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Modification of soil enzyme activities as a consequence of replacing meadows by pine plantations under temperate climate

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ABSTRACT

Changes in land use frequently modify the capacity of ecosystems to provide services. The purpose of this study was to investigate the effects of a specific land-use change, i.e. from meadows to pine plantations under temperate climate, on soil enzyme activities. To this aim, the variation of five key soil enzyme activities (dehydrogenase, β-glucosidase, arylsulphatase, acid phosphatase and urease) was evaluated in different sites located in the Urdaibai Reserve of the Biosphere (northern Spain). Lower values of dehydrogenase [effect size, computed as 100 × (1 − mean value from pine plantations/mean value from meadows), was 82.9%], β-glucosidase (52.9%) and urease (52.5%) activity were observed in soils from pine plantations versus meadows. Acid phosphatase and arylsulphatase activity showed a pattern of variation that was not dependent on land-use. The largest variation in enzyme activity values was due to changes at the small scale, not between the studied sites, an encouraging finding for the suitability of enzyme activities as bioindicators of the impact of land-use changes on soil functioning. Our results suggest that nutrient cycling (as reflected by the values of soil enzyme activities) might have been modified as a consequence of replacing meadows by pine plantations.

Introduction

Although the main purpose of deliberate land-use changes is generally to enhance the capacity of the land to support the human enterprise, instead, many land-use changes end up reducing such capacity. Actually, changes in land use can undermine the capacity of ecosystems to provide goods and services in the long term, even on regional and global scales (Foley et al. 2005). In this respect, the capacity of soils to recycle nutrients is an ecosystem service essential for maintaining fertility in terrestrial ecosystems.

Microbial communities play a key role in many soil processes and the delivery of essential ecosystem services. Soil microorganisms are, to a great extent, responsible for soil fertility owing to their involvement in the cycling of nutrients required for plant growth. Indeed, microbial communities have a critical role in regulating soil ecosystem processes, like decomposition of the organic matter entering the soil, and therefore in soil nutrient cycling. Microbial communities can be considered as a dynamic biological property of the soil ecosystem which most frequently shifts in response to land use and management and can provide a direct measure of soil functioning (Garbisu et al. 2011). Most relevantly, it has been explicitly acknowledged (Acosta-Martínez et al. 2008) that differences in soil enzyme activities among different ecosystems can be reflected in the soil's functional integrity and associated ecosystems services, and then should be taken into account when changing land uses to, for instance, less conservative practices in a given region.

Among the land-use changes with global consequences, highly managed tree plantations are replacing forested and non-forested ecosystems worldwide. In particular, in the Basque Country (northern Spain), pine trees (*Pinus radiata*) have been extensively planted in the last decades as timber trees. Actually, a vast reforestation program was put into practice in the Basque Country: especially during the second half of the 20th century, the Atlantic Basque Country became one of the most active regions in Europe regarding the plantation of fast-growing tree species. Nowadays, close to 85% of the low mountains in the Atlantic Basque Country are covered by forest ecosystems, and ca. 50% of this area is occupied by *P. radiata* plantations. On the other hand, the surface occupied by agrarian land-uses in the Basque Country represents a small fraction (ca. 31% of the total surface): among these, Atlantic meadows are one of the most important uses (16.8% of the total).

We hypothesized that the abovementioned land-use change may have affected soil enzyme activities and, hence, the capacity of soils to recycle nutrients. Therefore, the aim of this study was to investigate the effects of a specific land-use change, i.e. from meadows to pine plantations under temperate climate, on soil enzyme activities. For this purpose, the variation of five key soil enzyme activities (dehydrogenase, β-glucosidase, arylsulphatase,

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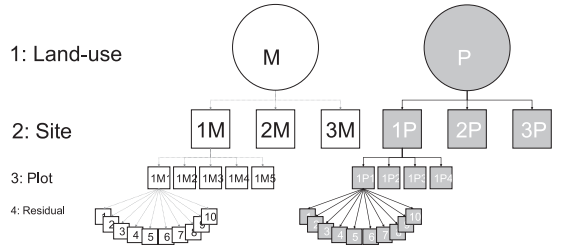

Fig. 1. Schematic showing the three main factors included in the hierarchical mixed effects design: (1) *land-use*, a fixed-effects factor with two levels explicitly stated: meadows (M) and pine plantations (P); (2) *site*, nested in land-use, a first random-effects factor with three levels: three sites for M and three additional sites for P; and (3) *plot*, nested in site, a second random-effects factor with five levels in M and four levels in P. In all plots, 10 soil samples were randomly taken and analyzed. For clarity, the arrangement of samples is only displayed for plot 1 in site 1 under both land-uses, but the same arrangement applies for the remaining combinations of sites and plots.

acid phosphatase and urease) was evaluated in different sites located in the Urdaibai Reserve of the Biosphere (Basque Country).

Materials and methods

The study was carried out in a set of Monterrey pine (*Pinus radiata*) plantations and seminatural managed meadows located in the Urdaibai Reserve of the Biosphere – URB (Basque Country), an area characterized by a temperate humid climate. Although, in the past, the landscape in this ecoregion included large extensions of diverse mixed forests, much of the original wilderness has been replaced by a fragmented landscape, which shows a variety of patches under different management practices combining agriculture, forestry and cattle grazing. During the last decades, profound socioeconomic changes and land abandonment in the Basque Country have resulted in massive modifications of its landscape. The same pattern holds true for the URB, where tree plantations are clearly dominant: 55.6% of the 21,941 ha present in the URB are covered by plantations of *P. radiata* and other minor species; in turn, smaller patches of Atlantic meadows cover 22.3% of the URB.

