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Intrapopulation genotypic variation in leaf litter chemistry does not control microbial abundance and litter mass loss in silver birch, *Betula pendula*

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Abstract

Background and aims Differences among plant genotypes can influence ecosystem functioning such as the rate of litter decomposition. Little is known, however, of the strength of genotypic links between litter quality, microbial abundance and litter decomposition within plant populations, or the likelihood that these processes are driven by natural selection.

Methods We used 19 *Betula pendula* genotypes randomly selected from a local population in south-eastern Finland to establish a long-term, 35-month litter decomposition trial on forest ground. We analysed the effect of litter quality (N, phenolics and triterpenoids) of senescent leaves and decomposed litter on microbial abundance and litter mass loss.

Results We found that while litter quality and mass loss both had significant genotypic variation, the genotypic variation among silver birch trees in the quantity of bacterial and fungal DNA was marginal. In addition, although the quantity of bacterial DNA at individual tree level was negatively associated with most secondary metabolites of litter and positively with litter N, litter chemistry was not genotypically linked to litter mass loss.

Conclusions Contrary to our expectations, these results suggest that natural selection may have limited influence on overall microbial DNA and litter decomposition rate in *B. pendula* populations by reworking the genetically controlled foliage chemistry of these populations.

Keywords: litter quality, bacteria, fungi, phenolic compounds, nitrogen, triterpenoids, decomposition, natural selection

Introduction

Plant litter decomposition, one of the fundamental ecosystem processes, is determined by the interaction of litter quality, the decomposers that colonize the litter, and environmental conditions. Plant species are known to differ in the quality of litter they produce (Bardgett and Wardle 2010; Wardle 2002), and as a legacy of these differences, communities of litter degrading microbes (Grayston and Prescott 2005; Kang and Mills 2004; Templer et al. 2003; Weand et al. 2010) and rates of litter decomposition (Cornelissen 1996; Cornwell et al. 2008; Wardle et al. 1998) vary by plant species. Within ecosystems, this can create spatial variation of soil organisms and processes (Bardgett and Wardle 2010). Similar variation can also be created by intraspecific genetic variation, however, and this variation is increasingly recognized as an important driver of the structure and dynamics of plant associated communities and ecosystem functioning (Pastor 2017; Whitham et al. 2006; Whitham et al. 2008).

Microbes, i.e. fungi and bacteria, are the main decomposers of plant litter and account for ca. 95% of soil decomposer biomass and respiration (Chapin et al. 2011). High nitrogen (N) concentration is assumed to enhance microbial growth and litter decomposition (Heal et al. 1997; Melillo et al. 1982). Secondary metabolites, which remain in senescent leaves as a highly diverse group (Paaso et al. 2017), differ as microbial resources due to differences in their chemical structure. Soluble low-molecular weight phenolics are relatively easily utilized by soil microbes (Bowman et al. 2004; Schimel et al. 1996), whereas the phenolic polymers, such as lignin and condensed tannins (proanthocyanidins) can retard microbial activity (Kraus et al. 2003; Madritch and Hunter 2003; Makkonen et al. 2012; Schimel et al. 1996). In general, it appears that litters that have low concentrations of nutrients and high concentrations of lignin and other phenolic compounds are characterized by fungal-dominated microbial communities and slow decomposition rates and nutrient release (Bardgett and Wardle 2010; Wardle 2002). Supporting the importance of genotypic variation in driving ecosystem functioning, many studies have shown how plant genotypes vary in litter quality and decomposition rate (Crutsinger et al. 2009; LeRoy et al. 2012; Madritch et al. 2006; Silfver et al. 2007, 2015). Especially for *Populus*, evidence has accumulated of the biomass, activity and composition of microbial communities varying remarkably among the litters of different genotypes (Madritch et al. 2009; Schweitzer et al. 2008a). What is still partly lacking, however, is the

evidence that leaf litter quality, microbial abundance and litter decomposition rate are genotypically linked within local plant populations, i.e. at the scale of intraspecific variation where green leaf traits are subjected to natural selection. It has also been argued that the role of genetic variation may be overestimated in the current literature because most studies have focused on systems with particular ecological characteristics, such as hybrid zones and clonal plant species (Tack et al. 2012). In addition, the examined genotypes are often collected from a wide area to maximize genetic variation, whereas the experiments are performed in common gardens to minimize environmental variation (Tack et al. 2012). More studies that use non-clonal plant species and intrapopulation genotypic variation in an experimental setting, where the environmental and genotypic variation represent equal spatial scale, are therefore needed.

Our study species, *Betula pendula* Roth, has a wide distribution in Europe, being particularly abundant in the eastern parts (Atkinson 1992; Hynynen et al. 2010). Using genotypes randomly selected from a *B. pendula* population in south-eastern Finland, significant intrapopulation genotypic variation has earlier been found for many *B. pendula* traits, including green foliage secondary chemistry (Laitinen et al. 2000), leaf N resorption efficiency (Mikola et al. 2018) and litter decomposition rate (Silfver et al. 2007, 2015). The green foliage chemistry of tree populations is a reflection of various selection forces that act on the genotypic structure of populations, and we have recently shown that most secondary metabolites of *B. pendula* foliage, and their intrapopulation genotypic variation, can remain in the senescent leaves and partly decomposed leaf litter (Paaso et al. 2017). As secondary metabolites can affect litter decomposition (Hättenschwiler and Vitousek 2000; Schweitzer et al. 2008b), this should allow natural selection to influence ecosystem functioning through acting, e.g. in terms of herbivore defense (Bryant et al. 2009), on the green leaf chemistry of *B. pendula* populations. On the other hand, we found that the concentrations of lignin and condensed tannins, which both can restrict decomposition (Hobbie et al. 2006; Melillo et al. 1982; Schweitzer et al. 2008b; Talbot and Treseder 2012; Vaieretti et al. 2005), had a negative genotypic correlation with each other in the senescent leaves and that the heritable variation in lignin concentrations vanished during decomposition (Paaso et al. 2017). These patterns might counteract a straightforward genotypic link between the green leaf chemistry and litter decomposition rate.

