

## Root-surface phosphatase activity in shrublands across a European gradient: Effects of warming

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**Abstract:** Root-surface phosphatase activities were measured in natural and semi-natural shrublands across an European climatic gradient of temperature and rainfall including Wales (WL), Denmark (DK), Netherlands (NL), Hungary (HU), Italy (IT) and Spain (SP). In each site a warming experiment was conducted since 1999 or 2001 by means of passive night-time warming using reflective curtains that covered the vegetation at night. The treatments increased yearly average soil temperatures around 0.8°C in most of sites. Root-surface phosphatase activity values ranged between 56 mg PNP g<sup>-1</sup> h<sup>-1</sup> in IT and 3.5 mg PNP g<sup>-1</sup> h<sup>-1</sup> in HU. Warming had no effect on root-surface phosphatase activity across the sites and only in Hungary a slight increase was detected. Plants at Mediterranean sites (IT, SP) showed a higher root-surface phosphatase activity than plants at temperate sites (WL, NL, DK). We suggest it might be an adaptation of plant species evolved under Mediterranean climate that allows them a) to compensate in wet period for the decrease in phosphatase activity, and thus P uptake, during drought periods, and/or b) to benefit from soluble organic P flushes following the frequent drying-rewetting episodes experienced by soils in Mediterranean ecosystems.

**Key words:** Root-surface phosphatase, Climate change, Drought, Warming, Shrublands  
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### Introduction

Global climate is expected to warm 1.4 to 5.8°C over this century, with the greatest increases expected at northern latitudes (IPCC, 2001). A warmer climate may affect several biogeochemical processes due to effects on plant growth and microbial activity which may eventually lead to a change in the plant and microbial demand for nutrients such as phosphorus (P). P is the key limiting macronutrient for plant growth in many ecosystems (Raghothama, 1999) and its fate under climate change may determine whole ecosystem response to future climatic conditions.

Experimentation has shown that warming stimulates aboveground plant productivity (Rustad *et al.*, 2001). The positive effects to warming are stronger in colder ecosystems (Rustad *et al.*, 2001) where temperature is the most limiting factor for plant growth (Kömer and Larcher, 1988). Warming can also stimulate rate of soil microbial activity as reflected in a 46% increase in net N mineralization rates in a metaanalysis of experimental results (Rustad *et al.*, 2001).

If warming increases plant growth, it will also increase plant nutrient demands, whereas possible interferences with leaf senescence may reduce nutrient resorption (Gusewell, 2004). If warming increases microbial activity it will also increase microbial nutrient demands, which will reduce the nutrient supply to plants under nutrient-limiting conditions (Raghothama, 1999). Consequently, P may become scarcer for plant demands.

The form of phosphorus preferentially assimilated by plants is the orthophosphate anion (Duff *et al.*, 1994). In most soils, the concentration of available inorganic P in soil solution is approximately 2 µM, which is several orders of magnitude lower than that in plant tissues (5-20 mM) (Raghothama, 1999). Because of the low weathering rates of P-containing minerals, the main source of P to sustain plant and microorganisms growth in natural ecosystems is the mineralization of organic P (Schlesinger, 1991; Ravikumar *et al.*, 2007). Most of the soluble P in soils is in organic forms that may represent as much as more than 90% of water soluble P (Turner



and Haygart, 2001). Plant roots and microorganisms secrete phosphatases which release inorganic P by hydrolysis of ester bonds between organic carbon and phosphorus. Some of the phosphatases produced by roots remain bound to root surface (Sahu *et al.*, 2007).

