Decomposition of Forest Litter and Feces of Armadillidium vulgare (Isopoda: Oniscidea) Produced from the Same Litter Affected by Temperature and Litter Quality

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Abstract: To explore the question how litter and macrofauna feces respond to temperature and how respiration differs for litter with a different CN ratio, we compared the decomposition rates of leaf litter (Alnus glutinosa, Salix caprea, and Acer campestre) and isopod (Armadillidium vulgare) feces produced from the same litter in response to three constant (8, 16, and 24 °C) and one fluctuating (first week 8 °C, the other week 24 °C) temperatures in a 50 week laboratory experiment and in a field trial. Microbial respiration of litter with lower CN ratio (alder and willow) was significantly higher than respiration of feces, no significant difference was found for maple litter with higher CN ratio. This was supported by field litter bag experiments where alder and willow litter decomposed faster than feces but the opposite was true for maple litter. Litter respiration was significantly affected by temperature but feces respiration was not. Fluctuating temperature caused either lower or equal respiration as compared to mean constant temperature. The content of phenolics was significantly higher in intact litter in comparison with decomposed litter and feces, either fresh or decomposed. The CN ratio decreased as litter turned to feces in maple and alder litter but increased in willow litter. In conclusion, microbial respiration of both litter and feces were substantially affected by litter quality; the litter was more sensitive to temperature than feces.

Keywords: CN ratio; feces; isopods; litter decomposition; microbial respiration; phenolics

1. Introduction

Soil contains three times more carbon (C) than the atmosphere. Carbon storage in terrestrial ecosystems depends on the balance between the gain from net primary production and the loss through decomposition [1–3]. Hence, litter decomposition exhibits a critical function in the C budget of terrestrial ecosystems [4,5].

Leaf litter decomposition and mineralization are regulated by an array of abiotic factors, of which the most important are climate, mainly temperature and moisture [6], and the chemical nature of litter [7]. Increasing temperature directly accelerates metabolic and biochemical processes [8], therefore accelerating decomposition rates as well. However, different soils, in which organic matter is bound in different ways, show highly variable responses to temperature [5]. Ecological stoichiometry describes how macroelements C, nitrogen (N), phosphorus (P), and their ratios are critical for organisms to build biological structures and regulate physiological processes [9]. Microbial growth requires a balance among those elements. Therefore, the relative abundance of C and nutrients in organic...
matter should regulate microbial activity and, thus, influence litter decomposition processes [9]. Besides stoichiometry, compounds that play a fundamental role in the chemical defense of plants against herbivores and pathogens, such as phenolic compounds, may substantially affect litter decomposition [10]. Some researchers have shown that the total phenolic content differs between early successional-tree species and old-growth species [11]. The phenolic content also changes with plant growth and is affected by abiotic factors, such as temperature and radiation [12]. Phenolic compounds may affect the activity of microbial and faunal decomposers. [13,14] showed that phenolics can inhibit enzyme-catalyzed reactions or bind and precipitate proteins. Similar findings were presented by Frouz et al., 2011 [15]; they observed the polymerization of organic substances and the inclusion of proteins and phenols into humic acids in the digestive tracts of bibionid larvae and earthworms. Many decomposers have specific adaptations to deal with phenolic compounds. Some, such as Bibionidae larvae, use a highly alkaline gut to make phenol protein complexes more soluble [16,17], while isopods are capable of oxidizing [18] and hydrolyzing ingested phenolics [13,19]. Other studies observed a specific activity of peroxidase in earthworms [20]. However, there is little information about relationships between the changes in the content of phenolics during decomposition and decomposition rate.