100 To examine (1) if the high intrapopulation genotypic variation of N and secondary metabolites in *B.*
101 *pendula* senescent leaves (Paaso et al. 2017; Mikola et al. 2018) have predictable, long-term effects on litter
102 decomposition rate when the litter is placed on heterogeneous forest ground, and particularly, (2) if these
103 effects can be understood by the effects of metabolites on bacterial and fungal abundances, we established a
104 35-month litter decomposition trial using the same genotypes, originating from a single *B. pendula*
105 population, which were previously studied by Paaso et al. (2017) and Mikola et al. (2018). We
106 supplemented the data available from these studies by measuring litter N concentration after early
107 decomposition, and predicted that microbial abundance and litter mass loss would follow the variation in
108 the concentrations of N and secondary metabolites in the senescent leaves. Due to the persistence of
109 genotypic variation in litter chemistry through decomposition (Paaso et al. 2017), we further predicted that
110 the variation in overall quantity of fungal and bacterial DNA and litter mass loss would exhibit a significant
111 genetic component. This would effectively link natural selection with ecosystem functioning if those traits
112 that were originally selected for other functions in live trees (such as protection against herbivores) would
113 also have an effect on litter-dwelling microbes and decomposition.
114
115 **Materials and methods**
116 *Plant material, growing site and leaf litter collection*

117 The mother trees of the 19 *B. pendula* genotypes used in this study were originally selected from a naturally
118 regenerated 0.9-ha *B. pendula* – *B. pubescens* Ehrh. forest stand in Punkaharju, southeast Finland (61°48'
119 N, 29°18' E) and thus represent the genotypic variation of a local *B. pendula* population. The trees we used
120 were micropropagated from the mother trees in the spring 1998 (Laitinen et al. 2005) and were planted at
121 the Kuikanniitty growing site in June 1999. The Kuikanniitty site (61°47′ N, 29°21′ E, 79 m above sea
122 level) is an abandoned, agricultural field with a soil defined as fine sandy till (Laitinen et al. 2005). When
123 established, the site was divided into six replicate blocks, each of which had plots of four identical saplings
124 randomly selected from the genotypes. Two of the trees in each plot were later harvested, leaving more
125 space for the remaining two, and one of these trees was randomly selected for our study (n=6 for each
126 genotype).
127

Leaf litter was collected by enclosing two south-facing branches of each tree at the height of 1.4-3 m in white polyethylene mesh bags (150 cm × 60 cm, mesh size 2 mm) before autumn leaf abscission (September 8 to 10) . The bags were removed after leaves had fallen in all trees (October 28 to 30), the litter was pooled within trees, stored at ambient temperature, and from each litter sample twenty random leaves were collected for microbial and chemical analyses. These sub-samples, hereafter called senescent leaves, were ground in liquid N and stored at -80 ºC. The remaining litter material was used for the decomposition trial.

Litter decomposition trial

The decomposition trial was established in November 2008 at a forest site in Loppi, south Finland (60°36' N, 24°24' E, 140 m above sea level), instead of the Kuikanniitty agricultural field, to ensure that decomposer microbes adapted to tree leaf litter decomposition would colonize the litter. The site was clear-cut in early 2008 to allow planting of *B. pendula* saplings for the purposes of other experiments (Mikola et al. 2014; Silfver et al. 2015). Before the clear-cut, the site was covered by a mixed *Pinus sylvestris – B. pendula* forest. The soil at the site is post-glacial sorted fine sand, topped by a few centimeters of humus, with a pH of 5.0 and total C and N concentrations of 6 and 0.3%, respectively, in the upper 0–5 cm layer (Mikola et al. 2014). The ground layer vegetation is dominated by a fern *Pteridium aquilinum* (L.) Kuhn, grasses *Calamagrostis arundinacea* (L.) Roth and *Deschampsia flexuosa* (L.) Trin., and dwarf shrubs *Vaccinium myrtillus* L. and *Vaccinium vitis-idea* L. (Mikola et al. 2014). The site has six replicate blocks, each divided into 2×2 m planting plots (Mikola et al. 2014), and for the present study, a litter patch (diameter ca. 30 cm, 10 g of litter as dry mass equivalent) was established in a random selection of the plots for each of the trees sampled in the Kuikanniitty site (Mikola et al. 2018). Allocation of tree individuals to field blocks followed the blocking at the Kuikanniitty growing site, and within each block the litter of different genotypes was randomly allocated to the planting plots.

Before the patches were established, four litter bags (10×10 cm; mesh size 0.5 mm), one for each of the four consecutive harvests, were produced for each patch using the patch litter. Each bag included five to eight randomly picked and weighed leaves. The litter bags were buried in their corresponding patches and

156 the patches were covered, but not enclosed by white polyethylene mesh (2 mm). To mimic the annual input
157 of fresh litter, each patch was augmented with 25 g of newly collected litter (as a dry mass equivalent) in
158 autumns 2009 and 2010. The litter used for the patches and the litter bags was not dried for initial dry mass
159 measurements to preserve the microbes such as endophytes (Saikkonen et al. 2003), which naturally grow
160 on the falling litter. Instead, a subsample of eight random leaves was picked from each litter sample and
161 dried, and the water content was used to estimate the amount of dry litter added into each patch as well as
162 the initial litter dry mass used in the litter bags.
163
164 Litter bags were harvested for measuring mass loss in June 2009, October 2009, July 2010 and October
165 2011, i.e. after decomposition of 7, 11, 20 and 35 months. The intervals from Nov 2008 to June 2009, from
166 Oct 2009 to July 2010 and from July 2010 to October 2011 include 4 to 5 months of mean air temperature
167 < 0 °C. In each harvest, litter samples were dried at 60 ºC for 72 h and weighed for dry mass. Litter
168 chemistry was analyzed for 7-month old litter and bacterial and fungal abundance for 7- and 11-month old
169 litter. In each case, ten to twenty random leaves were picked from the patch and transported to a laboratory,
170 where they were ground in liquid N and stored in -80 ºC. Litter chemistry included concentrations of N,
171 condensed tannins, lignin, intracellular phenolics, epicuticular flavone aglycones and epicuticular
172 triterpenes, which were available from the studies by Paaso et al. (2017) and Mikola et al. (2018), except
173 for the N concentration of the 7-month old litter, which was analyzed for this study. Nitrogen concentration
174 was analyzed using a LECO CNS-2000 Analyzer (LECO Corporation, USA) and the concentration of
175 condensed tannins using the acid butanol assay (Hagerman 2002). Lignin concentrations were determined
176 using the acetylbromide method (Brinkmann et al. 2002), with slight modifications, and those of low
177 molecular phenolic compounds using high-performance liquid chromatography-mass spectrometry (Paaso
178 et al. 2017).
179
180 The microbial abundances, i.e. quantities of fungal and bacterial DNA in the senescent leaves and in the
181 litter after 7 and 11 months of decomposition, were analyzed using the real-time quantitative PCR (qPCR).
182 DNA was isolated from 25-125 mg of ground litter using FastDNA@Spin Kit for Soil (Obiogene, USA).
183 The same extraction method was used for the pure cultures of bacteria (*Escherichia coli*, own collection)

