Notes

Leaf N resorption efficiency and litter N mineralization rate have a genotypic tradeoff in a silver birch population

Juha Mikola [iD],[1,5] Tarja Silfver,[1] Ulla Paaso,[1] Boy J. M. H. Possen,[2,4] and Matti Rousi[3]

[1]*Faculty of Biological and Environmental Sciences, Ecosystems and Environment Research Programme, University of Helsinki, Niemenkatu 73, 15140 Lahti, Finland*
[2]*Natural Resources Institute Finland (Luke), Finlandiantie 18, 58450 Punkaharju, Finland*
[3]*Natural Resources Institute Finland (Luke), Latokartanonkaari 9, 00790 Helsinki, Finland*

Abstract. Plants enhance N use efficiency by resorbing N from senescing leaves. This can affect litter N mineralization rate due to the C:N-ratio requirements of microbial growth. We examined genotypic links between leaf N resorption and litter mineralization by collecting leaves and litter from 19 *Betula pendula* genotypes and following the N release of litter patches on forest ground. We found significant genotypic variation for N resorption efficiency, litter N concentration, cumulative three-year patch N-input and litter N release with high broad-sense heritabilities ($H^2 = 0.28$–0.65). The genotype means of N resorption efficiency varied from 46% to 65% and correlated negatively with the genotype means of litter N concentration, cumulative patch N-input and litter N release. NH_4^+ yield under patches had a positive genotypic correlation with the cumulative patch N-input. During the first year of litter decomposition, genotypes varied from N immobilization (max 2.71 mg/g dry litter) to N release (max 1.41 mg/g dry litter), creating a genotypic tradeoff between the N conserved by resorption and the N available for root uptake during the growing season. We speculate that this tradeoff is one likely reason for the remarkably wide genotypic range of N resorption efficiencies in our birch population.

Key words: Betula pendula; *broad-sense heritability; genotype; leaf litter; mineralization; nitrogen; population; resorption efficiency; resorption proficiency; tradeoff.*

Introduction

Efficient nitrogen (N) use and conservation in leaves, combined with effective N uptake from decomposing litter are the corner stones of plant N economy and growth. The majority of N in plant leaves is used for photosynthetic enzymes (Evans 1989) and, in general, species that have high leaf N concentrations have high rates of photosynthesis (Wright et al. 2004). Perennial plants can enhance leaf N use efficiency by reducing leaf tissue turnover and resorbing N from leaves before they are shed at senescence (Aerts and Chapin 2000). Leaf life span of woody evergreens can be >10 yr, and due to N resorption, the mean residence time of N in woody deciduous plants can be years despite the mean leaf life span being only a few months (Eckstein et al. 1999). No more than 60% of green leaf N can be conserved in resorption (Vergutz et al. 2012), however, and the remaining N needs to be recycled through soil decomposers before it is again available for plant uptake. This decomposer pathway is an important, but a costly way to retain N as uptake of nutrients consumes 30–50% of the root carbon budget

(Lambers et al. 2008). Furthermore, in contrast to N resorption, mineralized N is susceptible to uptake by other plants while cycling through the soil. However, N resorption from senescing leaves is not free either, but has energetic costs, including a shorter growing season (Tateno 2003).

Nitrogen resorption efficiency (the proportion of green leaf N resorbed during leaf senescence) and proficiency (the concentration to which N is reduced in senescing leaves) vary among plant functional groups (Aerts 1996, Killingbeck 1996, Vergutz et al. 2012), but are also known to show considerable intraspecific variation (Eckstein et al. 1999, Covelo et al. 2008). Deciduous trees and shrubs typically have a higher leaf N concentration and a higher N resorption efficiency than evergreens, while evergreens tend to have a higher N resorption proficiency, i.e., they produce litter with lower N concentration (Aerts 1996, Eckstein et al. 1999). Leaf N concentration and N resorption also respond to environmental variation. In a global data set of almost five hundred plant species, leaf N concentration increases with increasing soil N mineralization (Ordoñez et al. 2009). Similarly, although N resorption efficiency was long thought to be insensitive to soil N fertility within species (Chapin and Moilanen 1991, Aerts 1996) and across species (Del Argo et al. 1991, Wright and Westoby 2003), recent meta-analyses of interspecific comparisons (Kobe et al. 2005, Vergutz et al. 2012) and fertilization treatments (Yuan and Chen 2015) suggest that the efficiency decreases with increasing soil N fertility and leaf N

concentration. This means that plants growing in fertile environments produce leaf litter of high N concentration, not only because they have high green leaf N concentrations (Ordoñez et al. 2009), but also because they resorb relatively less of the green leaf N (Kobe et al. 2005, Yuan and Chen 2015). In species comparisons though, low N resorption efficiency does not necessarily signify high litter N concentration as those species that have low green leaf N concentrations tend to produce litter with low N concentration despite having a low N resorption efficiency (Aerts 1996).

Differences in litter N concentrations can have significant effects on litter N mineralization and soil N availability. Using agricultural settings and crop residues, Vigil and Kissel (1991) showed that around 75% of the variation in litter N mineralization could be explained by variation in litter N concentration. They further calculated that the break point between net N mineralization and net N immobilization was 1% N of litter dry mass. Above this concentration, microbial N requirements are met and decomposition leads to net N mineralization, whereas below the concentration, microbes need to absorb additional N to grow and litter decomposition leads to N immobilization. The generality of this pattern was verified in a global long-term decomposition trial, where the net immobilization and net release of N in leaf litter were shown to be tightly driven by the initial litter N concentration, regardless of the variation in other litter quality and site attributes (Parton et al. 2007). In this trial, net N release started when the C:N ratio of litter decreased below 40. Considering that the C concentration of plant leaf litter is typically slightly above 40% (Vergutz et al. 2012), this corresponds well with the notion of 1% N being a threshold of litter N mineralization (Vigil and Kissel 1991). Based on these observations, it is clear that by affecting litter N concentrations, the green leaf N concentration and N resorption efficiency have a great potential to control soil N availability. In particular, it has been suggested that N resorption, which is highly beneficial for plant growth (May and Killingbeck 1992), could create a tradeoff between root N uptake and plant growth by means of reducing soil N availability (Aerts 1997, Aerts and Chapin 2000).