Soil fauna [21] affects leaf litter decomposition. The woodlice (Isopoda) belong to abundant macro-decomposers together with millipedes (Diplopoda), earthworms (Oligochaeta), dipteran larvae, and termites [22]. While they make only little direct contribution to the decomposition process per se (mineralization of organic compounds into carbon dioxide), the soil macrofauna significantly indirectly affects decomposition by modifying the habitat for microorganisms [2,6]. Macrofauna increases the surface area available for microbial decomposition through the mechanical breakdown of leaf litter into smaller particles [23]. Macrofauna also helps to mix the litter into a homogenous state and transports it to more favorable microclimatic conditions in deeper, moister soil [24], accelerates microbial inoculation to materials [22], and influences the density and composition of the soil microflora responsible for fine-scale decomposition [2,25]. On the contrary, it has been shown that feces of macrofauna decompose more slowly than litter [26] and that soil fauna can promote the stabilization of organic matter in soil [21,26].

In this study, we used the isopod Armadillidium vulgare (Latreille). This terrestrial isopod represents a common and abundant member of the saprophagous soil macrofauna in deciduous woodlands in Europe [25] and is invasive in other parts of the world. It may reach field densities as high as 10,000 individuals per m² [27]. Isopods typically occur in leaf litter and the uppermost soil layer, where they consume a substantial proportion of annual leaf litter fall. Because of their low assimilation efficiency, isopods return large amounts of the consumed litter as feces, which can form a substantial part of litter and top soil layers, providing increased surfaces that are readily colonized by microbial populations [25]. Isopods are also known for preferring to feed on partially decayed litter, which may reflect a preference for litter with a reduced content of tannin and phenolics [10]. Contrary to the phenolic content in fresh litter, which is negatively correlated to decomposition, a decrease of the phenolic content during decomposition may be associated with a decrease in decomposition rate [26].

This experiment was designed to explore if the conversion of litter to macrofauna feces affects organic matter decomposition, if feces respond to temperature differently than litter, and how respiration differs for litter with different CN ratio and phenolic content. Specifically, we addressed the following hypotheses: (1) In the long-term, microbial respiration of all litter types will be higher compared to microbial respiration of feces obtained from the same litter. (2) Litter respiration will increase with a decreasing CN ratio and the feces effect will be more pronounced in litter with a lower CN ratio. (3) Litter respiration will be more sensitive to temperature than feces respiration. (4) There will be a larger variation in the content of phenolics due to gut passage and decomposition; the content of phenolic compounds in more decomposed litter and in isopod feces will be lower than in the fresh leaf litter.
2. Material and Methods

2.1. Materials

Three types of leaf litter, alder (Alnus glutinosa), willow (Salix caprea), and maple (Acer campestre) were used. These litter types represent a broad gradient of litter with various CN ratio and phenolic content. Alder and willow litter were collected from a brown coal post-mining site near Sokolov (NW Bohemia, Czech Republic). Alder and willow represent the predominating trees at the reclaimed and non-reclaimed habitats at which the field manipulation experiment was carried out. On the contrary, maple litter was collected in deciduous woodland located in an urban area on Petřín hill in Prague (Czech Republic). All types of litter were collected from the soil surface in October 2012. Green litter and litter which was not in contact with soil was excluded because soil macrofauna generally prefers litter with microbial conditioning [3]. The collected litter was approximately 3–5 weeks old (after fall). The leaf litter was carefully separated by hand from other components, such as small branches and woody debris, air-dried, and stored in paper bags in a dark, dry location before usage.

The terrestrial isopod Armadillidium vulgare (Latreille) was collected in a deciduous woodland at the same place as the maple litter in October 2012 and kept in a clime-box at 16 °C. About 120 individuals of A. vulgare were divided into three plastic containers and supplied with rewetted leaf litter of alder, willow, or maple for three months. Every third day, the feces were collected and new rewetted litters were provided. Collected feces were immediately air-dried and stored in paper bags in a dark, dry location before usage.

Rewetting of all material was done one day before the material was used in the experiment [28] by placing it in nylon mesh bags (0.02 mm mesh size) and submerging these bags in distilled water for 2 h. Afterwards, the water was shaken out of the bags and litter was wrapped in the filter paper and kept in a refrigerator for 24 h. The litter used in the feeding experiment was removed from the paper wrap immediately before use as food for isopods.