184 and fungi (*Saccharomyces cerevisiae*, commercially available yeast), which served as positive controls in
185 the qPCR analysis. Sterilized water and the reaction mixture without the template served as negative
186 controls. The samples were amplified using the LightCycler Quantitative real-time PCR machine (Roche
187 Diagnostics Penzberg, Germany). The primers pE (5'-AAA CTC AAA GGA ATT GAC GG-3') and pF'
188 (5'-ACG AGC TGA CGA CAG CCA TG-3') were used for the domain Eubacteria (Edwards et al. 1989),
189 and the primers ITS3 (5'-GCA TCG ATG AAG AAC GCA GC-3') and ITS4 (5'-TCC TCC GCT TAT
190 TGA TAT GC-3') for fungi (Manerkar et al. 2008). The total reaction volume was 20 µl, which included 2
191 µl of diluted template (dilution for bacteria 1:100 and for fungi 1:1000), 10 µl of reaction mixture (Dynamo
192 HS SYBR Green qPCR Kit), 0.5 µl of each bacterial or 0.25 µl of each fungal primer, and 7 µl or 7.5 µl of
193 water (for bacterial and fungal analysis, respectively). The PCR temperature program for the bacteria
194 included initial denaturation of 10 min at 94 °C, 30 cycles of 10 s at 94 °C followed by annealing for 20 s at
195 57 °C and extensions for 30 s at 72 °C and for 1 s at 81 °C. For the fungi, the program consisted of initial
196 denaturation of 15 min at 95 °C, 41 cycles of 60 s at 95 °C followed by annealing for 60 s at 58 °C and
197 extensions for 60 s at 72 °C and for 1 s at 77 °C. For both microbial groups, the melting curve analysis for
198 the amplicon was performed at 60-95 °C with measurements of the fluorescence signal at every 0.2 °C for 1
199 s. A standard curve with four to five dilutions of positive standards was used to calculate the number of
200 copies in the original template. This value was then divided by the dry weight of the litter sample used in
201 the DNA extraction.
202
203 *2.3. Statistical analysis*
204 The broad-sense heritabilities (H^2) (Falconer and Mackay 1996) of litter N concentration, microbial DNA
205 quantity and litter mass loss were calculated according to equation 1, where σ_G^2 and σ_E^2 are variance
206 components for genotypes and environment (or error), respectively. Calculating broad-sense heritabilities
207 allowed us to estimate how large a proportion of the total variation in microbial DNA quantity and litter
208 mass loss could be explained by the genotypic variation of our study population. The variance components
209 were calculated using the SPSS GLM Variance components procedure (ANOVA, Type III Sum of
210 Squares). In the calculation model, the genotype was treated as a random factor and the field block,
211 following a common practice in forest breeding, as a fixed factor. This differs from some of our earlier

studies (Mikola et al. 2014; Silfver et al. 2015), where we were interested in the size of the block-scale environmental variation and treated the block as a random factor. Coefficients of genotypic variation (CV_G) were further calculated according to equation 2, where $\bar{x}$ is the phenotypic mean.

Eq. 1 $$H^2 = {\sigma_G^2}\Big/{(\sigma_G^2 + \sigma_E^2)}$$

Eq. 2 $$CV_G = {\sqrt{\sigma_G^2}}\Big/{\bar{x}}$$

The statistical significance of genotypic variation in litter N concentration, microbial DNA quantity and mass loss was tested using the Analysis of Variance (ANOVA; SPSS statistical package, version 22; IBM SPSS Statistics). In the ANOVA models, the genotype was treated as a random factor and the field block as a fixed factor, thus following the procedure in the calculations of variance components. The qPCR run was included in the models of microbial DNA as a fixed factor, but the effects of the qPCR run and the field block were not fully distinguishable as we analyzed the microbial samples block by block. Moreover, although the field block was a statistically significant source of variation for many response variables, its meaningful interpretation is difficult as it retains variation from two undistinguishable sources, i.e. the variation originating from the tree growing site and that arising from the litter patch location. For these reasons, neither the block nor the qPCR run effect is presented in the ANOVA table. To fulfil the assumptions of normality and homoscedasticity, the data were log(x+1) or square root transformed when necessary. Equality of variances was tested using a median-based Levene's test as suggested by Nordstokke and Zumbo (2007).

The associations among the attributes of litter chemistry (N, condensed tannins, lignin, intracellular phenolics, surface flavone aglycones and surface triterpenes), microbial DNA quantity and litter mass loss were examined both at the level of genotypes (genotype mean values used in calculations of genotypic correlations) and individual trees (values for individual trees used in calculations of phenotypic correlations) and using Spearman's rank correlation test. In these correlations, the chemistry attributes were always contrasted with microbial DNA quantity and mass loss of one harvest further (e.g. the N

concentration of senescent leaves was contrasted with the mass loss of the 7-month old litter and the N concentration of the 7-month old litter with the mass loss of the 11-month old litter). The associations between microbial DNA quantity and mass loss were tested both within the harvests and between the harvests.