The evidence of links between leaf and litter N concentrations, N resorption and soil N availability mostly comes from interspecific comparisons (e.g., Aerts 1996, Kobe et al. 2005, Chávez-Vergara et al. 2015). These are valuable for understanding how plant species respond to and affect N availability in ecosystems (Hobbie 2015). Much less is known about intraspecific, and particularly, intrapopulation genetic links between these traits. Such links can, however, reveal how selection and adaptation can drive ecosystem functioning (e.g., Fitzpatrick et al. 2015) and whether such ecosystem level tradeoffs exist, which could direct and constrain plant trait adaptation (e.g., Fischer et al. 2006). Here we report results from a study, where we used a tree stand of 19 replicated silver birch, *Betula pendula* genotypes to address these questions in a tree population. *Betula pendula* is one of the most common broad-leaved tree species in Europe (Atkinson 1992) and is a typical pioneer species that rapidly colonizes disturbed forest habitats (Atkinson 1992, Fischer et al. 2002). We previously showed that our *B. pendula* population has significant genotypic variation in the decomposition rate of leaf litter and that

this variation is positively associated with the genotypic variation in litter N concentration (Silfver et al. 2007). In the present study, we focused on the causes of the variation in litter N concentration (i.e., green leaf N concentration vs. N resorption), the consequences of variation in litter N concentration for litter N release, and the potential tradeoff between efficient N resorption and soil N mineralization. We hypothesized that (1) these traits, including the extended phenotype of litter N mineralization (sensu Whitham et al. 2003), have significant heritable variation in our population, and (2) the genotypic correlations at the population level follow the correlations found earlier in interspecific, intraspecific and treatment comparisons, i.e., (a) green leaf and litter N concentrations are positively correlated (Aerts 1996, Kobe et al. 2005), (b) genotypes that have higher green leaf N concentration have lower N resorption efficiency (Kobe et al. 2005, Yuan and Chen 2015), (c) genotypes with higher N resorption efficiency produce litter of lower N concentration (Eckstein et al. 1999), and (d) litter N concentration is positively correlated with litter N release (Vigil and Kissel 1991, Parton et al. 2007). We also expected that (3) the heritable variation in N resorption efficiency can lead to a genotypic tradeoff between efficient N resorption and soil N mineralization as suggested by species comparisons (Aerts 1997). Finally, to test if (4) efficient N resorption nevertheless is under selection in our study population, we contrasted the genotype means of N resorption efficiency with those of tree growth.

METHODS

Plant material

The mother trees of the 19 *B. pendula* genotypes were selected from a naturally regenerated 0.9 ha forest stand in Punkaharju, south-east Finland (61°48′ N, 29°18′ E) in 1997 and represent the random genetic variation of a local *B. pendula* population. To estimate genetic variation, the cloned progeny (produced using a micropropagation technique) of trees was planted into six replicate blocks in an experimental agricultural field in Kuikanniitty, Punkaharju in 1999 (Laitinen et al. 2005). The soil of the site is classified as fine sandy till (Laitinen et al. 2005) and in the region, the thermal growing season starts in the beginning of May and ends in the middle of October (Finnish Meteorological Institute 2018). Among the planted trees, the mean date of bud burst varies from April 24th to May 10th (measured for 2002–2005 and 2010–2012) and the start of autumn senescence from September 9th to September 24th (2011–2012) (Possen et al. 2014). At the time this study was carried out, the trees were on average 11 m tall. Tree growth for summers 2008 and 2009 was calculated using height measurements carried out at the end of growing seasons in 2007–2009.

Leaf and litter collection

To estimate green leaf N concentration, 30 leaves were collected from five replicate trees of each of the 19 genotypes at the end of June 2008. For each tree, the leaves were randomly picked (excluding damaged leaves) from one south-facing

branch at the height of 1.5 m. The leaves were frozen in liquid N and stored frozen until processed in the laboratory. Leaf litter was collected from all six replicate trees of each genotype in 2008–2010 by enclosing two to four 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) well before onset of leaf abscission. The number of replicate trees was increased by one to fit the number of replicate blocks in the site of litter decomposition and part of the litter material had to be collected from higher branches to achieve sufficient amount of litter for each tree. After leaf fall, in late October or early November, the mesh bags were removed from the trees and the collected litter was stored at ambient temperature until 30 leaves were randomly picked from each bag for N analysis. Leaf and litter samples were ground in liquid N and dried in the oven at 70°C before their N concentration was determined using a LECO CNS-2000 analyzer (LECO Corporation, Saint Joseph, MI, USA). Data of 2008 leaf and litter N concentrations have earlier been used for calculating total N export from senescing leaves in a study that examined reasons for the genotypic variation in the autumnal aphid load of *B. pendula* (Sinkkonen et al. 2012). In the present study, these data were utilized to calculate N resorption efficiency, RE_N for each tree using Eq. 1. It is good to note that this equation underestimates the level of resorption efficiency by ~10% since it ignores mass loss during leaf senescence (van Heerwaarden et al. 2003), and besides this systematic error, some error could also arise from possible genetic variation in mass loss.

