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Effects of liming on ectomycorrhizal community structure in relation to soil horizons and tree hosts

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ABSTRACT

Liming is a forestry practice used to correct tree cation deficiency induced by soil acidity. Ectomycorrhizal (ECM) community structure and functioning is closely linked to soil nutrient availability, which is strongly affected by liming. The aim of this study was to assess the impact of liming on ECM community structure depending on soil horizon and tree host. Acidophilic species occurring in untreated plots, such as *Russula ochroleuca*, were absent from limed plots and were replaced by more generalist morphtoypes. The abundance of ECM root tips in the untreated plots was higher in topsoil layers, whereas most of the ECM root tips in the limed plots were in the organomineral layer, whatever the tree host. Liming was the major determinant of fungal community structure, then tree host.

Introduction

Forest decline on acidic soils has been reported in the East of France and in central Europe for the past 30 years (Ulrich *et al.* 1979; Erland & Taylor 2002). Symptoms of spruce needle yellowing or beech decline were due to soil cation starvation, especially calcium and magnesium, which are leached in drainage water because of increased acidic deposition (Hüttl, 1989). Compensatory liming (i.e. the direct input of calcium and magnesium oxides in forest soil by hand or by helicopter) proved to be efficient in restoring tree mineral nutrition (Ulrich *et al.* 1979; Renaud JP *et al.* pers. com). Liming increases pH, Ca and Mg supplies, soil base saturation, and shifts humus type from moder to oligomull (Kreutzer 1995). Liming is a common practice in European forests to improve calcium and magnesium supply and to correct soil acidification, and was intensely used in the 1980s to counteract forest decline following acid rain. It is now once again a topical subject for forest management as a tool to improve the production of wood biomass, because of its supposed mild impact on the functioning of forest ecosystems (Hüttl 1989).

In temperate and boreal forests, tree growth and nutrition is highly dependant on mutualistic associations with fungi; more than 95 % of fine tree roots are in symbiotic association with mycelium, forming mixed organs called ectomycorrhizas (ECM; Smith & Read 1997). The fungal partners (Ascomycota or Basidiomycota) provide the tree with nutrients, such as nitrogen, phosphorus, calcium or magnesium, derived from both mineral and organic sources.

Liming reduces humus accumulation and, as a consequence, C and N contents in soil (Kreutzer 1995; Rosenberg *et al.* 2003). Liming also increases soil pH and reduces soil Al^{3+} concentration (Kreutzer 1995; Renaud JP *et al.* pers. com). These soil parameters influence ECM diversity (Erland & Taylor 2002) and, in Norway spruce experiments, liming increased two-fold ECM root abundance in the humus layers (Nowotny *et al.* 1998) and disturbed the ECM community structure (Qian *et al.* 1998), by decreasing the abundance of

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Russula ochroleuca and *Tylospora* sp. root tips and increasing those of *Piceirhiza nigra* and *Tuber puberulum*. In other experiments, liming caused a replacement of the ECM species, and had no effect on ECM diversity at high doses (8.75 ton ha^{-1}), whereas diversity decreased at lower doses (3.25 and 4.28 ton ha^{-1}) (Taylor & Finlay 2003). In contrast, Blaise & Garbaye (1983) found a reduced ECM abundance in limed beech plots. It also appears that liming has a gradually decreasing influence on pH with soil depth (Kreutzer 1995). The aim of this study was, thus, to assess the consequences of liming on the structure of ECM communities.

Material and methods

Study site

The experimental site of Humont (48°00′00″N, 6°29′28″E, Altitude: 570 m, Vosges forest, North-Eastern France) consists of moderately declining stands of 35-year-old Norway spruce (*Picea abies*) and 60-year-old beech (*Fagus sylvatica*). The liming treatment was carried out by helicopter in 1991 with 757 kg ha^{-1} of $CaCO_3$ and 380 kg ha^{-1} of $MgCO_3$, which was a relatively low dose compared to what is often applied in central Europe (Hüttl 1989; Kreutzer 1995).

The allocrisol (typic dystrochrept, USDA, 1999) is formed on sandstone. Fifteen years after the treatment, liming had restored tree health, mineral nutrition and vegetation diversity, had shifted humus type from moder to oligomull and had strongly enhanced earthworm colonisation (Renaud JP *et al.* com. pers). Liming also increased soil pH and divalent cations (Ca^{2+} and Mn^{2+}) concentration, whereas Al^{3+} concentration had decreased (Table 1). The concentration of exchangeable Mg^{2+} was higher in the beech limed plot than in the untreated one, whereas it remained unchanged in the spruce limed plot (Table 1).

