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Liming impacts *Fagus sylvatica* leaf traits and litter decomposition 25 years after amendment

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ABSTRACT

Liming is a common technique used to restore forest health in acidified areas but little is known on the remanence of this amendment in forest ecosystems. We thus investigated how calcareous amendments impact internal biogeochemical cycling of beech stands (*Fagus sylvatica*), especially the chemistry and traits of leaves and leaf litter and the subsequent rate of litter decomposition 25 years after applications. We also wanted to know if leaf features provide easy predictive tools of litter quality. In both limed and unlimed stands, we compared functional traits (SLA, LDMC, stomatal density), nutrients and fiber contents (hemicellulose, cellulose and lignin) of green and senescent leaves. Additionally, litter bags were used to infer litter decomposition rate. Our results clearly showed that liming impacts leaf chemistry but not morphological leaf traits many years after treatment. In limed stands, green leaves had significantly higher content of Ca and lignin, but lower Mg and cellulose. For senescent leaves, limed stands had greater Ca, Na and cellulose, but lower K, Mn, and N. Resorption rates of K, Na and Mg, in senescent leaves were also affected by limed treatment. We also found a higher litter decomposition rate in limed stands than in unlimed stands. We thus demonstrated that the effect of liming on soil nutrient availability was weak but that changes in beech leaf quality could be recorded 25 years after treatment. These mid-term liming effects on leaf litter quality likely alter soil properties, soil biota and their interactions and thus litter decomposition rate. Lastly, the use of functional leaf traits as a proxy of ecosystem functioning (such as litter decay) is not sufficiently sensitive to detect changes in sites with smooth environmental stress variation.

1. Introduction

Forest ecosystems in the Northern hemisphere have been exposed to severe atmospheric depositions of acidifying compounds due to human activities during the four last decades (Driscoll et al., 2001; Rice and Herman, 2012). Anthropogenic acidifications have drastically affected chemical and biological properties of European forest soils (Formanek and Vranova, 2002) with severe consequences on tree physiology and health (Packham et al., 2012). This is particularly true when trees grow on acidic soils that are not adequately buffered against additional acid deposition (Ling et al., 1993). Liming with calcite ($CaCO_3$) or dolomite ($CaMg(CO_3)_2$) is a practice that has been intensely used since the early 1980s in beech European forests to counteract anthropogenic or natural soil acidification (Godbold, 2003), to improve supplies in base cations and the subsequent production of wood biomass (Pawlowski, 1997; Formanek and Vranova, 2002; Lofgren et al., 2009). However, it is not clear how calcareous amendments impact internal biogeochemical cycle of stands, especially the chemistry and features of leaves and leaf litter and the subsequent rate of litter decomposition several years after applications.

A possible way to investigate liming impact on ecosystem functioning lies in the measurement of leaf traits, i.e. leaf characteristics measurable at the individual level such as leaf morphology, chemistry or phenology (Violle et al., 2007). These traits provide information such as plant ecophysiological response to abiotic factors (Abrams et al., 1994) or mechanisms affecting growth patterns and competitive interactions (Reich et al., 1990). For instance, rapid acquisition of resources is generally correlated with high Specific Leaf Area (SLA), Leaf Nitrogen and Phosphorus Content (respectively LNC and LPC) or pH of foliar extracts (Cornelissen et al., 2006), while high Leaf Dry Matter Content (LDMC), lignin content or carbon (C)-to-N ratio reflect a resource conservation strategy. These leaf economics trade-offs have been captured at the interspecific levels but recent studies (Albert et al., 2011,

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2012) suggest that these functional variations are robust to individual trait values. These authors thus recommend testing intraspecific trait variation more systematically.

Functional traits not only predict vegetation responses to environmental changes, but they also influence key ecosystem processes. For instance, the chemical and physical properties of plant litter have a major influence on nutrient cycling and accumulation of organic matter in soil (Cornwell et al., 2008; Kurokawa et al., 2010). Decomposition rates are partly controlled by tissue nutrient concentration and by the density of structural material in the leaf (Cornwell et al., 2008). As a consequence, SLA is positively correlated with litter decomposition rate, while cellulose and lignin have negative effects on litter decomposition rate and therefore nutrient turnover. Functional traits of living leaves can also be good predictors of litter decomposability (Cornelissen and Thompson, 1997; Kazakou et al., 2006). Some chemical traits in green leaves, such as nitrogen, carbon or phosphorus content, are likely to be linked with litter nitrogen content or with the litter lignin: nitrogen ratio (Wright et al., 2004, 2005). Structural traits such as LDMC might reflect structural support and defense against herbivores. Indeed, positive correlations were observed between LDMC and lignin content (Fortunel et al., 2009). A variation of these functional leaf traits might have impacts on litter quality and thus on litter decomposability (Cornelissen et al., 2004; Kazakou et al., 2006; Quested et al., 2007; Fortunel et al., 2009).