$$RE_N = 100 \times \left(1 - \frac{\text{leaf litter N\%}}{\text{green leaf N\%}}\right) \qquad (1)$$

Litter patches and their N dynamics

Litter decomposition and N dynamics were examined at a forest site in Loppi, southern Finland (60°36′ N, 24°24′ E), instead of the Kuikanniitty agricultural field, to ensure that litter was exposed to decomposer microbes adapted to tree leaf litter decomposition. In the Loppi region, the long-term (1981–2010) mean daily temperatures of the warmest (July) and coldest (February) month are 17 and −7°C, respectively, and the mean annual precipitation is 660 mm (Finnish Meteorological Institute 2018). The soil in the site is post-glacial sorted fine sand. The upper 5-cm soil layer has a 15% loss on ignition, total C and N concentrations of 6% and 0.3%, respectively, and a pH of 5.0 (Mikola et al. 2014). Before the area was cut in 2008, it was covered by forest stands of different mixture of *B. pendula* and *Pinus sylvestris*.

To study litter N dynamics, a litter patch (diameter ca. 30 cm, 10 g of litter as dry mass equivalent) was established at the site for each tree in the autumn of 2008. The patches were allocated to six replicate blocks following the blocking factor in the Kuikanniitty site. To prevent litter disappearance, but still ensuring direct contact with ground surface, the patches were covered, but not enclosed, by white polyethylene mesh (2 mm). Each patch was augmented with 25 g of new litter (as a dry mass equivalent) in the autumn

of 2009 and 2010. In a few cases in each autumn, the litter sample of a particular tree was not sufficiently large (i.e., did not reach 10 or 25 g) and was completed using litter from a tree of the same genotype from another replicate block. To estimate litter mass loss rate, part of the litter in each patch was in 2008 enclosed in four 10 × 10 cm litter bags (mesh size 0.5 mm), on average 0.45 g (as dry mass equivalent) in each. To preserve the microbes that naturally grow on senescent leaves, the litter that was placed in the patches and within the litter bags was not dried for dry mass measurements. Instead, an independent sub-sample was dried (3 d at 70°C) and used for estimating the moisture content of the litter. One of the litter bags in each patch was sampled in the following autumn (middle October 2009) to quantify litter mass loss (data not shown). To estimate the N concentration of decomposed litter, 20 leaves were collected from each litter patch, ground in liquid N, dried and analysed using a LECO CNS-2000 analyzer. The amount of N released from the litter (mg N per g dry litter) was then calculated using the data of mass loss and N concentration.

The accumulation of N into litter patches through the three autumns, i.e., the total patch N-input, was calculated using the N concentration and dry mass of the added litter. To estimate N mineralization under the litter layer during a growing season, two mesh bags (5 cm × 5 cm, mesh size 200 μm) filled with 1 g of ion exchange resin (Amberlite® MB-150 Mixed Bed Exchanger, Rohm and Haas, France) were placed under each patch for the 2011 growing season. In the autumn of 2011, the bags were transferred to the laboratory, rinsed using distilled water and the two bags of each patch were pooled and their NH_4^+ contents extracted in 50 mL of 2 mol/L KCl. The KCl solution was filtered through a glass microfiber filter (Whatman, GE Healthcare Europe GmbH, Freiburg, Germany) and the NH_4^+ concentrations were analyzed using a Lachat QuikChem 8000 analyzer (Zellweger Analytics, Inc., Lachat Instruments Division, Milwaukee, WI, USA).

Statistical analysis

The broad-sense heritabilities (H^2) of the measured traits were calculated on an individual plant basis according to Eq. 2, where σ_G^2 and σ_E^2 are variance components for genotypes and environment, respectively (SPSS GLM Variance components procedure; IBM SPSS Statistics 23, New York, NY, USA). Following a common practice in tree breeding, the field block was interpreted in the calculation model as a fixed factor. This differs from some of our earlier studies (Mikola et al. 2014, Silfver et al. 2015), where we were also interested in the magnitude of the block-scale environmental variation and treated block as a random factor. Coefficients of genotypic variation (CV_G) were calculated according to Eq. 3, where $\bar{x}$ is the phenotypic mean.

$$H^2 = \frac{\sigma_G^2}{\left(\sigma_G^2 + \sigma_E^2\right)} \qquad (2)$$

$$CV_G = \frac{\sqrt{\sigma_G^2}}{\bar{x}} \qquad (3)$$

The effects of genotype and block on tree and litter patch attributes were analyzed using ANOVA, and the genotypic correlation among the attributes was estimated using a Pearson correlation coefficient of genotype means. For the heritability calculations and ANOVA, the concentrations of NH_4^+ were log-transformed to correct the log-normal distribution of model residuals and in 2008, one value of litter N concentration was omitted as an outlier (genotype 26, litter N 1.52% of dry mass). For ANOVA, the homogeneity of variances was tested using Levene's test and the normality of model residuals was checked using frequency distributions and Shapiro-Wilk normality test.

RESULTS

Genotypic variation was a key determinant of green leaf N concentration, N resorption efficiency and litter N concentration in 2008 (Fig. 1A–C, Table 1). Broad-sense heritabilities (H^2) were high (0.28–0.34) and the coefficients of genotypic variation (CV_G) varied between 0.063 and 0.080 for these variables (Table 1). The genotype means of N resorption efficiency had a weak, positive correlation with the genotype means of green leaf N concentration (Fig. 2A) and a strong, negative correlation with the genotype means of litter N concentration (Fig. 2B), while green leaf N concentration and litter N concentration were not correlated (Fig. 2C). The genotypic variation in litter N concentration was consistent among years: the genotype means of 2008 litter N concentration correlated positively with those in 2009 ($r = 0.75$, $P < 0.001$, $n = 19$) and 2010 ($r = 0.60$, $P = 0.007$).