2.2. Microcosms and Incubation

Collected materials (three types of litter and feces from these litters) were divided into two parts; the first part was stored in paper bags for chemical analyses of initial materials and the second part was used for long-term respiration experiments.

The leaf litter was cut into small pieces (1 cm²) and placed in the nylon litter bags (2 × 2 cm, mesh size 0.02 mm); about 0.2 g was placed in each bag. As isopods were observed to not consume large veins of leaves [29] the thick veins were removed prior to the experiment. This ensured that we were comparing feces with the material from which they were initially derived. Similar litter bags were used for feces (0.2 g per bag). The litter and feces material was rewetted by submerging all bags in distilled water for 1 h. After that, the bags were removed, an excess of water was shaken out, and the litter bags were placed on the surface of wet sand.

The laboratory microcosm was placed in a 250 mL glass bottle (one bag per bottle) with 40 g of fine sand on the bottom. The sand contained no organic matter and was moistened in a way that there was no visible water level in the sand but provided a constant capillary fridge. This has been checked by changes in sand color (caused by local disappearance of water) by applying pressure to the sand surface (wet sand effect). Distilled water was added bi-weekly in each bottle (1 mL per bottle) to maintain constant moisture. To prevent litter leaching, water was added to microcosms along the wall. Litter bags with litter or feces were maintained under three constant (8, 16, 24 °C) and one fluctuating (one week 8, the other week 24 °C) temperature in clime-boxes. For the measurement of microbial respiration, bottles were sealed for 6 days every week and supplied with 4 mL of 0.5 M NaOH in a small beaker. The CO₂ produced in the bottle was trapped in the NaOH and the quantity trapped was determined by titration with 0.05 M HCl after the addition of 2 mL of BaCl₂ [30]. As there were no other mechanisms by which organic matter may have left the experimental system (e.g., leaching), we may use the cumulative amount of C loss by respiration as only C loss from the decomposing material...
in this case. Respiration measurements were repeated for 50 weeks; after this time, the respiration experiment was completed.

The field experiment was performed in the mixed forest where all three species occur. The litter bags (the same parameters as in the laboratory experiment) with samples were fixed to the ground in March 2013. As the material was in contact with moist soil, no rewetting was necessary during the experiment. After 50 weeks, the litter bags were removed, immediately transported to the laboratory, and then oven-dried at 60 °C for 48 h to determine the remaining dry mass. In all cases, three replicates were measured.

2.3. Substrate Analyses

After 50 weeks, the experiment was terminated. All material was oven-dried at 60 °C for 48 h to a constant weight. The oven-dried material was then ground using a centrifugal mill to obtain a uniform particle size of <1 µm and divided into two portions; one for the measurement of total C and N concentrations and the other for the determination of phenolic content. Also, a portion of the material used to prepare feces was kept.

The total C and N concentrations in all materials at the start and at the end of the respiration experiment were measured with a CN analyzer (The Elemental Analyzer 1108, Carlo Erba Instruments, Milano, Italy). We performed triplicate measurements of each sample and the results were averaged.

The amount of total soluble phenolics (TSP) in all materials at the start and at the end of the respiration experiment was determined according to Singleton and Rossi, 1965 [31]. Frozen materials were homogenized in liquid N. Phenolics were extracted three times in 80% methanol (v/v) in a water bath (55 °C) and concentrations were determined spectrophotometrically at a wavelength of 750 nm, using a Helios spectrophotometer (Unicam, Cambridge, UK) in a methanol extract with a Folin-Ciocalteau phenol reagent (Sigma-Aldrich) and gallic acid (Sigma-Aldrich) as a standard. Material from the field experiment was additionally weighted to establish dry matter content.

2.4. Data Analyses

All computations were done in Statistica 10.0 (manufacturer, city, abbreviation of state, country). For the respiration data, a three-way ANOVA was used to determine the effect of substrate type, litter or excrements, tree species, and four temperature treatments, on respiration rate. Two-way ANOVA was used to compare C loss in litters and feces within each treatment. For factors that had a significant effect and no interactions, LSD post hoc tests (p < 0.05) were calculated. For the phenolics data and field respiration experiment, two-way ANOVA was used.