Results

Litter N concentration

The genotypic variation in litter N concentration was statistically significant after 7 months of decomposition, with the genotype explaining 20% of total phenotypic variation (Table 1, Fig. 1). The genotype means of N concentration in 7-month old litter correlated positively with the genotype means of N concentration in senescent leaves (ρ=0.63, P=0.004, n=19). Concentrations of N and secondary metabolites did not correlate at the genotype level in either senescent leaves or decomposed litter, except for the weak negative correlation in senescent leaves between N and intracellular phenolics (ρ=-0.463, P=0.046, n=19) .

Bacterial and fungal DNA

The quantity of DNA on decomposing leaves in comparison to senescent leaves was on average 2- and 4-fold higher for bacteria after 7 and 11 months of decomposition, respectively, and 1.3- and 2-fold higher for fungi after 7 and 11 months of decomposition, respectively (Fig. 2). In senescent leaves, the genotype explained 10% of the total variation in bacterial and fungal DNA, but statistically, the genotype effect was only marginally significant (Table 1). After 7 and 11 months of decomposition, the genotype effect was not statistically significant for either microbial group, although after 7 months the genotype could still explain 4% of the total variation in the amount of fungal DNA (Fig. 2, Table 1). The quantities of bacterial and fungal DNA did not correlate with each other at the level of tree genotype in the senescent leaves (ρ=0.075, P=0.759, n=19) or after 7 (ρ=0.28, P=0.238) or 11 months of litter decomposition (ρ=-0.10, P=0.679). The quantities of bacterial and fungal DNA did not correlate with each other at the level of individual trees in the senescent leaves (ρ=0.07, P=0.475, n=112) or after 7 months of litter decomposition (ρ=0.18, P=0.058), but had a weak negative correlation after 11 months of decomposition (ρ=-0.20, P=0.035).

Litter mass loss

On average 9, 24, 28 and 51% of litter mass was lost during the 7, 11, 20 and 35 months of decomposition, respectively (Fig. 3). After 7 months of decomposition, the genotype explained 25% of the total variation in mass loss and the genotype effect was statistically significant (Table 1). In the later stages of decomposition, the heritability estimates were considerably smaller (0.5-7%) and the genotype effect was not statistically significant (Table 1). The genotype means of litter mass loss were, however, positively correlated between the 7- and 11-month old litter (ρ=0.43, P=0.069, n=19) and between the 7- and 20-month old litter (ρ=0.70, P=0.001), but not between the 7- and 35-month old litter (ρ=0.14, P=0.571).

Associations among litter chemistry, microbes and mass loss

At the genotype level, the quantity of bacterial DNA had a positive correlation with litter mass loss at the 11-month harvest when contrasted within and between the harvests, whereas no significant correlation was found for fungi (Table 2). At the level of individual trees, the quantity of bacterial DNA had a positive correlation with litter mass loss at the 11-month harvest, whereas the quantity of fungal DNA correlated negatively with litter mass loss both in the senescent leaves and 11-month old litter (Table 3).

No genotypic correlation was found between litter chemistry and microbial DNA quantity or mass loss (Table 4). At the level of individual trees, however, the N concentration in senescent leaves was positively and concentrations of intracellular phenolics and epicuticular flavonoid aglycones negatively correlated with the quantity of bacterial DNA in the 7-month old litter (Table 5). These patterns were mostly repeated later as the concentrations of lignin and N in the 7-month old litter were positively correlated and intracellular phenolics and condensed tannins negatively with the quantity of bacterial DNA in the 11-month old litter (Table 5). In contrast, none of the senescent leaf chemistry attributes were associated with the fungal DNA or litter mass loss at the early stage of decomposition (Table 5). However, N and lignin concentrations in the 7-month old litter were negatively associated with the quantity of fungal DNA (Table 5), and the concentration of condensed tannins was negatively and the concentration of lignin positively correlated with litter mass loss (Table 5).

Discussion

Litter chemistry and microbial abundance

In line with our earlier observations of high and persistent intrapopulation genotypic variation of N and secondary metabolites in *B. pendula* senescent leaves (Mikola et al. 2018; Paaso et al. 2017), we found that the N concentration of partly decomposed litter had substantial genotypic variation. In the senescent leaves, the genotypic variation was found to explain 34% of the total phenotypic variation (Mikola et al. 2018), which corresponds with the earlier estimates of 28 and 27% of green leaf N concentrations explained by genotype in *Populus trichocarpa* (Guerra et al. 2016) and *Pinus radiata* (Li et al. 2015), respectively. Although the estimates of heritability and CV_G decreased during the first 7 months of decomposition (H^2 from 0.34 to 0.20 and CV_G from 0.080 to 0.050), the ranks of genotype means of N concentration were strongly positively correlated between the senescent leaves and decomposed litter, thus giving strong support to the earlier suggestions that the genotypic variation of foliage chemistry persists through the early stages of decomposition (Paaso et al. 2017).

The effect of tree genotypic variation on the quantities of bacterial and fungal DNA found on senescent leaves (CV_G 0.087 and 0.093, respectively) is in line with earlier observations of genotypic structure of tree populations controlling fungal infections in green leaf foliage (Barbour et al. 2009). The genetic variation we found may be due to microbes of senescent leaves originating from the epiphyte and endophyte communities of the green foliage (Busby et al. 2016; Peñuelas et al. 2012; Saikkonen et al. 2003) as the variation disappeared during the first 7 months of decomposition, i.e. during the period when the litter microbes presumably became more dominated by soil decomposers (for the endophyte–saprotroph fungal continuum see U´Ren and Arnold 2016). Our results thus seem to suggest that even though the genotypic variation of foliage chemistry persists through the senescence and early decomposition of litter, it is the green leaf microbial community that is responsive to this variation rather than the decomposers that later colonize the litter. In fact, this is not surprising considering the high metabolic flexibility of soil communities to decompose litters of different origin (Lavelle 2002; Makkonen et al. 2012). However, we did not use any amplicon-sequencing method to quantify variation at finer taxonomic resolution of