Mean litter N concentration increased from 1.02 to 1.39% during the first year of decomposition (Fig. 1C, D) and the mean litter N release was negative (Fig. 1E, Table 1). Litter N release had a significant genotypic component, however, and the genotype means varied from N release to N immobilization (Fig. 1E, Table 1). Litter N release had a negative genotypic correlation with N resorption efficiency (Fig. 2D) and a positive correlation with litter N concentration ($r = 0.82$, $P < 0.001$). Genotypic variation decreased in litter N concentration during decomposition (Fig. 1C, D, Table 1) and the genotype means of litter N concentration and N concentration of decomposed litter were not significantly correlated ($r = 0.25$, $P = 0.301$).

Genotypic variation was highly significant in the cumulative patch N input (Fig. 1F, Table 1), which had a positive, genotypic correlation with first-year N release ($r = 0.60$, $P = 0.006$) and a negative, genotypic correlation with N resorption efficiency ($r = -0.46$, $P = 0.048$). Variation in resin NH_4^+ yield contained genotypic variation, although this was not statistically significant (Fig. 1G, Table 1), and the genotype means of NH_4^+ yield correlated positively with the genotype means of cumulative patch N input ($r = 0.45$, $P = 0.051$).

Genotypic variation in tree growth was statistically significant in 2009 and marginally significant in 2008 (Fig. 1H, Table 1). Broad-sense heritabilities of growth remained low, but the coefficients of genotypic variation were higher than for N attributes (Table 1). The genotype means of N resorption efficiency did not significantly correlate with the

genotype means of 2008 ($r = -0.04$, $P = 0.864$) or 2009 growth ($r = -0.29$, $P = 0.231$). The genotypic correlation of growth between the two years was positive, but weak and statistically non-significant ($r = 0.12$, $P = 0.623$).

DISCUSSION

Genotypic variation in leaf N traits

As we hypothesized, plant traits related to N use and conservation had a significant genotypic component in our *B. pendula* population. We have earlier shown that green leaf and litter N concentrations are genetically controlled in *B. pendula* (Silfver et al. 2007, Sinkkonen et al. 2012), but the role of N resorption in the genotypic variation of litter N concentration has remained unexplored. Our results show that *B. pendula* N resorption efficiency has a significant genotypic component ($H^2 = 0.28$, $CV_G = 0.065$). The magnitude of this variation is moderate if compared to the CV_G estimates of growth in the present study (0.10–0.11), in an earlier study of the same population (0.10–0.19) (Mikola et al. 2014) and other *B. pendula* populations (0.05–0.13) (Stener and Hedenberg 2003, Stener and Jansson 2005), but the range of mean genotype N resorption efficiencies (46–65%) is remarkably high. When compared to the mean difference between evergreen (47%) and deciduous (54%) woody plants (Aerts 1996), it appears that the intrapopulation range of N resorption efficiencies among *B. pendula* genotypes is almost triple the range of mean efficiencies between the main functional groups of woody plants. In line with the former, reviews have concluded that intraspecific variation accounts for a significant proportion of the total variation in leaf chemical traits within plant communities (Siefert et al. 2015) and particularly in N resorption efficiency can almost rival interspecific variation within and among plant functional groups (Eckstein et al. 1999).