Plants respond to P deficiency by morphological changes of the roots as well as several responses at molecular, biochemical and physiological levels like, for example, activation of phosphatase genes as well as enhanced production and external secretion of phosphatases (Raghothama, 1999). The production of phosphatases, both extracellular and intracellular, in response to inorganic P deficiency is an integral part of plant response to inorganic P deficiency (Duff *et al.*, 1994; Raghothama, 1999). Induction of phosphatase secretion by inorganic P deficiency has been documented in suspension cell cultures and roots and proteoid roots (Bozzo *et al.*, 2002). Besides nutritional inorganic P deficiency, phosphatase synthesis is also induced by water deficiency and salinity stress (Duff *et al.*, 1994). Addition of organic P also stimulates the acid phosphatase activity in roots (Tarafdar and Claassen, 2003). The possible effects of warming, decreasing P availability and the phosphatase activity induction by P deficiency, may conduct to warming-driven increases in phosphatase activity.

We aimed to study warming effects on root-surface phosphatase activity in plants from six different shrubland ecosystems across a European gradient of temperature and water availability. We used a set of climate experiments at all six sites involving temperature manipulation that have been running mostly since 1999 (Beier *et al.*, 2004). In a previous work it was found that moderate warming tended to decrease the foliar P concentration in four of the sites with significant decreases in *Deschampsia flexuosa* in DK and *Globularia alypum* in SP (Penuelas *et al.*, 2004), indicating less soil potential to supply P to plants. We aimed to study whether root-surface phosphatase may respond to warming, which could be related to the mentioned decreases in foliar P. As far as we know only a few experiments have actually studied the effects of warming on root-surface phosphatase. We also aimed to reveal trends across the European climatic gradient comparing ecosystems with contrasting temperature and soil moisture regimes, a possibility, as far as we know, not yet explored in literature.

### Materials and Methods

The study was carried out at the experimental sites of the EU project Vulcan located in Denmark (DK), Netherlands (NL), Wales (WL, United Kingdom), Italy (IT), Hungary (HU) and Spain (SP). In each site, six 20 m<sup>2</sup> plots of local shrubland were selected and three plots assigned to warming while three plots served as untreated controls (Table 1). The treatments were initiated in 1999 in four of the sites (WL, DK, NL, and SP) and in 2001 in the other two (IT and HU) and have been continuously working since those dates. Details on experimental system and sites are described in Beier *et al.* (2004) and in Table 1.

The warming treatment was performed as passive night-time warming by reflective aluminium curtains covering the vegetation

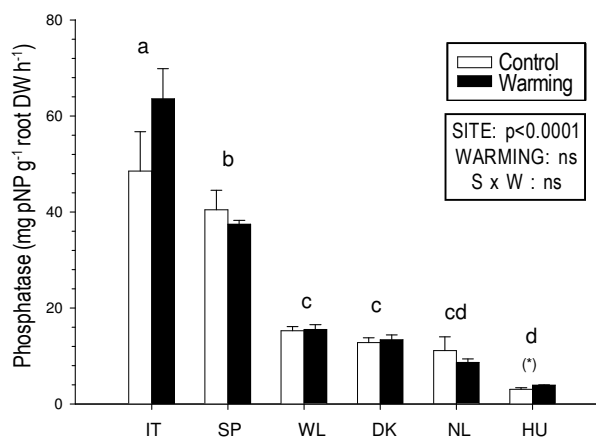
at night (Beier *et al.*, 2004). Solar energy is accumulated in the ecosystem during the day and a fraction of the energy is re-radiated back to the atmosphere at night as long wave IR-radiation. The covering of the ecosystem with the reflective curtains reduces the loss of IR radiation. In order to avoid influencing the hydrological cycle, the covers are automatically removed in case of rain. The warming plots consisted of 20 m<sup>2</sup> of shrubland area covered by a light scaffolding carrying the reflective aluminium curtain. The reflective curtains were coiled on a beam supported by a scaffolding and connected to a motor. The motor was activated automatically according to preset climatic conditions (*eg.* curtains were removed automatically if the wind speed exceeded 10 m s<sup>-1</sup>). Temperature increase is not constant through the year and is also variable between days, for example covering the plots with roofs during cloudy nights has no effect on T. The effect of warming treatment is quantified as the difference between warming and control annual average temperatures since the treatment started in 1999 or 2001 depending on the site. Warming plots had annual average temperatures 0.8°C warmer than control across all the sites, excepting IT where the warming effect was lower (Table 1).