Sampling and sample processing

The limed area is a mixed forest stand composed of spruce (*P. abies*), beech (*F. sylvatica*) and fir (*Abies alba*) monospecific patches. We chose, separately for spruce and beech, two pairs of plots of about 400 m^2, one plot in the limed area the other outside (untreated area), forming four plots: spruce untreated, spruce limed, beech untreated and beech limed. Plot couples were set in areas homogeneous in terms of topography, soil water flow, tree age and sylviculture.

To measure the effect of liming depending on the soil horizons, we collected three soil cores (8 cm diameter, 15 cm deep, 750 cm^3) in each plot, before leaf fall on October 9, 2006. The spatial arrangement of these soil cores was randomly arranged. Then, soil cores were sliced into three subsamples corresponding to the fragmented litter, humic and organo-mineral layers.

Each subsample was sieved separately through a 0.5 mm screen and then gently washed with tap water, and observed under stereomicroscope. Ectomycorrhizal morphotypes were first identified morphologically (Supplementary Table 1) using Agerer's (1987–1998) descriptions. For each collected morphotype, seven tips were frozen at −80 °C and used for confirming the morphotype identification by DNA sequencing of the ITS region (Gardes & Bruns 1993). All soil cores were stored at 4 °C.

To measure the effect of liming on the ECM community structure depending on soil depth, ECM tips of each species were exhaustively counted in each subsample of the three cores in each plot.

Molecular identification

The total genomic DNA of 7 pooled ECM tips stored at −80 °C for each morphotype was extracted with the Dneasy Plant Mini Kit (Qiagen SA, Courtaboeuf, France) following the manufacturer's instructions. Morphotypes were not molecularly identified for each subsample and plot, because of cases of double bands and unsuccessful amplifications (Table 2). Nevertheless, even if some morphotyping errors might have occurred, visually similar morphotypes usually belonged to the same fungal taxon according to DNA sequencing. The Internal Transcribed Spacer (ITS) region of the fungal nuclear rDNA was specifically amplified using the primer couples ITS1F/ITS4 (Invitrogen, Cergy Pontoise, France), or ITS1F/ITS2 (producing shorter DNA amplicons) for recalcitrant sequences. The PCR reactions were performed in a Gene Amp 9600 thermocycler (Perkin Elmer instruments, Norwalk, Conn.) in a mix composed of 2.5 μl of each primer, 20 μl of Eppendorf mastermix (Dutscher, Brumath, France) and 25 μl of milliQ water. The PCR reactions were done using the following parameters: initial denaturation at 94 °C for 3 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s and extension at 72 °C for 1 min, with a final extension at 72 °C for 10 min. Amplification success was checked in a 1 % agarose electrophoresis gel in 1 % TBE (Tris-Buffer-EDTA) stained with ethidium bromide

Table 1 – Chemical composition of the topsoil of the four sampling plots. Concentrations of C, N and P_2O_5 are given in g kg^{-1}

Tree host	Treatment	Code	N	C/N	C	pH	H^+	Al^{3+}	Ca^{2+}	Fe^{3+}	Mg^{2+}	Mn^{2+}	K^+	Na^+	P_2O_5
Spruce	Untreated	SU-A	4.89	21.10	103.00	4.24	1.44	8.10	0.80	0.14	0.44	0.18	0.41	0.05	0.17
	Limed	SL-A	4.83	19.90	95.90	4.27	1.00	6.74	0.93	0.10	0.44	0.24	0.39	0.04	0.19
Beech	Untreated	BU-B	4.28	15.80	67.60	4.02	0.84	8.20	0.20	0.11	0.22	0.14	0.35	0.03	0.23
	Limed	BL-B	3.07	17.50	53.70	4.53	0.80	5.22	1.57	0.02	0.47	0.43	0.29	0.03	0.20

The P_2O_5 was extracted following the Duchaufour method. Concentrations in H^+, Al^{3+}, Ca^{2+}, Fe^{3+}, Mg^{2+} and K^+ are given in cmol kg^{-1} and correspond to the exchangeable fraction of these cations.