Genotypic correlations

Both interspecific comparisons and manipulation experiments have shown a positive correlation between green leaf and litter N concentrations (Aerts 1996, Kobe et al. 2005). Our results suggest that this association may not hold at the intrapopulation genotype level. Instead, we show genotypic variation of *B. pendula* litter N concentration to be strictly controlled by the genotypic variation in N resorption efficiency, with the genotype means of litter N concentration decreasing with increasing means of N resorption efficiency. This association is not inevitable although it may appear so. For instance, if genotypes differ in green leaf N concentration, but have equal N resorption proficiency (i.e., produce litter with equal N concentration), the genotype means of N resorption efficiency and litter N concentration do not correlate. We also failed to find evidence that genotypes with higher green leaf N concentration have lower N resorption efficiency as suggested by interspecific comparisons and experiments (Kobe et al. 2005, Yuan and Chen 2015). If anything, this association seems to be weakly positive at the genotype level, which could simply be explained by higher
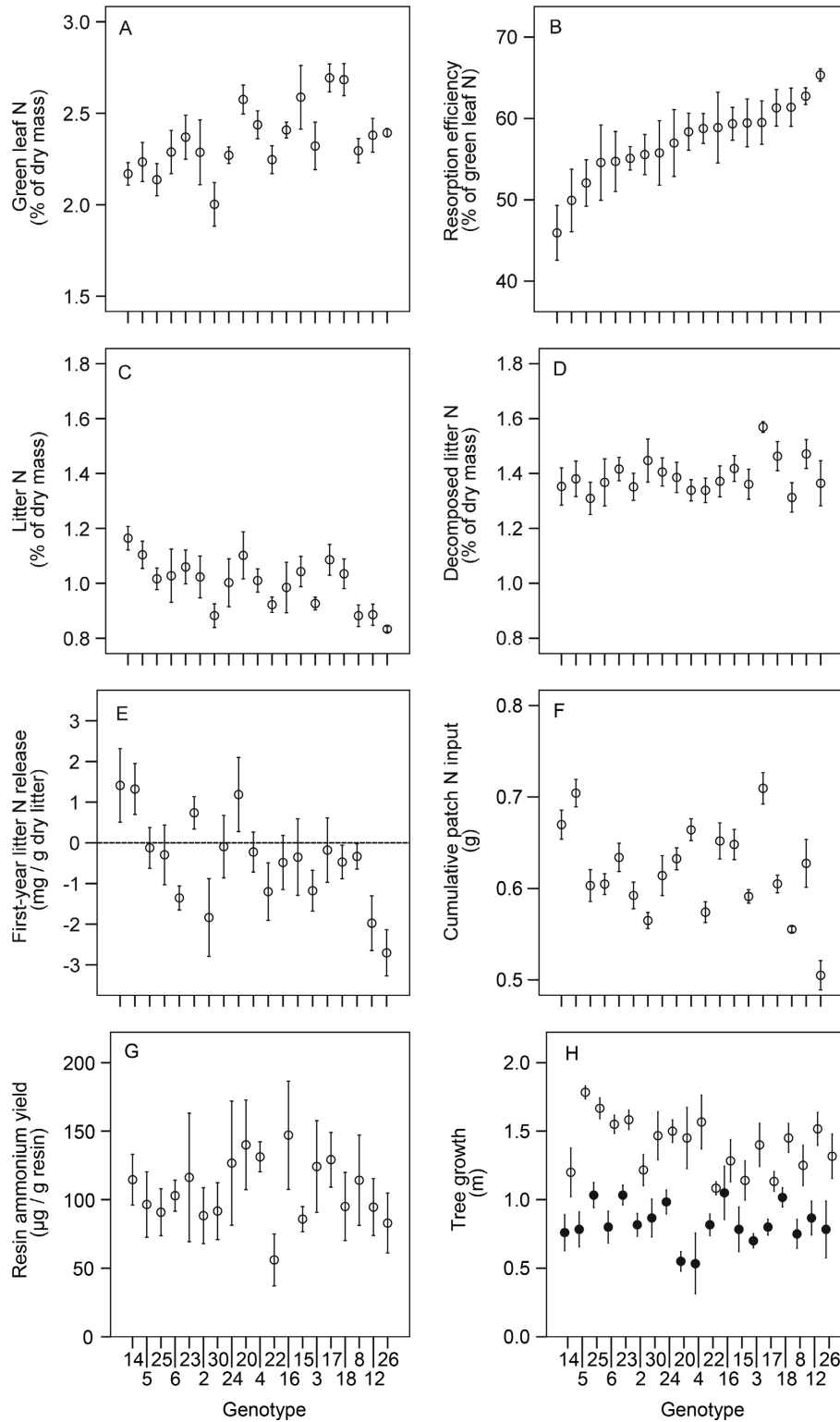
FIG. 1.   Genotype means (±1 SE, *n* = 5–6) of (A) green leaf N concentration (summer 2008), (B) N resorption efficiency (autumn 2008), (C) litter N concentration (autumn 2008), (D) litter N concentration after a year of decomposition (autumn 2009), (E) quantity of N released from litter during the first year of decomposition (autumn 2008–autumn 2009), (F) cumulative 3-yr litter patch N input (autumns 2008–2010), (G) resin NH_4^+ yield under the litter patch during the third growing season since patch establishment (summer 2011) and (H) tree growth in summer 2008 (black symbols) and 2009 (open symbols); the genotypes are arranged in an increasing order of N resorption efficiency.

TABLE 1. Number of observations (N), phenotype mean ($\bar{x}$), variance components (σ^2; G = genotype, E = environment), broad-sense heritability (H^2), coefficient of genotypic variation (CV_G) and the statistical significance of the genotype effect for *Betula pendula* growth and litter N attributes.

|							Genotype effect	
	N	$\bar{x}$	σ^2_G	σ^2_E	H^2	CV_G	F	P
Green leaf and leaf litter								
Green leaf N (% of dry mass, summer 2008)	94	2.37	0.022	0.051	0.30	0.063	3.25	<0.001
N resorption efficiency (% of green leaf N, 2008)	93	57.1	13.6	34.4	0.28	0.065	2.93	0.001
Litter N (% of dry mass, autumn 2008)	111	1.02	0.0067	0.0130	0.34	0.080	4.04	<0.001
Litter N (% of dry mass, autumn 2009)	98	1.08	0.0085	0.0075	0.53	0.085	6.81	<0.001
Litter N (% of dry mass, autumn 2010)	107	0.99	0.0056	0.0132	0.30	0.076	3.39	<0.001
Litter patch								
Cumulative patch N input (g, 2008–2010)	112	0.62	0.0024	0.0013	0.65	0.079	11.8	<0.001
Decomposed litter N (% of dry mass, autumn 2009)	111	1.39	0.0010	0.0175	0.05	0.023	1.34	0.183
First-year litter N release (mg/g dry litter)	109	−0.41	0.83	2.07	0.29		3.40	<0.001
Resin NH_4^+ yield (µg/g resin, summer 2011)†	112	1.97	0.0082	0.060	0.12	0.045	1.07	0.400
Tree growth								
Height increment (m, summer 2008)	113	0.83	0.0082	0.082	0.09	0.109	1.56	0.078
Height increment (m, summer 2009)	112	1.40	0.020	0.108	0.16	0.101	2.09	0.013