323 microbes across our genotypes. It is therefore possible that even though microbial abundance, i.e. the
324 overall DNA quantity, was not affected by *B. pendula* genotype in partly decomposed 7- and 11-mo old
325 litters, the composition of fungal and bacterial communities varied across the genotypes as shown in an in-
326 stream *Populus* study by Marks et al. (2009).
327
328 Earlier studies of the variation of microbial abundances in plant litter among plant phenotypes and
329 genotypes have produced mixed results. No difference was found in microbial activity and biomass among
330 litters originating from *Quercus laevis* phenotypes in an oak forest after 3–36 months of decomposition
331 (Madritch and Hunter 2002, 2005). In contrast, Le Roy et al. (2007) found that genotypic variation in both
332 *P. angustifolia* and *P. fremontii* affected the fungal biomass in the litter after 7 days of decomposition in an
333 aquatic environment, but similarly to our study, the variation disappeared in *P. fremontii* during early
334 decomposition. When microbial abundances have been analyzed in the humus layer beneath 7- to over 20-
335 year old trees, significant genotype effects on microbial abundances have been found for *B. pendula*
336 (Kasurinen et al. 2005), *Populus angustifolia* (LeRoy et al. 2007; Schweitzer et al. 2008a) and *P.*
337 *tremuloides* (Madritch et al. 2009; Madritch and Lindroth 2011), but not for *P. fremontii*, which generally
338 seems to express much less variation in many studied traits (e.g. leaf secondary chemistry, litter
339 decomposition) than other *Populus* crosstypes (Schweitzer et al. 2008a and references therein). Altogether
340 these results suggest that soil microbial decomposers can respond to the genotypic variation in leaf litter
341 characteristics, but the response may take years to develop and for some tree species the connection may
342 not exist or be weak. The strength of response might also depend on the composition of bacterial and fungal
343 communities at the study site, which could be tested using reciprocal litter transplant experiments.
344
345 There was no genotypic link between litter chemistry and the overall quantity of bacterial and fungal DNA,
346 which was most probably due to the vanishing genotypic variation in microbial abundances during litter
347 decomposition. Considering that bacteria and fungi are the primary decomposers of plant litter, this would
348 suggest that the high genotypic variation of *B. pendula* litter chemistry (Paaso et al. 2017; Mikola et al.
349 2018) may have little influence on litter decomposition. On the other hand, when looking at this
350 relationship on the phenotypic level of individual trees, our results show that litter chemistry and the

quantity of microbial DNA were connected, the quantity of bacterial DNA being negatively associated to the concentration of phenolics and positively to the concentrations of N and lignin during the first year of decomposition. The soluble low-molecular weight secondary compounds are often considered as a suitable resource for microbes (Bowman et al. 2004; Schimel et al. 1996), but our results suggest that bacterial abundance may in general be retarded by these compounds. The negative association between the quantity of bacterial DNA and the concentration of condensed tannins was anticipated based on earlier studies (Kraus et al. 2003; Madritch and Hunter 2003; Makkonen et al. 2012; Schimel et al. 1996), whereas the positive association between the quantity of bacterial DNA and the concentration of lignin was not (Sariyildiz and Anderson 2003; Vaieretti et al. 2005). This positive correlation may, however, be related to the fact that lignin and tannin concentrations were negatively correlated in the senescent leaves (Paaso et al. 2017). The positive association between N concentration and the quantity of bacterial DNA was expected and supports the idea that N rich litter induces a decomposer community that is dominated by bacteria (Bardgett and Wardle 2010; Wardle 2002). In a stark contrast to the quantity of bacterial DNA, the quantity of fungal DNA had no significant connection to phenolic concentrations, but instead was negatively associated with N and lignin concentrations. In general, the contrasting responses of the two microbial groups to litter characteristics may partly mirror the fact that fungi are the main decomposers of carbohydrates, whereas bacteria are adapted to digesting substrates with higher protein contents and low C:N ratios (Lavelle and Spain 2001).

Litter mass loss and links to litter chemistry and microbial abundance

The high genotypic variation in the early litter mass loss diminished in our study when the decomposition proceeded and practically no genotypic variation was left after three years. The positive genotypic correlation of mass loss between the 7- and 20-month old litters, however, implies that despite the diminishing genotypic variation, the genotypic rank of mass loss rate remained the same through the first 20 months of decomposition. Most earlier studies that have examined the intraspecific genotypic variation in plant litter decomposition at field conditions have been short-term and lasted no more than one year. In some of these studies, genotypic variation may have been overemphasized by the use of hybrid zones and clonal plant species or common garden approaches with genotypes originating from different populations

379 (Tack et al. 2012), but the genotypic effects and heritability estimates they report (Crutsinger et al. 2009;
380 LeRoy et al. 2012; Madritch et al. 2006) are near to those measured in our study. For instance, in an in-
381 stream decomposition trial, LeRoy et al. (2012) found that 30% of the total variation in litter decomposition
382 rate was explained by *P. tremuloides* genotype. This is well in line with our observation of genotype
383 explaining 25% of the variation in *B. pendula* litter mass loss (H^2=0.248) during the first seven months of
384 decomposition. By contrast, the few long-term trials, lasting over 18 months, have reported non-significant
385 genotypic or phenotypic effects on litter decomposition (Korkama-Rajala et al. 2008; Madritch and Hunter
386 2005). For example, similarly to our findings, Madritch and Hunter (2005) found significant phenotypic
387 differences in the decomposition rate of *Quercus laevis* leaf litter after 18 months of decomposition, but no
388 difference after 36 months of decomposition. Together with our results, these results seem to indicate that
389 genotypic and phenotypic variation in decomposition rate disappear after the initial phases of
390 decomposition. On the other hand, Madritch and Hunter (2005) found that long-term nutrient fluxes can be
391 influenced by plant phenotype, suggesting that the genotypic and phenotypic variation in nutrient dynamics
392 may persist longer than the variation in litter decomposition rate.
393
394 We found no genotypic correlation between litter chemistry and the quantity of microbial DNA and litter
395 mass loss. It thus appears that while *B. pendula* litter quality and litter mass loss both have significant
396 genotypic variation, these variations are not linked by the abundance of decomposer microbes. This
397 suggests that the genotypic variation in the concentrations of N and secondary compounds in *B. pendula* is
398 not a good predictor of the genotypic variation in litter mass loss. What could be the reason for such
399 apparent lack of genotypic link between litter chemistry and litter mass loss? First, it is possible that the
400 physical attributes of litter, such as leaf toughness and specific leaf area, instead of chemistry, drive the
401 variation in litter decomposition. There is some evidence that leaf toughness can better explain interspecific
402 differences in litter decomposition than litter N content and the C/N-ratio (Li et al. 2009; Pérez-
403 Harguindeguy et al. 2000). Second, as lignin concentration is among the most important factors regulating
404 litter decomposition (Hobbie et al. 2006; Melillo et al. 1982; Vaieretti et al. 2005), the quickly diminishing
405 genotypic variation of lignin concentrations in our litter (Paaso et al. 2017) could be part of the explanation.
406 Third, as we already earlier speculated, the negative genotypic correlation between lignin and condensed