In spring 2004 three soil cores within the main rooting depth for each site were sampled in each plot at a distance of 15 cm of the selected plants, to ensure identity of roots. HU samples were obtained in late spring as reflected by higher soil T at sampling in Table 1. Sampled plant species were the dominant plants in the different sites: *Calluna vulgaris* in NL, a mixture of *C. vulgaris*, *Empetrum nigrum* and *Vaccinium myrtillus* in WL, *Deschampsia flexuosa* in DK, *Globularia alypum* in SP, *Cistus monspeliensis* in IT and *Populus alba* (shrubby *Populus* resprouts) in HU. Species differ among sites because the climatic gradient is wider than the climatic envelope for a single species. At every location characteristic shrubby vegetation was selected. In DK *D. flexuosa* was chosen because a beetle attack strongly damaged *C. vulgaris* plants. Fine roots (diam. ≤ 1 mm) were picked either after sieving soil at 2 mm, or directly with forceps when in organic layers.

Collected roots were rinsed with water, excess water was removed, and 50-100 mg of fresh roots were sampled. Roots were immersed in 10 ml of 0.1M citric acid buffer adjusted to the soil pH of each site (Table 1). Phosphomonoesterase activity was measured by the hydrolysis of p-nitrophenylphosphate (p-NPP, Sigma-Aldrich) to p-nitrophenol (p-NP, Sigma-Aldrich) as described by Tabatabai and Bremner (1969) and actualised by Kroehler and Linkins (1988). 0.1 µl of p-NPP stock solution was added to the 10 ml of buffer, to yield a 4mM solution of p-NPP, and incubated at 37°C for 60 minutes. The reaction was stopped by adding 0.1 ml of a terminating solution (0.1 M Tris made to pH 12 by adding 0.1 M NaOH). Absorbance was read at 410 nm and p-NP production was obtained with a standard curve obtained using pure p-NP (Sigma-Aldrich). After the assay, root samples were oven dried at 65°C until constant weight. Root phosphatase activity was expressed as µg p-NP per g root dry weight per hour.

**Table 1:** Average values for climatic, soil and plant variables for each site of the studied European gradient. Climatic data were obtained from the period 1998-2005 for WL, NL, DK and SP and for the period 2001-2005 in IT and HU. Average annual air temperature (°C), average annual warming treatment effect (°C) in soil at 5 cm depth resulting from the difference between warming and control average annual temperatures, average soil T (°C) for the seven days prior to sampling date in spring 2004, average annual precipitation (mm), soil type, sampling depth (cm), soil pH, and soil organic matter (SOM, %), aboveground biomass (g plant m<sup>-2</sup>) of the shrublands in summer 2004 and aboveground net primary productivity (ANPP, g plant m<sup>-2</sup> y<sup>-1</sup>) of the shrublands in 2004

	WL	DK	NL	HU	IT	SP
Average air T	8.7	8.7	10.1	10.8	15.6	15.5
Annual warming effect	0.7	1.0	0.6	0.9	0.12	0.95
Soil T at sampling	10.9	10.8	10.2	20.6	12.6	10.7
Precipitation	1126	718	848	509	511	580
Sampling depth	0-10	0-7	+4-0	0-20	0-10	0-12
Soil type	Peaty podzol	Sandy podzol	Sandy podzol	Calcaric arenosol	Luvi and litosol	Petrocalcic calcixercept
pH	3.9	3.7	3.7	7.9	7.7	8.1
% SOM	89.0	8.3	65.0	0.74	7.8	3.5
Aboveground biomass	3546	998	557	341	439	643
ANPP	201	293	185	86	112	161



**Fig. 1:** Root-surface phosphatase activity in spring 2004 in the six studied sites, (\*) p<0.1 between treatments. Different letters indicate significant differences between sites (p<0.05). P value for SITE and WARMING and its interaction (S x W) included.