†Log$_{10}$-transformed.
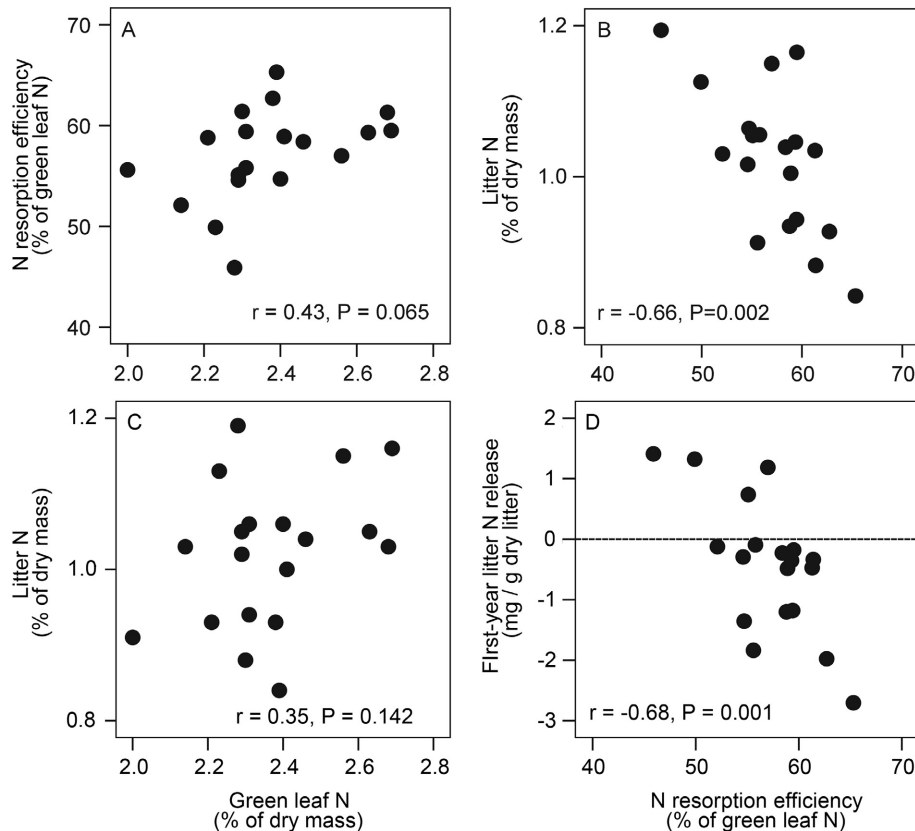
FIG. 2. Genotypic correlations (*n* = 19) among green leaf N concentration, N resorption efficiency, litter N concentration and the quantity of N released from litter during the first year of decomposition.

amounts of leaf N being available for resorption in genotypes with higher green leaf N concentration. Similar associations – a negative correlation between resorption efficiency and litter N concentration, and a positive correlation between green leaf N concentration and resorption efficiency – were also found among trees in a field study of mountain birch, *B. pubescens* subsp. *czerepanovii* (Nordell and Karlsson 1995, Eckstein et al. 1999). Nordell and

Karlsson (1995) speculated that these associations and the phenotypic variation in their study were genetic rather than due to plastic responses to environmental variation. Our results confirm their speculation; these intraspecific associations seem to be under genetic control.

The wide genotypic range of resorption efficiencies that we found in our population is expected to have a significant impact on N cycling. When we contrasted litter N concentration, microbial growth and litter mass loss using the same litter material as in the present study, we found that litter N concentration affected bacterial and fungal growth at individual tree level, but surprisingly had no effect on litter mass loss (data not shown). The present results, however, show that litter N release during the first year of decomposition is strongly positively linked to litter N concentration. This is in good agreement with earlier findings of litter N concentration controlling litter N release (Vigil and Kissel 1991, Parton et al. 2007). On average, the first-year N release was negative, suggesting that microbes added N from the environment to the decomposing litter instead of releasing N from the litter. The mean N concentration of our litter, 1.02% N of dry mass, is exactly at the threshold of N mineralization observed in earlier studies (Vigil and Kissel 1991, Parton et al. 2007). Because of this and the wide range of N resorption efficiencies among the genotypes, two sets of genotypes producing either N releasing or N absorbing litter appeared. Based on a large data set of deciduous and evergreen woody species, Killingbeck (1996) concluded that leaf N resorption is complete when litter N concentration reaches values <0.7% and incomplete when it remains >1.0%. This suggests that none of our genotypes had complete N resorption and more than half clearly had incomplete resorption. This could be explained by the pioneer character of *B. pendula* in boreal forest succession (Atkinson 1992, Fischer et al. 2002). After a disturbance, typically a forest fire, and in early forest succession, competition for nutrients is not severe and it is likely that complete nutrient resorption is not selected for in such conditions. During the first year of decomposition, mean litter N concentration increased to 1.39% N of dry mass, which suggests that during the second year of decomposition, N mineralization is positive. However, Parton et al. (2007) found that even for litter that had a N concentration >1, major N release started only after 40% of litter mass was lost. In our case, only 24% of mass was lost during the first year of decomposition (data not shown), suggesting that major N release of birch litter may not yet start after a year of decomposition.

Our results show that genotypic variation and genotype rank for litter N concentration were consistent across years, which is remarkable considering that the positive correlation for genotype means of growth between the two years was weak and statistically not significant. As a result, cumulative litter patch N input had a highly significant genotypic component ($H^2 = 0.65$, $CV_G = 0.079$) and the highest genotype N input was 40% higher than the lowest input. This variation was also reflected in soil NH_4^+ availability as the genotype means of cumulative N input and resin NH_4^+ yield were positively correlated. Although the resin NH_4^+ yield had a lot of environmental variation and the genotypic variation was moderate and statistically not significant, these results suggest that mineralization of litter N can be considered as an extended phenotype of *B. pendula* (sensu Whitham et al. 2003), apparently genetically controlled through N resorption efficiency. Notably, the genotypic link between N resorption efficiency and litter N mineralization was detectable under the litter layer although the genotypic variation in litter N concentration decreased during decomposition. This shows that in a litter layer of regular autumnal input, the long-term effects of the genotypic variation of litter N concentration on litter N mineralization are not disguised by microbial processes that reduce genotypic variation in litter N concentration during decomposition.