407 tannins (Paaso et al. 2017) may counteract the link between the genotypic variation in the concentrations of
408 individual metabolites and litter mass loss. Fourth, our results suggest that bacterial and fungal abundance
409 can have contrasting responses to the variation in litter chemistry and differ in their link to decomposition
410 rate, with bacterial abundance having a positive and fungal abundance a negative correlation with litter
411 mass loss. In the same way as the negative correlations between metabolite concentrations, such a
412 discrepancy between the responses and effects of the two main groups of decomposers may explain why
413 litter chemistry does not appear to be connected to litter decomposition. Moreover, analyzing bacterial and
414 fungal community composition might further have revealed differences in the responses of microbial taxa
415 within communities. All in all, while there is several potential reasons that could explain our findings, the
416 evidence is accumulating that the chemistry and mass loss of *B. pendula* litter are surprisingly weakly
417 connected (cf. Silfver et al. 2015). Thus, in contrast to what we expected (Paaso et al. 2017), selection may
418 not be able to drive decomposition rate through acting on green leaf chemistry in *B. pendula* populations.
419
420 Nitrogen mineralization is a process closely linked to organic matter decomposition. Microbes break down
421 organic matter using exoenzymes, which liberates dissolved organic N (DON) in the soil (Chapin et al.
422 2011). Microbes absorb DON for their growth requirements and depending on whether microbial growth is
423 C or N limited, secrete surplus NH_4 into the soil (Chapin et al. 2011). We have recently shown that litter N
424 mineralization rate in *B. pendula* is tightly controlled by the genotypic variation in N resorption efficiency
425 (and the following senescent leaf N concentration), not by the genotypic variation in green leaf N
426 concentration (Mikola et al. 2018). Together with our current findings these results have three implications
427 for understanding the variation of litter decomposition and N mineralization within tree populations. First,
428 intrapopulation genotypic variation in green leaf chemistry may be a poor predictor of litter decomposition
429 and mineralization rates. Second, the links of plant foliage traits with the rates of litter mass loss and litter
430 N mineralization may be decoupled, the link with N mineralization being more prominent because of the
431 strong control by N resorption efficiency. Third, although these results leave little space for natural
432 selection to drive ecosystem functioning through acting on green leaf chemistry in tree populations, the
433 process is still possible through selection acting on other live plant traits such as the leaf N resorption
434 efficiency.

Conclusions

Our results show that while *B. pendula* litter chemistry and litter mass loss both have significant genotypic variation, the variation in chemistry of the litter may not trigger significant genotypic variation in the overall microbial DNA and may not be related to the variation in litter mass loss. In contrast to what we expected (Paaso et al. 2017), this suggests that selection may not be able to drive litter decomposition rate in *B. pendula* populations through acting on the green leaf chemistry of these populations. However, the link between selection and ecosystem processes is still possible through selection acting on other live plant traits such as the leaf N resorption efficiency that appears to be tightly correlated with the genotypic variation of *B. pendula* litter N mineralization rate (Mikola et al. 2018).

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Figure legends

Figure 1. The mean (+ SE, n = 5-6) of N concentration in the litter after 7 months of decomposition in 19 *Betula pendula* genotypes (the genotype order follows the 7-month mass loss in Fig. 3).

Figure 2. The mean (+ SE, n = 5-6) of the number of bacterial and fungal DNA copies in the senescent leaves and litter after 7 and 11 months of decomposition in 19 *Betula pendula* genotypes (the genotype order follows the 7-month mass loss in Fig. 3).

Figure 3. The mean (+SE, n = 4-6) of leaf litter mass loss after 7, 11, 20 and 35 months of decomposition in 19 *Betula pendula* genotypes (the genotypes are in the order of increasing mass loss after 7 months).

684 **Tables**

685 **Table 1.** Number of observations (N), the mean ($\bar{x}$), variance components (σ^2;G = Genotype, E =
686 Environment), broad-sense heritability (H^2), coefficient of genotypic variation (CV_G) and the statistical
687 significance of the genotype effect on mass loss, number of bacterial and fungal DNA copies and N
688 concentration of *Betula pendula* litter.

689

690

	N	$\bar{x}$	σ^2_G	σ^2_E	H^2	CV_G	Genotype effect	
							F	P
Litter mass loss								
7-mo old litter	111	8.84	5.099	15.44	0.248	0.255	2.93	< **0.001**
11-mo old litter	111	23.5	2.280	30.55	0.069	0.064	1.44	0.136
20-mo old litter	111	27.5	2.658	39.33	0.063	0.059	1.39	0.155
35-mo old litter	105	50.5	0.650	140.3	0.005	0.016	1.03	0.442
Bacterial DNA								
Senescent leaves[a]	110	1.1E+4	1.1E+6	1.0E+7	0.094	0.093	1.58	0.084
7-mo old litter[b]	112	8.33	0	0.084	0	0	0.76	0.739
11-mo old litter[b]	112	8.67	0	0.054	0	0	0.94	0.532
Fungal DNA								
Senescent leaves[a]	110	7.0E+4	3.7E+7	3.3E+8	0.102	0.087	1.58	0.086
7-mo old litter[b]	112	9.79	0.003	0.061	0.040	0.005	1.27	0.231
11-mo old litter[b]	112	9.97	0	0.041	0	0	0.75	0.748
N concentration								
7-mo old litter	112	1.16	0.003	0.013	0.202	0.050	3.53	< **0.001**

691 [a] square root transformed
692 [b] log(x+1) transformed
693

694 **Table 2.** Spearman's rank correlations (and their P-values) between the genotype means (n = 19) of *Betula*

695 *pendula* litter mass loss and the number of bacterial and fungal DNA copies found in the litter.