Soil organic matter (SOM) content was estimated by loss on ignition at 375°C for the WL, NL and DK soils, while for the HU, SP and IT soils SOM was estimated from organic carbon (OC) analysed by dichromate oxidation using the Van Bemmelen’s conversion factor of 58% OC content in OM (Nelson and Sommers, 1982).

Comparison of root surface phosphatase activity was performed by a two way ANOVA using treatment and sites as factors with post-hoc Fisher’s PLSD. Comparisons within sites were performed by t-test comparing control with warming.

### Results and Discussion

Root-surface phosphatase activity was dependent on site (Fig. 1), with highest values in IT (56.1 mg PNP g<sup>-1</sup> h<sup>-1</sup>) and lowest values in HU (3.5 mg PNP g<sup>-1</sup> h<sup>-1</sup>). Root-surface phosphatase was higher for plants growing in warmer Mediterranean sites and lower in more northern and colder ecosystems (Fig. 1, Table 1). This result could partially reflect a positive effect of temperature on root-

surface phosphatase activity. However, this was not the case in our experiments because warming treatment did not affect the enzyme activity considering all the sites together. Neither did the warming treatment affect enzyme activity for any particular site, except for a marginal increase in HU (p≤0.076) (Fig. 1). The lack of effect of warming in *G. alypum* in the same experiment has been confirmed by Sardans *et al.* (2006) through all the seasons of a growth cycle. Similarly, a lack of warming effect has also been reported by DeLucia *et al.* (1997) in *Pinus ponderosa*.

Contrary to our working hypothesis, the trend to lower leaf P concentration under warming detected in a previous study in some of the experiments (Peñuelas *et al.*, 2004), specially in *G. alypum* in SP and *D. flexuosa* in DK, was not reflected in root-surface phosphatase activity. In fact, results from later in the period of experimentation, in January 2005, did not show any effect of warming in P concentration in leaves, stems and roots of *G. alypum* (Sardans *et al.*, 2006), indicating interannual variability in warming effects. Warming induced a slight positive effect in net primary productivity (16% in WL and 15% in NL) (Peñuelas *et al.*, 2004), in agreement with predictions of positive effects on plant growth in northern ecosystems (Rustad *et al.*, 2001), but the assumed higher P demands by these ecosystems were not reflected in root-surface phosphatase activity. Higher P demands for growth without increased root-surface phosphatase activity would fit well with lower foliar P concentrations, but at the light of the interannual variability found in *G. alypum* (Peñuelas *et al.*, 2004; Sardans *et al.*, 2006), the effects at leaf level need further study to be confirmed.

The temperatures during the week previous to measurements were within a narrow range (10-12.5°C) in all the sites except for HU that was measured later in the growth period (20°C) (Table 1). Thus, differences among sites do not reflect differences among current temperatures, but among yearly average temperatures, indicating an effect of climatic region rather than an effect of current temperature with possible consequences under warming. Besides differences in temperature between



Mediterranean and temperate climates, Mediterranean climate is characterised by low and irregular rains and a high evaporative demand leading to prolonged periods of low soil moisture in summer months and alternation of dry-wet periods in spring and fall.

In SP, one of the Mediterranean sites, a strong between seasons variability in the activity of the enzyme has been reported (Sardans *et al.*, 2006). Sardans *et al.*, (2006) showed higher root-surface phosphatase activities in autumn and spring, when temperature and soil water were optimum for plant and soil activity, and lower in summer due to dry soil conditions, in winter due to low temperatures and in the dry spring 2005. They also showed negative effects of experimental drought in root-surface phosphatase activity, highlighting the importance of soil water availability for plant and soil activity in this ecosystem (Sardans *et al.*, 2006; Emmett *et al.*, 2004). In addition, a positive relation between root water content and root-surface phosphatase activity was also described for *G. alypum* (Sardans *et al.*, 2006).

