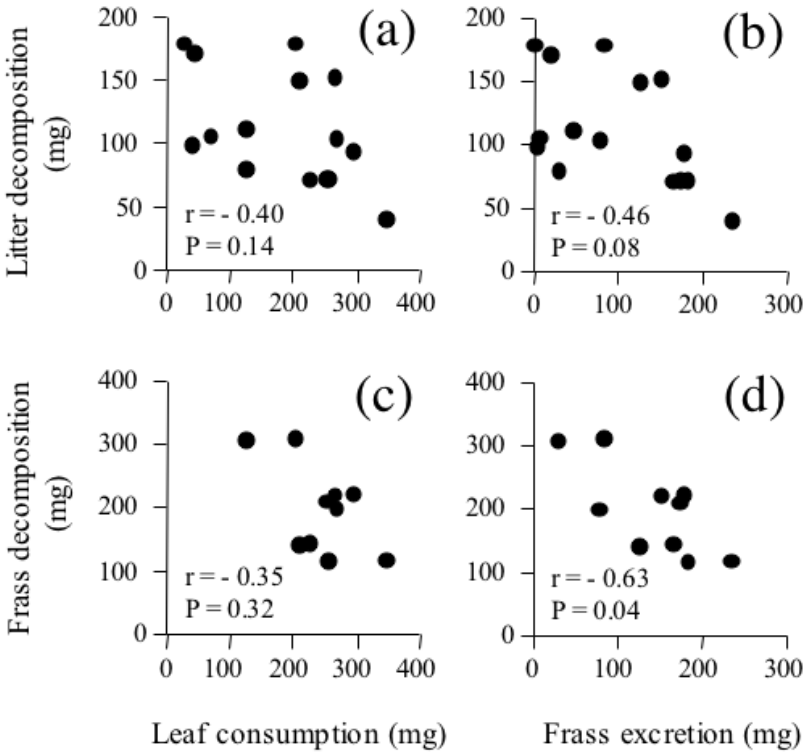

(a)
(b)
(c)
(d)
Magnolia obovata
Alnus sieboldiana
Castanea crenata
Quercus acutissima
Quercus serrata
Zelkova serrata
Prunus grayana
Prunus jamasakura
Wisteria floribunda
Mallotus japonics
Salix eriocarpa
Populus tremula
Rhus javanica
Clethra barbinervis
Gamblea innovans
Not tested
Not tested
Not tested
Not tested
0 100 200 300 400
0 100 200 300
0 50 100 150 200
0 100 200 300
Leaf consumption (mg)
Frass excretion (mg)
Litter decomposition (mg)
Frass decomposition (mg)

Fig.3


(a)
C:N ratio
60
50
40
30
20
10
0
Leaf
Litter
Frass
(b)
Decomposition (mg)
250
200
150
100
50
0
Litter
Frass

550

Fig.4


(a)
(b)
(c)
(d)
Litter decomposition (mg)
Frass decomposition (mg)
r = - 0.40
P = 0.14
r = - 0.46
P = 0.08
r = - 0.35
P = 0.32
r = - 0.63
P = 0.04
0
50
100
150
200
300
400
Leaf consumption (mg)
Frass excretion (mg)