696

	Mass loss	
	After 7 months	After 11 months
Bacterial DNA		
Senescent leaves	0.45 (0.054)	
7-mo old litter	0.31 (0.190)	<0.01 (1.00)
11-mo old litter		**0.48 (0.036)**
Fungal DNA		
Senescent leaves	0.03 (0.920)	
7-mo old litter	0.39 (0.099)	0.45 (0.056)
11-mo old litter		-0.11 (0.642)

697

Table 3. Spearman's rank correlations (and their P-values) between litter mass loss and the number of bacterial and fungal DNA copies extracted from the litter of individual *Betula pendula* trees (n = 110-111).

	Mass loss	
	After 7 months	After 11 months
Bacterial DNA		
Senescent leaves	0.14 (0.156)	
7-mo old litter	0.09 (0.362)	0.10 (0.281)
11-mo old litter		**0.27 (0.005)**
Fungal DNA		
Senescent leaves	**-0.21 (0.026)**	
7-mo old litter	0.10 (0.320)	0.03 (0.764)
11-mo old litter		**-0.24 (0.012)**

Table 4. Spearman's rank correlations (and their P-values) between genotype means (n = 19) of *B. pendula* in senescent leaf and litter chemistry and the number of bacterial and fungal DNA copies and litter mass loss measured one harvest further. Data of secondary metabolites and senescent leaf N are from Paaso et al. (2017) and Mikola et al. (2018), respectively.

	Bacterial DNA	**Fungal DNA**	**Litter mass loss**
Senescent leaves		*7-mo litter*	
Intracellular phenolics	-0.03 (0.909)	-0.24 (0.325)	0.01 (0.972)
Epicuticular flavonoid aglycones	-0.22 (0.371)	-0.13 (0.596)	0.33 (0.166)
Epicuticular triterpenoids	-0.04 (0.875)	-0.11 (0.658)	0.26 (0.290)
Condensed tannins	0.12 (0.627)	-0.28 (0.244)	-0.15 (0.528)
Lignin	0.02 (0.932)	0.05 (0.836)	-0.03 (0.920)
Nitrogen	0.38 (0.110)	-0.06 (0.814)	0.27 (0.267)
7-mo old litter		*11-mo litter*	
Intracellular phenolics	-0.42 (0.071)	0.26 (0.286)	-0.40 (0.094)
Epicuticular flavonoid aglycones	0.21 (0.379)	0.28 (0.251)	0.23 (0.351)
Epicuticular triterpenoids	0.17 (0.482)	0.34 (0.152)	0.14 (0.562)
Condensed tannins	-0.03 (0.920)	0.28 (0.251)	-0.16 (0.523)
Lignin	-0.28 (0.238)	-0.27 (0.273)	-0.18 (0.468)
Nitrogen	0.38 (0.110)	0.42 (0.074)	0.22 (0.359)

Table 5. Spearman's rank correlations (and their P-values) between individual *B. pendula* trees (n = 101-111) in senescent leaf and litter chemistry and the number of bacterial and fungal DNA copies and litter mass loss measured one harvest further. Data of secondary metabolites and senescent leaf N are from Paaso et al. (2017) and Mikola et al. (2018), respectively.

	Bacterial DNA	**Fungal DNA**	**Litter mass loss**
Senescent leaves		*7-mo litter*	
Intracellular phenolics	**-0.31 (0.002)**	-0.11 (0.265)	-0.09 (0.354)
Epicuticular flavonoid aglycones	**-0.27 (0.007)**	-0.02 (0.814)	<0.01 (0.994)
Epicuticular triterpenoids	-0.15 (0.130)	0.04 (0.717)	-0.04 (0.708)
Condensed tannins	-0.06 (0.492)	-0.05 (0.632)	-0.08 (0.385)
Lignin	-0.07 (0.451)	0.16 (0.084)	-0.06 (0.560)
Nitrogen	**0.26 (0.007)**	0.04 (0.663)	-0.01 (0.928)
7-mo old litter		*11-mo litter*	
Intracellular phenolics	**-0.28 (0.004)**	-0.09 (0.359)	-0.19 (0.058)
Epicuticular flavonoid aglycones	-0.05 (0.645)	-0.02 (0.884)	0.05 (0.624)
Epicuticular triterpenoids	0.07 (0.469)	-0.05 (0.629)	0.08 (0.426)
Condensed tannins	**-0.23 (0.017)**	0.09 (0.358)	**-0.23 (0.017)**
Lignin	**0.41 (<0.001)**	**-0.30 (0.001)**	**0.26 (0.006)**
Nitrogen	**0.21 (0.027)**	**-0.19 (0.041)**	0.14 (0.154)

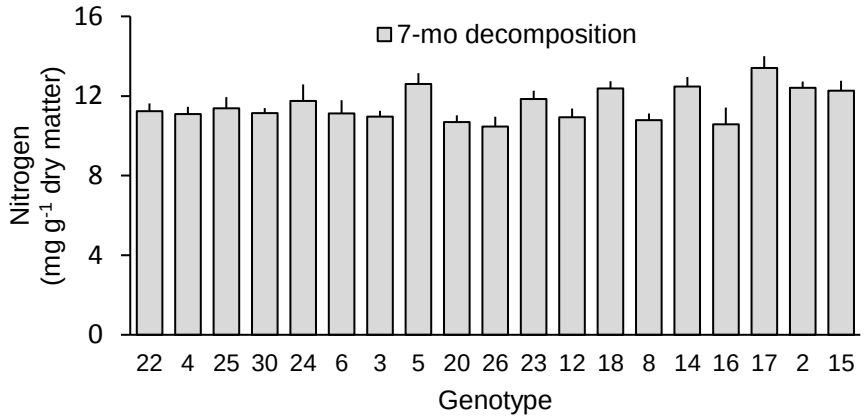
7-mo decomposition
Nitrogen
(mg g-1 dry matter)
16
12
8
4
0
22 4 25 30 24 6 3 5 20 26 23 12 18 8 14 16 17 2 15
Genotype

Figure 1.