Genotypic tradeoff between N conservation and mineralization

We predicted that heritable variation in N resorption efficiency can lead to a genotypic tradeoff between efficient N resorption and soil N availability as suggested by species comparisons (Aerts 1997). Our results confirm this prediction: those genotypes that had higher N resorption efficiency created a litter patch that released less NH_4^+ during litter decomposition. Leaf N resorption should be a target for natural selection due to its beneficial effects on plant fitness (May and Killingbeck 1992). It has also been suggested that N resorption proficiency is a better measure of N conservation than N resorption efficiency and therefore the ultimate target of selection (Killingbeck 1996, Wright and Westoby 2003). In our case, these two measures of N conservation were tightly correlated and the genotypes that showed highest efficiency also had the highest proficiency (i.e., the lowest litter N concentration). This suggests that selection acting to minimize N loss through senescent leaves in *B. pendula* populations will enhance both the efficiency and the proficiency of N resorption.

Why is there so much genotypic variation in N resorption then? Our results show that high N resorption efficiencies and proficiencies lower litter N release and soil N availability. Consequently, the benefit of efficient autumnal N resorption may be lost through declined root N uptake during growing seasons. Such genotypic tradeoffs between soil N availability and N resorption efficiency might be an explanation for the high genotypic variation of resorption efficiency and the wide range of efficiencies that we found in our population. This is in line with our finding that N resorption efficiency, despite being consistent across years (based on litter N concentrations), did not have a positive genotypic correlation with tree growth in our study population, i.e., there apparently was no selection for high N resorption efficiency. It is important to note, though, that trees in our study grew in an agricultural field, a favorable growing environment, where genotypes of high N resorption efficiency may neither significantly benefit from efficient N conservation nor significantly suffer from intense competition for N in the soil. Finally, gene transfer may also have a role in maintaining the genotypic variation in N resorption efficiency (cf. Mikola et al. 2014). *Betula* species are efficient producers of light pollen (Geburek et al. 2012) that can

travel long distances with air masses (Siljamo et al. 2008). If the N resorption efficiency of a genotype shows plasticity and varies with environmental conditions (i.e., there is a genotype × environment interaction), which is the case for *B. pendula* growth (Silfver et al. 2009), long-distance pollen transfer can maintain high genetic variation in N resorption efficiency in local populations.

Conclusion

Our results revealed high genotypic variation in N resorption efficiency within a local *B. pendula* population. By controlling litter N concentration and litter N release this variation cascaded down to soil N mineralization and created a genotypic tradeoff between N resorption efficiency and soil mineral N availability. These results have important implications for plant community and evolutionary ecology. First, they support the view that intraspecific variation can comprise a significant part of the total trait variation within local plant communities (Siefert et al. 2015) and suggest that genotypic variation can be a major source of this variation. Second, the tradeoff that we found offers a likely explanation, along with the energetic costs of N resorption (Tateno 2003), for the wide genotypic range of resorption efficiencies and poor resorption performance of some genotypes in our population, as well as for earlier findings of high intraspecific variation of N resorption efficiency in plants (Eckstein et al. 1999).

Literature Cited

Aerts, R. 1996. Nutrient resorption from senescing leaves of perennials: Are there general patterns? Journal of Ecology 84:597–608.

Aerts, R. 1997. Nitrogen partitioning between resorption and decomposition pathways: a tradeoff between nitrogen use efficiency and litter decomposability? Oikos 80:603–606.

Aerts, R., and F. S. Chapin III. 2000. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. Advances in Ecological Research 30:1–67.

Atkinson, M. D. 1992. *Betula pendula* Roth (*B. verrucosa* Ehrh.) and *B. pubescens* Ehrh. Journal of Ecology 80:837–870.

Chapin III, F. S., and L. Moilanen. 1991. Nutritional controls over nitrogen and phosphorus resorption from Alaskan birch leaves. Ecology 72:709–715.

Chávez-Vergara, B. M., A. González-Rodríguez, J. D. Etchevers, K. Oyama, and F. García-Oliva. 2015. Foliar nutrient resorption constrains soil nutrient transformations under two native oak species in a temperate deciduous forest in Mexico. European Journal of Forest Research 134:803–817.

Covelo, F., A. Rodríguez, and A. Gallardo. 2008. Spatial pattern and scale of leaf N and P resorption efficiency and proficiency in a *Quercus robur* population. Plant and Soil 311:109–119.

Del Argo, J. M., A. Escudero, and M. V. Garrido. 1991. Effects of site characteristics on nitrogen retranslocation from senescing leaves. Ecology 72:701–708.

Eckstein, R. L., P. S. Karlsson, and M. Weih. 1999. Leaf life span and nutrient resorption as determinants of plant nutrient conservation in temperate-arctic regions. New Phytologist 143:177–189.

Evans, J. R. 1989. Photosynthesis and nitrogen relationships in leaves of C3 plants. Oecologia 78:9–19.

Finnish Meteorological Institute. 2018. Electronic archive of climate in Finland for 1981–2010. http://en.ilmatieteenlaitos.fi/seasons-in-finland [last accessed 3 March 2018].

Fischer, A., M. Lindner, C. Abs, and P. Lasch. 2002. Vegetation dynamics in central European forest ecosystems (near-natural as well as managed) after storm events. Folia Geobotanica 37:17–32.

Fischer, D. G., S. C. Hart, B. J. Rehill, R. L. Lindroth, P. Keim, and T. G. Whitham. 2006. Do high-tannin leaves require more roots? Oecologia 149:668–675.

Fitzpatrick, C. R., A. A. Agrawal, N. Basiliko, A. P. Hastings, M. E. Isaac, M. Preston, and M. T. J. Johnson. 2015. The importance of plant genotype and contemporary evolution for terrestrial ecosystem processes. Ecology 96:2632–2642.