In order to investigate the variation of soil enzyme activities in both meadows and pine plantations of the URB, we used a mixed model (Fig. 1) combining one fixed-effects factor with two hierarchically nested, random-effects factors (Anderson et al. 2008). Land-use was considered as a fixed factor, with two explicit levels: meadows and pine plantations. Site was the first random factor (nested in land-use): three different sites per land-use were studied. Plot was the second random factor (nested in site): four (pine plantations) or five (meadows) plots per site were sampled in the field. Finally, at each plot, 10 randomly located soil samples were taken for analysis of enzyme activities. Within-plot variation was used as estimate of residual variation. Experimental sites, dispersed through the URB, were separated by several kilometers; plots (within sites) were hundreds of meters apart, and soil samples (within plots) were tens of meters apart.

In the Atlantic Basque Country, most of the areas currently covered by fast-growing, exotic tree species (e.g., *P. radiata*) were before covered by agrarian uses. In order to assure that the specific pine plantation plots studied here had previously been occupied by meadows, we asked plot owners for information on previous uses: in a large 75% of the pine plantations, we were guaranteed that plots had previously been occupied by seminatural managed meadows (no information was available regarding the remaining 25%). Then, we assumed that possible differences in soil enzyme activities between meadows and pine plantations would be caused by the abovementioned change in land-use (meadow to pine plantation).

Soil sampling and analysis

Soil samples were taken from 0 to 10 cm depth with a 3 cm diameter auger (the uppermost part of the soil contains the majority of soil microbial activity) (Taylor et al. 2002). For the analysis of enzyme activities related to the main biogeochemical cycles (i.e., carbon, nitrogen, phosphorus and sulphur cycles), soils were sieved to <2 mm and stored fresh at 4 °C until analysis (samples were analyzed within two months after sampling). For β-glucosidase, arylsulphatase and acid phosphatase activity, the methods developed by Dick et al. (1996) and Taylor et al. (2002) were used according to the following protocol: 1 g dry weight (DW) soil was mixed with 1.6 ml of buffer (20 mM modified universal buffer-MUB, pH 6.0, for β-glucosidase; 500 mM acetate buffer, pH 5.8, for arylsulphatase; 20 mM MUB, pH 6.5, for acid phosphatase) and 0.4 ml of substrate [4-nitrophenyl-β-D-glucopyranoside (1.5%, w/v) for β-glucosidase; potassium 4-nitrophenyl sulphate (1.3%, w/v) for arylsulphatase; 4-nitrophenyl phosphate disodium salt (1.85%, w/v) for acid phosphatase]. The mixture was incubated at 37 °C for 45 min and the reaction stopped with 0.4 ml of 500 mM $CaCl_2$ and 1.6 ml of 500 mM NaOH. After centrifugation (3500 × *g*, 3 min), the absorbance value of the samples was read at 410 nm.

For urease activity (Kandeler and Gerber 1988), 1 g DW soil was mixed with 1.75 ml of 100 mM borate buffer (pH 10.0) and 0.25 ml of 820 mM urea. The mixture was then incubated at 37 °C for 1 h and the reaction stopped with 6 ml of acidified 2 M KCl. After centrifugation (3500 × *g*, 3 min), 0.25 ml of the supernatant fraction was mixed with 3.75 ml of distilled water and 2 ml of a reagent composed of a sodium salicylate/sodium nitroprusiate mixture (17%, w/v and 0.12%, w/v, respectively), 0.3 M NaOH, and distilled water (1:1:1, v/v/v). Finally, 0.8 ml of sodium dichloroisocyanurate was added to the reaction mixture. After 30 min, the absorbance value of the samples was read at 670 nm.

Dehydrogenase activity was determined following a modification of Von Mersi and Schiner (1991): 1 g (wet weight) soil was mixed with 0.4 ml of 100 mmol l^{-1} Tris(hydroxymethyl) aminomethane buffer (pH 7.0) and 0.4 ml of iodonitrotetrazolium chloride-INT (0.5%, w/v). The mixture was incubated at 25 °C for 4 h, and the reaction was stopped with 8 mL of methanol. After centrifugation (1250 × *g*, 5 min), the absorbance value of the samples was read at 490 nm.

For physico-chemical analysis, the soil was air-dried at 30 °C for 48 h, sieved to <2 mm and stored at room temperature. Soil physico-chemical properties (Table 1) were determined following standard methods (MAPA 1994).

Statistical analyses

We applied multivariate methods to analyze the whole matrix of data (5 enzyme activities × 270 soil samples). First, in order to explore quantitative relationships between soil enzyme activities, we applied a Principal Component Analysis (PCA) to the correlation matrix (Ramette 2007). Subsequently, we displayed values of enzyme activities on the ordination diagram defined by the first two axes of the PCA, and fitted smoothed, local regression curves [locally (weighted) polynomial regression] (Zuur et al. 2007) to explore the main patterns of variation. Multivariate ordinations and related analyses were done by running Canoco for Windows 4.5 (ter Braak and Šmilauer 2002).

In order to analyze possible differences in enzyme activities due to the factors included in the design, we applied a Permutational Analysis of Variance (PERMANOVA). To assure the matching between the PERMANOVA and the PCA, variables were normalized before the analysis and Euclidean distance was used as a measure of resemblance. Land-use (2 levels) was introduced as a fixed factor, whereas both site (3 levels; nested in land-use) and plot (4–5