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Molybdenum and phosphorus limitation of asymbiotic nitrogen fixation in forests of Eastern Canada: Influence of vegetative cover and seasonal variability

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ABSTRACT

The limitation of asymbiotic dinitrogen (N_2) fixation by phosphorus (P) is well-documented. Studies on Mo limitation of asymbiotic N_2 fixation are, however, scarce. To what extent Mo limits asymbiotic N_2 fixation on the global scale is still unclear and the mechanisms controlling the emergence of Mo limitation remain elusive. The aim of this work was to investigate the effect of nutrient additions (P, Mo and P+Mo) on asymbiotic N_2 fixation activity in leaf litters from Eastern Canadian forests (cold temperate). We specifically tested how different vegetative covers (deciduous versus coniferous) respond to nutrient additions. We also evaluated on one site (coniferous litters) if nutrient (Mo, P) limitation change during the growing season. We report that the vegetative cover has a strong influence on the emergence of Mo and P limitations; while many sites under coniferous cover responded to different combinations of nutrient addition, none of the sites under deciduous cover responded to any nutrient addition. We also observed that nutrient limitation changed during the growing season; asymbiotic N_2 fixation in coniferous litters was limited by P in the early stage of the growing season, by Mo but not P in mid-season and in late season neither P nor Mo were limiting. This Seasonality in nutrient limitation might be an important factor affecting the biological N input in temperate and boreal ecosystems that remain to be fully described. Our results are discussed in the context of litter decomposition and nutrients cycling.

1. Introduction

Dinitrogen (N_2) fixation is one of the most important reactions of the biosphere. It is the major point of entry of new N to natural ecosystems (Cleveland et al., 1999; Galloway et al., 2004). This reaction is catalysed by the enzyme nitrogenase in few prokaryotes (Vitousek and Howarth, 1991). The most common form of the nitrogenase requires molybdenum (Mo) as a metal cofactor (Williams and Frausto da Silva, 2002). In terrestrial ecosystems, the contribution of asymbiotic N_2 fixation (ANF) and lichens to global N input has long been overshadowed by symbiotic N_2 fixation with legumes and actinorhizal plants. In recent years, ANF has gained interest in the scientific community and important efforts have been made to better integrate this N source into conceptual models of the N cycle (Reed et al., 2011). For instance, DeLuca et al. (2002) showed that N_2 fixation by feather mosses can account for up to 2/3 of the annual N input in boreal forests. However, our understanding of the parameters controlling ANF is still incomplete. It is well established that N_2 fixation is limited by the availability of phosphorus (P) (Vitousek and Howarth, 1991; Vitousek and Farrington, 1997; Mills et al., 2004). This is particularly true in tropical ecosystems where highly weathered soils are depleted in rock-derived resources (i.e. P). The availability of the micronutrient molybdenum (Mo) is well documented to limit N_2 fixation in agricultural systems (Hafner et al., 1992; Gupta, 1997; Srivastava et al., 1998; Vieira et al., 1998a, 1998b, 1998c). In unmanaged ecosystems, evidence for the limitation of N_2 fixation by Mo are scarce: Mo has been found to limit ANF in the coastal temperate forest of northwestern USA (Silvester, 1989). Mo has also been reported to limit ANF in cyanobacteria within lichen symbioses (Horstmann et al., 1982), in lakes (Glass et al., 2012; Romero et al., 2013) and in a tropical forest from Panama (Barron et al., 2009), the latter of which may also be co-limited by Mo and P (Wurzberger et al., 2012). However, to date our understanding of the mechanisms leading to Mo limitation of ANF remains sparse.

The question of the limitation of ANF by Mo is particularly acute in high latitude ecosystems. Cold temperate and boreal ecosystems are believed to be preferentially limited by N and ANF and lichens

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are critical components to the total N input (Wang et al., 2010). Contrary to tropical soils which are subject to intensive weathering, soils from cold temperate and boreal regions have been rejuvenated by the succession of the glacial eras. As a result, rock-derived nutrients, such as P and Mo, are expected to be less limiting in these soils than in tropical ones. However, few reports suggest that Mo limitation of ANF and lichens could occur in cold temperate and boreal ecosystems (Horstmann et al., 1982; Silvester, 1989). The mechanisms leading to this limitation are unclear. Mo and P are both present in soil solution under oxo-anionic forms (PO_4^{3-} and MoO_4^{2-} respectively) and thus sensitive to leaching. Their availability is reduced by sorption on the soil matrix (organic matter and oxides) especially at high pH. It is also important to bear in mind that Mo is the least abundant biometal in earth crust (Wedepohl, 1995). More research is required to determine the extent of Mo and P limitation of ANF in cold temperate and boreal ecosystems.