Although leaf traits are recognized as important tree features that are usually used to understand and predict long-term ecosystem functioning, the impact of liming on leaf traits has been poorly studied. Long et al. (2011) found that calcium and magnesium concentrations in sugar maple foliage remained significantly higher 21 years after lime application but they did not found significant effect on American beech (*Fagus grandifolia*) species comparatively to control stands. Thirty-three years after liming, Weis et al. (2009) found slightly higher concentrations of calcium and magnesium and lower potassium concentrations in twigs and needles of Norway spruce trees comparatively to control stands. In North-central Pennsylvania, Wargo et al. (2002) measured foliar polyamines, starch and soluble sugars in root tissues, and cambial electrical resistance (CER) to detect changes in stress and vitality indicators of limed sugar maple stands. They found that foliar putrescine, soluble sugars, and CER decreased, while starch increased in limed stands. All these studies analyzed element content in green leaves (e.g. C, N, P, S, Ca, Mg, Mn) but none of them explored structural or chemical traits directly associated with litter decomposition rate (e.g. lignin, cellulose).

Hence, the objective of the present study was to evaluate the mid-term effects of liming on green and senescent leaf litter traits in temperate French beech stands, including both chemical and morphological attributes. For that purpose, we compared the traits of both green leaves and senescent leaves in 25 years old beech stands that had received (or not) calcareous amendment before seed germination. We also measured the impact of liming on litter decomposition rate through litter bags experimentation (12 months). We hypothesized that liming (1) affects the functional composition and structure of green leaves 25 years after application, (2) alters nutrient resorption and leaf litter quality, and (3) impacts leaf litter decomposition rate.

2. Materials and methods

2.1. Study site

The study was carried out in the Eawy forest (France, Upper Normandy region, 7200 ha). The climate is temperate oceanic with a mean annual temperature of +10 °C and a mean annual pluviometry of 800 mm. A stand is a defined area of the forest that is uniform in species composition, tree age, silviculture and is managed by the French National Office (ONF) as a single unit. Four limed beech (*Fagus sylvatica* L.) stands were selected in such a way that they were independent (Fig. 1). In 1985, 7 tons per hectare of lime ($CaCO_3$) was applied using a spreading tractor. Four independent pure beech stands (Fig. 1) were also selected as control (no liming). The ages of trees within limed and unlimed stands were similar (25 years old).

The eight stands are managed as even-aged pure forests from natural regeneration by ONF (Table 1). All of them are set in a flat (plateau) topographic situation (205 m a.s.l.). The soil is an endogleyic dystric Luvisol (FAO, 2006) developed on more than 80 cm of loess (lamellated silt) lying on clay with flints. The understory vegetation is defined as a characteristic Endymio–Fagetum according to phytosociological classification (Durin et al., 1967).

In order to assess the effects of liming on soil chemical properties, three subsamples of the A horizon (first 10 cm above the organic layer) were collected in May 2011 and 2012 within frames (25 × 25 cm) in a central plot of each stand in order to avoid edge effect. In the laboratory, samples were sieved (2 mm) and air-dried. In dry samples, concentrations of total C and N were measured by gas chromatography with a CHN pyrolysis micro-analyzer. C-to-N ratio, pHwater and pHKCl (Baize, 2000), contents in available P (Duchaufour and Bonneau, 1959) and Cation Exchange Capacity (CEC) were also determined in samples, as well as total elements (Ca, Mg, K, Mn, Na, Al, Fe) by the cobaltihexamine exchangeable method (Ciesielski et al., 1997). ΔpH was computed corresponding to the difference between pH H_2O and pH KCl. For one type of soil, ΔpH is positively correlated with soil exchangeable acidity and soil base saturation (Baize, 2000). Means and standard errors were calculated by treatment and by variables ($n = 8$ replications). Non parametric tests (Kruskall test) were performed on soil variables (Table 2). Liming increased the availability of exchangeable cations, especially calcium and reduced the concentration of aluminium. Such results are commonly found in literature (Kreutzer, 1995; Long et al., 1997).

2.2. Green and senescent leaves sampling

Green leaves of three individuals were collected per stand, with a minimum of 20 meters between beech trees. Trees were located in the center of the stand in order to avoid edge effect. For each individual, green leaves were collected from 3 to 4 sun-exposed twigs (sun leaves) of beech trees in June 2010. Relatively young but fully expanded and hardened beech leaves were chosen, if possible without pathogen and herbivore damage. When collected, each leaf sample (30–40 leaves) was wrapped in moist paper, sealed in plastic bags and transported to the laboratory in cooler boxes to prevent weight loss. At the laboratory, the leaves were stored in plastic bags at 4 °C until further measurement (24 h maximum of storage).

Senescent beech leaves were collected within each stand between September and November every two weeks. Litter material consisted of freshly fallen leaves. We sampled freshly fallen leaves using litterfall collectors with plastic net (1 m^2, 30 cm deep installed at 1 m above the forest floor). Three collectors were placed close to the stand center and to trees sampled for green leaves, along a transect at 0, 20 and 40 m from the center in order to take a representative sample at the stand level (random orientation). Litter was air-dried at room temperature and stored in hermetic plastic box before the chemical analysis. One subsample of each collector was dried at 60 °C until constant in order to determine the initial dry mass (Gardner, 2006).