3. Results

3.1. Respiration Data

During the 50 week experiment, the microbial respiration of both litter and feces gradually decreased with time (Figure 1). The cumulative microbial respiration after 50 weeks of the experiment was used in further statistical analyses.
Figure 1. Cumulative changes in carbon (C) loss from the materials during the experiment. Example of average data for alder litter and feces at 16 °C. Bars represent SD.

The three-way ANOVA, comparing the effect of litter type, temperature, and litter vs. feces, found significant effects of all three factors. The highest respiration was found at 24 °C and the lowest at 8 °C, the respiration at fluctuating temperatures between 8 and 24 °C did not differ significantly from the mean temperature of this treatment, i.e., 16 °C. Carbon loss due to microbial respiration was significantly greater from litter than from *A. vulgare* feces (Figure 2; Table 1).

Figure 2. The cumulative loss of C at the end of the experiment from treatments with litter (L) and feces (F) kept at various temperatures (three constant 8, 16, 24 °C, and one fluctuating 8–24 °C). Statistically homogenous groups of columns are marked by the same letters according to LSD tests. Letters are ordered alphabetically. In groups of more than four letters, only the first and last letters are given.
Table 1. Output of three-way ANOVA of total C loss from individual litter and feces treatments experienced at various temperatures (8, 16, 24 °C and one fluctuating 8–24 °C). For the full data see Figure 3. Because of significant differences between litter type and litter transformed into feces, two-way ANOVA was performed separately for litter and feces. For factors that had a significant effect and no interactions, LSD post-hoc tests (p < 0.05) were calculated. Parameters are ordered from lowest to highest significance, statistically homogenous groups are marked by the same letter.

| df      | F     | p          | Post Hoc            |
|---------|-------|------------|---------------------|
| 3-way ANOVA all data |       |            |                     |
| 1–temperature | 3 26.5 | <0.0001 | 8a 8–24b 16bc 24c  |
| 2-litter type   | 2 7.5  | 0.0015    |                     |
| 3-litter vs. feces | 1 90.6 | <0.0001 |                     |
| interactions 1 × 2 | 6 1.5  | 0.1926    |                     |
| interactions 1 × 3 | 3 2.2  | 0.0968    |                     |
| interactions 2 × 3 | 2 7.0  | 0.0021    |                     |
| 2-way ANOVA litter only |       |            |                     |
| 1–temperature | 3 13.6 | <0.0001 | 8a 8–24b 24bc 16c   |
| 2-litter type | 2 10.9 | <0.0001 | maple a, alder b, willow b |
| interactions 1 × 2 | 6 1.3  | 0.2782    |                     |
| 2-way ANOVA feces only |       |            |                     |
| 1–temperature | 3 1.7  | 0.1913    |                     |
| 2-litter type | 2 1.2  | 0.3186    |                     |
| interactions 1 × 2 | 6 1.7  | 0.1704    |                     |

Figure 3. Remaining mass in litter types and feces in the field experiment after 50 weeks. Statistically homogenous groups are marked by the same letter.

Because of the significant interaction between litter type and litter vs. feces treatments (F = 7, p < 0.5), we used two-way ANOVA to explore the effect of treatment separately for litter and feces (Table 1). Litter decomposition was affected by temperature (F = 13.6, p < 0.05) but the decomposition of feces was not. For litter, there was also a significant effect of litter type but litter type did not affect feces. The highest litter respiration was found at 16 °C, which, however, did not differ significantly from that at 24 °C. Fluctuating temperatures (8–24 °C) resulted in significantly lower respiration than a constant mean temperature (16 °C). Respiration was greater in alder and willow litter than in maple litter.

When comparing all litter, feces, and temperature combinations by one-way ANOVA, the respiration of litter was significantly higher than the respiration of feces produced from the same litter at all investigated temperatures in alder and willow. However, the respiration of maple litter was significantly higher at 16 °C only but not at other temperatures (Figure 2).