Geburek, T., K. Hiess, R. Litschauer, and N. Milasowszky. 2012. Temporal pollen pattern in temperate trees: Expedience or fate? Oikos 121:1603–1612.

Hobbie, S. E. 2015. Plant species effects on nutrient cycling: revisiting litter feedbacks. Trends in Ecology and Evolution 30:357–363.

Killingbeck, K. T. 1996. Nutrients in senesced leaves: keys to the search for potential resorption and resorption proficiency. Ecology 77:1716–1727.

Kobe, R. K., C. A. Lepczyk, and M. Iyer. 2005. Resorption efficiency decreases with increasing green leaf nutrients in a global data set. Ecology 86:2780–2792.

Laitinen, M.-L., R. Julkunen-Tiitto, J. Tahvanainen, J. Heinonen, and M. Rousi. 2005. Variation in birch (*Betula pendula*) shoot secondary chemistry due to genotype, environment, and ontogeny. Journal of Chemical Ecology 31:697–717.

Lambers, H., F. S. Chapin III, and T. L. Pons. 2008. Plant physiological ecology. Second edition. Springer, New York, New York, USA.

May, J. D., and K. T. Killingbeck. 1992. Effects of preventing nutrient resorption on plant fitness and foliar nutrient dynamics. Ecology 73:1868–1878.

Mikola, J., U. Paaso, T. Silfver, M. Autelo, K. Koikkalainen, S. Ruotsalainen, and M. Rousi. 2014. Growth and genotype × environment interactions in *Betula pendula*: Can tree genetic variation be maintained by small-scale forest ground heterogeneity? Evolutionary Ecology 28:811–828.

Nordell, K. O., and P. S. Karlsson. 1995. Resorption of nitrogen and dry matter prior to leaf abscission: variation among individuals, sites and years in the mountain birch. Functional Ecology 9:326–333.

Ordoñez, J. C., P. M. van Bodegom, J.-P. M. Witte, I. J. Wright, P. B. Reich, and R. Aerts. 2009. A global study of relationships between leaf traits, climate and soil measures of nutrient fertility. Global Ecology and Biogeography 18:137–149.

Parton, W., et al. 2007. Global-scale similarities in nitrogen release patterns during long-term decomposition. Science 315:361–364.

Possen, B. J. H. M., M. Rousi, T. Silfver, M. J. Anttonen, S. Ruotsalainen, E. Oksanen, and E. Vapaavuori. 2014. Within-stand variation in silver birch (*Betula pendula* Roth) phenology. Trees 28:1801–1812.

Siefert, A., et al. 2015. A global meta-analysis of the relative extent of intraspecific trait variation in plant communities. Ecology Letters 18:1406–1419.

Silfver, T., J. Mikola, M. Rousi, H. Roininen, and E. Oksanen. 2007. Leaf litter decomposition differs among genotypes in a local *Betula pendula* population. Oecologia 152:707–714.

Silfver, T., H. Roininen, E. Oksanen, and M. Rousi. 2009. Genetic and environmental determinants of silver birch growth and herbivore resistance. Forest Ecology and Management 257:2145–2149.

Silfver, T., U. Paaso, M. Rasehorn, M. Rousi, and J. Mikola. 2015. Genotype × herbivore effect on leaf litter decomposition in *Betula pendula* saplings: ecological and evolutionary consequences and the role of secondary metabolites. PLoS ONE 10:e0116806.

Siljamo, P., M. Sofiev, E. Severova, H. Ranta, J. Kukkonen, S. Polevova, E. Kubin, and A. Minin. 2008. Sources, impact and exchange of early-spring birch pollen in the Moscow region and Finland. Aerobiologia 24:211–230.

Sinkkonen, A., E. Somerkoski, U. Paaso, J. K. Holopainen, M. Rousi, and J. Mikola. 2012. Genotypic variation in yellow autumn leaf colours explains aphid load in silver birch. New Phytologist 195:461–469.

Stener, L.-G., and Ö. Hedenberg. 2003. Genetic parameters of wood, fibre, stem quality and growth traits in a clone test with *Betula pendula*. Scandinavian Journal of Forest Research 18:103–110.

Stener, L.-G., and G. Jansson. 2005. Improvement of *Betula pendula* by clonal and progeny testing of phenotypically selected trees. Scandinavian Journal of Forest Research 20:292–303.

Tateno, M. 2003. Benefit to N_2-fixing alder of extending growth period at the cost of leaf nitrogen loss without resorption. Oecologia 137:338–343.

van Heerwaarden, L. M., S. Toet, and R. Aerts. 2003. Current measures of nutrient resorption efficiency lead to a substantial underestimation of real resorption efficiency: facts and solutions. Oikos 101:664–669.

Vergutz, L., S. Manzoni, A. Porporato, R. F. Novais, and R. B. Jackson. 2012. Global resorption efficiencies and concentrations of carbon and nutrients in leaves of terrestrial plants. Ecological Monographs 82:205–220.

Vigil, M. F., and D. E. Kissel. 1991. Equations for estimating the amount of nitrogen mineralized from crop residues. Soil Science Society of America Journal 55:757–761.

Whitham, T. G., et al. 2003. Community and ecosystem genetics: a consequence of the extended phenotype. Ecology 84:559–573.

Wright, I. J., and M. Westoby. 2003. Nutrient concentration, resorption and lifespan: leaf traits of Australian sclerophyll species. Functional Ecology 17:10–19.

Wright, I. J., et al. 2004. The worldwide leaf economics spectrum. Nature 428:821–827.

Yuan, Z. Y., and H. Y. H. Chen. 2015. Negative effects of fertilization on plant nutrient resorption. Ecology 96:373–380.