In parallel to the warming experiment described up to now, we have been running a drought experiment with similar plots, that showed that drought reduced leaf P concentration (Peñuelas *et al.*, 2004). This reduction indicates a decrease in P uptake by plants, a possibility that is supported by the accumulation of total P in soils under drought treatments in the SP site (Sardans *et al.*, 2006). The higher root-surface phosphatase activities in the water limited Mediterranean sites (SP and IT) measured in spring, with non-dry soils and relatively warm temperature, can be an adaptation to compensate the low activity during dry periods. Lower root-surface phosphatase activities under drought is a possible cause for this decrease in P uptake by plants. Thus, we suggest that higher activity in Mediterranean plants could be an adaptation to shorter optimal periods for root-surface phosphatase activities caused by dry climate.

There is another possible explanation for higher root-surface phosphatase activities in Mediterranean sites related with the dynamics of soil organic P in such ecosystems. Due to more frequent rains and/or less evapotranspiratory demand, soils of the northern locations do not experience the same high frequency of drying and rewetting cycles as the Mediterranean soils. Soil drying and rewetting releases organic P to soil solutions (Turner and Haygart, 2001) and leachates (Turner and Haygart, 2000; Turner *et al.*, 2003). Lysis of bacterial cells is the source of most of this organic P (Kieft *et al.*, 1987; Turner and Haygart, 2001) although release of occluded organic matter after aggregates destruction may also contribute. At least around a 90% of the increase in water-extractable phosphorus following soil drying comes from lysed bacterial cells (Turner *et al.*, 2003) of which a 30% is inorganic orthophosphate whereas the remaining fraction includes mainly organic P (Turner *et al.*, 2003).

Several reports describe that drying and rewetting stimulate microbial activity and mineralization of C and N (Haynes, 1986) and of P (Chepkwony *et al.*, 2001). Olsen and Court (1982), found that 11 drying and rewetting cycles increased resin extractable P up to 37%, and the biggest increases were recorded in soils with the

highest organic matter contents. Turner and Haygart (2001) reported that drying of moist soils before extraction increased the water soluble P by 185-1900%. Due to the mentioned frequent succession of drying-rewetting episodes in soils of Mediterranean ecosystems in some periods of the year, frequent pulses of soluble organic P occur in soils of these ecosystems, and mainly during the growth season of spring when stronger nutrient demand by plants occurs. Drying-rewetting episodes are less frequent in soils of more temperate ecosystems.

We hypothesize differences in root-surface phosphatase activity between Mediterranean and temperate ecosystem plant species could also be a consequence of differences in soil water regime that drive availability of soil soluble organic P content. We suggest that plants evolved under Mediterranean soil water regime have a high root-surface phosphatase activity that maximizes P supply at root surface during short wet periods among dry periods, given that drying-rewetting events promote the release of organic P to soil solution.

In summary, warming around 0.8°C had no effect on root-surface phosphatase activity in a set of European shrubland ecosystems. However, climate effects on this enzyme activity may be inferred from higher activities in Mediterranean vs. temperate ecosystems. The higher root-surface phosphatase activity for Mediterranean plants allow them to compensate during wet and relatively warm seasons the decrease in activity during dry months. Higher root-surface phosphatase activity for Mediterranean plants can be considered an adaptation to maximize root-surface phosphatase activity, and thus P uptake, during short wet periods optimal for root-surface phosphatase activities. On the other hand, frequent drying and rewetting cycles in Mediterranean soils lead to frequent pulses of soluble organic P and high root-surface phosphatase activity in Mediterranean plant species allow them to be benefited from such organic P pulses.

However, further studies including more similar set of species across the gradient are necessary to confirm the hypothesis and to draw stronger conclusions.

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