For the field conditions, two-way ANOVA indicated a significant effect of litter type, litter vs. feces as well as a significant interaction between these two factors (Table 2).
The remaining mass of litter increased with increasing CN ratio. Alder litter had significantly lower remaining mass than willow litter, which had significantly lower remaining mass than maple litter (Figure 3). No significant differences were found in the mass loss of feces produced from various litters. Field mass loss of feces produced from alder and willow litter was significantly lower than that of litter alone, while the opposite was true for maple (Figure 3).

3.2. Chemical Changes of Litter and Feces

The CN ratio was strongly affected by the litter type (Table 3; Figure 4). There were also significant differences between litter and feces but this was accompanied by an interaction with litter type (Table 3). The CN ratio decreased as litter turned to feces (Figure 4).

Two-way ANOVA indicated no significant difference in the content of phenolics among individual litter types ($F = 0.5, p = 0.64$) but showed a significant effect of treatments (feeding as litter transformed into feces and decomposition at various temperatures) ($F = 250.7, p < 0.0001$) (Table 4). Because of this, the effect of litter vs. feces before and after the decomposition experiment was compared for all...
litter types pooled. The content of phenolics was significantly higher in initial litter than in all other treatments (Table 4).

Table 4. Content of phenolics in individual litter types, two-way ANOVA tests on the effect of litter type and treatment (feeding as litter transformed into feces and decomposition at various temperatures). The effect of treatments was compared by LSD post-hoc tests. Statistically homogenous groups are marked by the same letter.

|                | Alder | Willow | Maple | Mean            |
|----------------|-------|--------|-------|-----------------|
| Litter before  | 188   | 221    | 189   | 199.2 ± 15.4a   |
| Feces before   | 19    | 12     | 11    | 14.1 ± 3.6b     |
| Litter after 8 °C | 10    | 7      | 13    | 9.8 ± 2.7b      |
| Litter after 16 °C | 8     | 4      | 4     | 5.5 ± 1.9b      |
| Litter after 24 °C | 7     | 5      | 2     | 4.4 ± 2.2b      |
| Litter after 8–24 °C | 7     | 7      | 10    | 7.8 ± 1.4b      |
| Feces after 8 °C | 12    | 8      | 5     | 8.1 ± 3.1b      |
| Feces after 16 °C | 6     | 4      | 4     | 4.7 ± 1.0b      |
| Feces after 24 °C | 5     | 4      | 6     | 4.7 ± 0.8b      |
| Feces after 8–24 °C | 6     | 4      | 3     | 4.5 ± 1.0b      |

4. Discussion

A slower decomposition of macrofauna feces compared to intact litter in the long-term was observed by several authors [24,26,29,32]. In contrary to these results, Špaldoňová and Frouz, 2014 [29] observed significantly lower respiration in feces than in litter, even in A. vulgare feces produced from maple litter. The fact that feces decomposed more slowly than unaffected litter may result from the compact structure of fecal pellets, which can inhibit microbial decomposition [33,34], and from the depletion of readily assimilable C compounds by intestinal microflora [13] associated with increased concentrations of recalcitrant compounds, such as lignin. As presented by Špaldoňová and Frouz, 2014 [29] in a previous study on isopods, a lower decomposability of feces may correspond with an increased proportion of lignin and changes in lignin quality, such as an increased ratio of syringyl to guaiacyl in feces as compared to litter.

The respiration of litter significantly increased with increasing temperature but this increase was not significant for feces. This suggests that litter respiration is more sensitive to temperature than feces respiration. Under field conditions, this effect may be enhanced by the fact that feces usually occur in deeper parts of the soil profile, which tend to be cooled as compared to the surface parts that are dominated by litter [35]. This may be more complicated when higher temperatures reduce soil moisture (which was likely not the case at the sites used, which are generally characterized by high soil moisture). Fluctuating temperatures, however, did not cause higher respiration than the mean temperature. Špaldoňová and Frouz, 2014 [29] found some effect of temperature fluctuations, however, these fluctuations included the freezing point and, hence, freezing and thawing cycles. This suggests that not temperature fluctuations per se but freezing and thawing cycles may have caused an increase of respiration in the experiment by Špaldoňová and Frouz, 2014 [29].