In the context of the leaf litter, the chemical composition of the litter likely influences the dynamics of P and Mo (total content, availability) and thus N_2 fixation. The role of organic matter on Mo speciation in top soil has recently been highlighted by Wichard et al. (2009). The composition of litter is the result of complex interactions between the vegetative cover and soil. Molybdenum and P limitation could emerge as a function of vegetative cover composition, the soil properties, or both. The importance of soil properties has been highlighted in tropical forests by Wurzberger et al. (2012). The influence of vegetative cover on the development of Mo and P limitation of ANF in the leaf litter remains mostly unexplored.

Another factor that could determine Mo and P limitation in cold temperate and boreal ecosystems is seasonality. In contrast to tropical forests in which the litter is continuously produced and degraded, in temperate and boreal ecosystems biological activity, litter deposition and nutrient cycling are strongly impacted by the seasons. Thus, it is not excluded that Mo and P limitations of ANF present a temporal dimension that has not been fully explored so far.

Here we studied the effect of Mo and P addition on ANF in leaf litters collected from cold temperate forests in Eastern Canada (Quebec). We selected 5 sites in which both coniferous and deciduous covers were sampled (Scheme 1). Our aim was to evaluate whether limitation of ANF by Mo and P depended on landscape-scale variation in vegetative cover. For each site and vegetative cover we monitored the response of ANF to four treatments; control, Mo only (+Mo), P only (+P) and both Mo and P combined (+Mo+P). In addition, we evaluated the variation in Mo and P limitation over the course of the growing season; on one selected site, we repeated the nutrient additions at various times of the growing season. Further analyses were performed on soils and leaf litters. We analysed total C, N, Mo and P contents, extractable Mo and P using resin beads extractions and pH in order to identify potential factors involved in the response of ANF to nutrient additions.

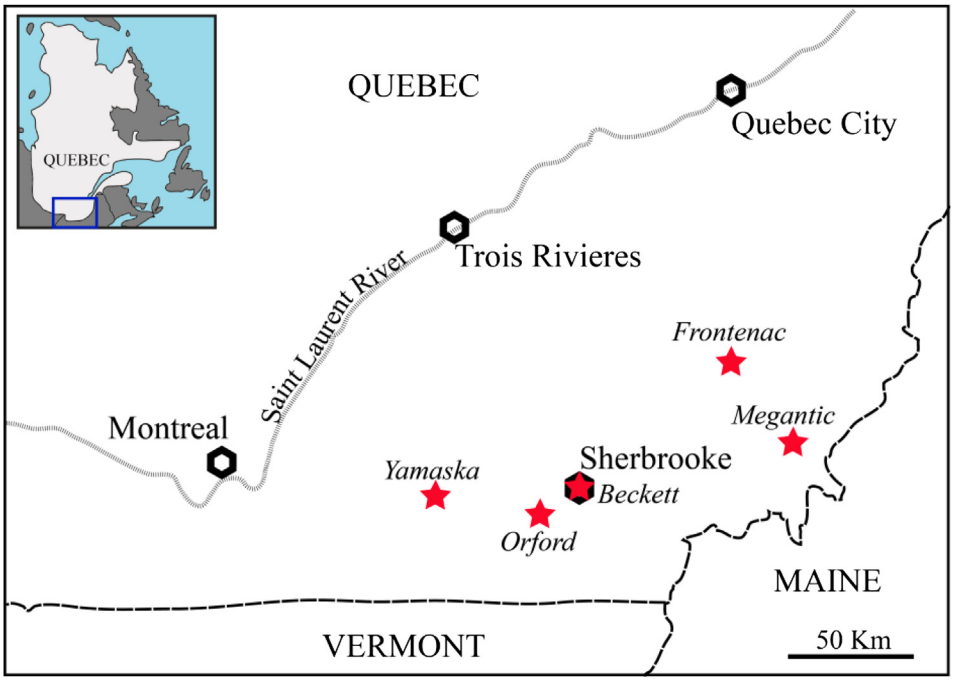
2. Materials and methods

2.1. Sites description and sampling

We collected leaf litter at 5 sites. Four were located in national parks managed by SEPAQ (Societe des Etablissements de Plein Air du Quebec): Mont Orford, Mont Megantic, park de la Yamaska and park de Frontenac. The fifth site (Bois Beckett) was located in Sherbrooke and is managed by the city. All these sites are located in the south of the St-Laurent River in the Estrie area (Scheme 1). This area is located between the southern limit of the Canadian shield and the northern edge of the Appalachian mountains. The climate is cold-temperate and continental humid. Soils are mostly podzolic and brunosolic and are representative of soils found in Eastern-Canada. Each site was divided in two sub-sites: one under deciduous cover (dominated by maples and birches) and the other under coniferous cover (dominated by black spruce and pines). The sub-sites were located close to each other (less than 3 km). Samples were collected between May and October in 2011 and 2012 (see Sup. Info Table S1 for details).

2.2. Nitrogen fixation assays

N_2 fixation activity was assayed by acetylene reduction assay (ARA) as previously described (Barron et al., 2009; Wurzberger



Scheme 1. Localization of the sites.