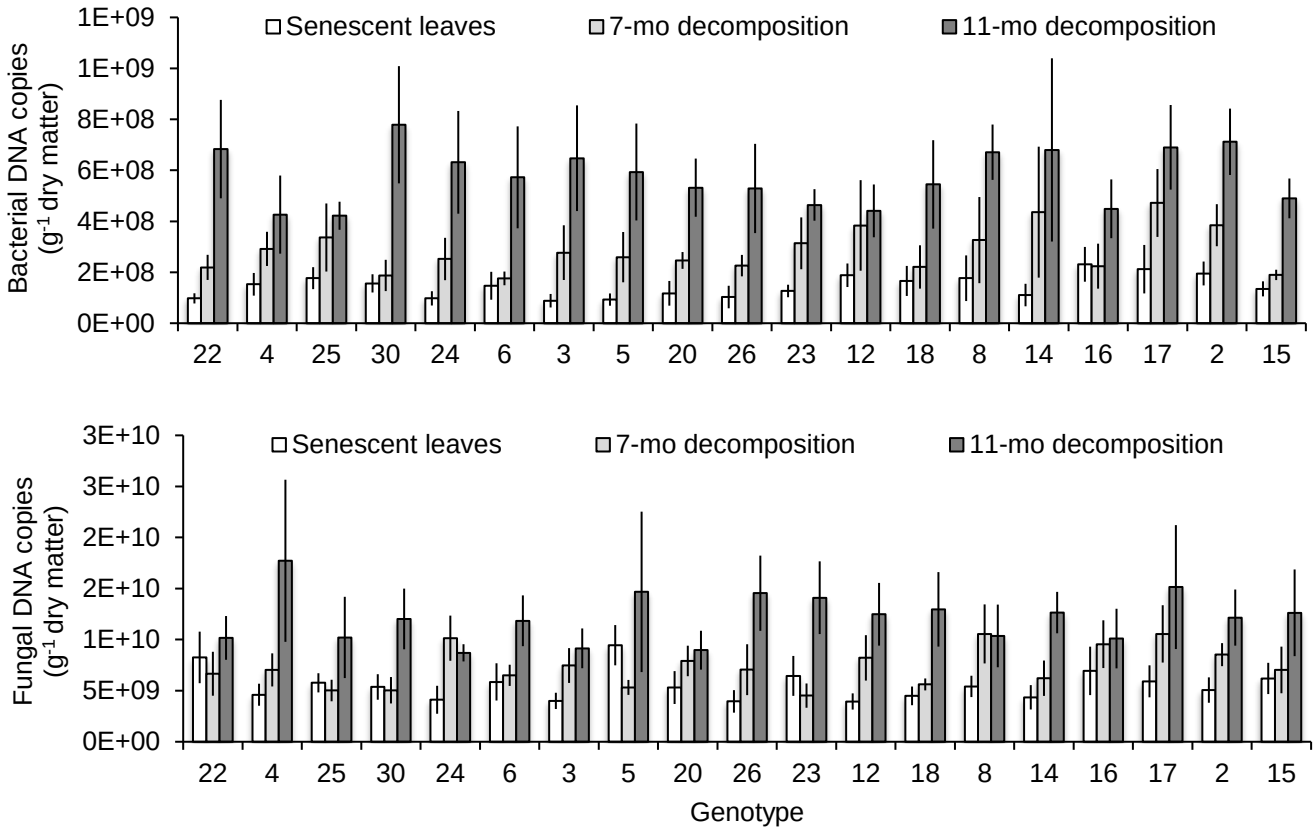
Senescent leaves
7-mo decomposition
11-mo decomposition
Bacterial DNA copies (g-1 dry matter)
1E+09
1E+09
8E+08
6E+08
4E+08
2E+08
0E+00
Fungal DNA copies (g-1 dry matter)
3E+10
3E+10
2E+10
2E+10
1E+10
5E+09
0E+00
22 4 25 30 24 6 3 5 20 26 23 12 18 8 14 16 17 2 15
Genotype

Figure 2.

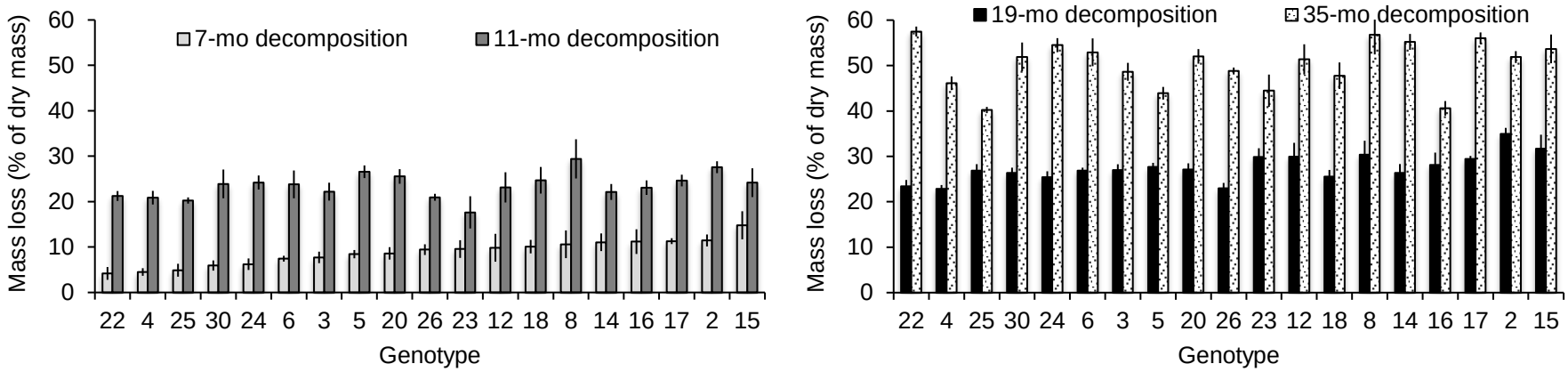
Mass loss (% of dry mass)
7-mo decomposition
11-mo decomposition
19-mo decomposition
35-mo decomposition
Genotype
22 4 25 30 24 6 3 5 20 26 23 12 18 8 14 16 17 2 15

Figure 3.