Significant differences in microbial respiration were also found among litter types. The litter with a lower CN ratio decomposed more rapidly than the litter with a higher CN ratio [35]. This agrees with the observation in this study that microbial respiration was significantly higher in alder and willow litter than in maple litter. Maple is a typical old-growth forest species with a low N content in its litter and, thus, shows lower attractiveness to microbial communities [3]. On the contrary, plant litter with a high N content and low CN ratio, such as alder and willow litter, shows higher microbial activity, mainly because N in the early stages of decomposition supports microbial growth and activity [36].
In feces, the CN ratio often decreases in comparison to litter. However, this decrease in CN ratio in feces does not correspond with higher respiration. This is in agreement with the conclusion of Frouz et al., 2015b [26] that fresh litter with a low CN ratio in early stages of decomposition corresponds with a faster decomposition, while a decrease in the CN ratio of feces or during litter decomposition does not stimulate a faster decomposition. Frouz et al., 2015b [26] even found a negative correlation between the decrease in the CN ratio of feces and their decomposition. This result was supported by field litter bag experiments. This agrees with Cleveland and Liptzin, 2007 [9], who demonstrated that the dissimilarity in decomposition rate between litter and feces is related to different CN ratios. This can be caused by the different nature of N in various stages of decomposition. In early stages of decomposition, most of the N is in a form available for microorganisms and a lower CN ratio indicates a higher N availability. As litter decomposes, the CN ratio decreases, most likely because C is used and lost as CO₂, while N gets recycled by the microbial community [37]. Consequently, the decrease in the CN ratio during decomposition can be assumed as an indicator of the decomposition process and, thus, more decomposed material (with lower CN ratio) has a lower decomposition (respiration) rate.

In contrary to other studies, showing large differences in the content of phenolics between early and late succession tree species [11,38], our results show a similar content of phenolics in all three tree species (Table 3). The initial litter had the highest phenolic content in comparison to the other substrates. We observed a considerable decrease in the content of phenolics when litter was transformed into feces. Phenolic compounds may reduce the utilization of proteins. The soil fauna has various mechanisms how to deal with that [17,20,26]. For the isopod Porcellio scaber, Zimmer (1999) [19] described the ability to hydrolytically degrade and detoxify phenolic compounds during digestion and the capability to oxidize ingested phenolics [13]. As revealed by Wood (2012) [39], Balloniscus sellowii (Isopoda: Oniscidea) might be efficient in using phenolic compounds as antioxidant agents.

We observed a significant decrease in the phenolic content in all types of substrates during the 50 weeks of incubation at all temperatures. This may be partly caused by leaching of phenolics during incubation [13] or by microbial activity. Microbial decomposers are responsible for the decomposition of simple phenols, catechin, flavones, or some oligo and polymers [40]. We also observed a substantial decrease in free phenolic compounds when litter was converted to feces. Here, leaching may be excluded because feces were not subject to any leaching. Some phenolic compounds may be decomposed in the gut [13]. However, it is also possible that some of the free phenolic compounds form insoluble complexes, e.g., with protein in a similar way as described by Frouz et al., 2011 [15]. These bounded polyphenols cannot be extracted as free phenols, which decreases the concentration of free phenols in feces. This may be also one of the mechanisms explaining how the N gets unavailable in the feces [15].

5. Conclusions

As expected by our hypotheses, litter respiration was positively affected by increasing temperature, while respiration of Isopoda feces seemed to be unaffected by temperature. Microbial respiration of both litter and feces was substantially affected by litter quality, being higher in litter with a lower CN ratio. As expected, the phenolic content of leaf litter considerably decreased as a result of Isopoda feeding but decreased also during microbial decomposition.

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