Short communication

Influence of pea root traits modulating soil bioavailable C and N effects upon ammonification activity

Nicolas Romillac [a, b], Séverine Piutti [a, b], Bernard Amiaud [c, d], Sophie Slezack-Deschaumes [a, b, *]

[a] Université de Lorraine, Laboratoire Agronomie et Environnement, UMR 1121, TSA 40602, F-54518 Vandœuvre Cedex, France
[b] INRA, Laboratoire Agronomie et Environnement, UMR 1121, TSA 40602, F-54518 Vandœuvre Cedex, France
[c] Université de Lorraine, UMR 1137, Ecologie et Ecophysiologie Forestière, F-54500 Vandoeuvre lès Nancy, France
[d] INRA, UMR 1137, Ecologie et Ecophysiologie Forestière, F-54280 Champenoux, France

ARTICLE INFO

ABSTRACT

Plant functional traits are useful tools for understanding plant impacts on soil nitrogen (N) mineralization. The objective of this study was to examine the root traits that govern the influence of *Pisum sativum* L. on potential protease and ammonification activities, which are two key microbial activities involved in N mineralization. Ammonification activity was greater during pea reproductive than vegetative stages, whereas potential protease activity did not vary along pea development. Ammonification activity was more strongly affected by root architecture traits (total root length and percentage of fine roots) than by root growth traits (root dry matter content). Pea root traits appear to affect ammonification activity in a complex manner involving variations in rhizodeposition that modulate carbon and N availability for soil microorganisms.

© 2015 Elsevier Ltd. All rights reserved.

Plants influence soil nitrogen (N) mineralization during their growth by controlling N uptake as well as the release of carbon (C) and N rhizodeposits to the soil (van der Krift and Berendse, 2001; Laungani and Knops, 2012). These rhizodeposits can be used as an energy source by soil microorganisms to produce enzymes implicated in soil organic N degradation, including protease and ammonification activities (Dijkstra et al., 2013; Bardgett et al., 2014). The subsequent increase in soil N mineralization may therefore have a positive effect on the resulting soil N availability. However, the release of these rhizodeposits may also improve net N immobilization, thus decreasing soil N availability (Orwin et al., 2010; Dijkstra et al., 2013). Therefore, identifying the mechanisms by which plants modify the microbial activities involved in N mineralization is important for better predicting how plant species alter soil N availability. Plant trait-based approaches may be helpful for understanding such mechanisms (Powell et al., 2013). In particular, the analysis of root traits may be a critical tool because roots are the key organs that drive nutrient acquisition, nutrient cycling and rhizodeposition (York et al., 2013; Bardgett et al., 2014).

The aim of our study was to identify the root traits that govern the potential influence of pea (*Pisum sativum* L.) on soil potential protease and ammonification activities. Pea was chosen because compared with other crops, this plant species releases readily mineralizable N and easily metabolizable C rhizodeposits that are assumed to have a positive effect on soil N mineralization (Evans et al., 1996; Mayer et al., 2003; Wichern et al., 2007a, 2007b). Therefore, according to the "economic rules" regulating microbial enzyme production (Allison and Vitousek, 2005), the elevated assimilable N resources in pea rhizodeposits would not require that microorganisms produce proteolytic enzymes to decompose proteinaceous organic N from soil organic matter. As opposed to vegetative stages, during pea reproductive stages, the relative excess in N (*i.e.*, amino acids) rather than C in the rhizodeposits would promote mineralization of amino acids via ammonification activities for their C content rather than their N (Farrell et al., 2013, 2014). Thus, we hypothesized that root traits modulating N rhizodeposition processes (Roumet et al., 2008) and soil C availability more strongly influence potential protease and ammonification activities than do traits related to root growth.

* Corresponding author. Université de Lorraine, Laboratoire Agronomie et Environnement, UMR 1121, TSA 40602, F-54518 Vandœuvre Cedex, France. Tel.: +33 3 83 59 58 96; fax: +33 3 83 59 57 99.
E-mail addresses: nicolas.romillac@univ-lorraine.fr (N. Romillac), severine.piutti@univ-lorraine.fr (S. Piutti), bernard.amiaud@univ-lorraine.fr (B. Amiaud), sophie.deschaumes@univ-lorraine.fr (S. Slezack-Deschaumes).
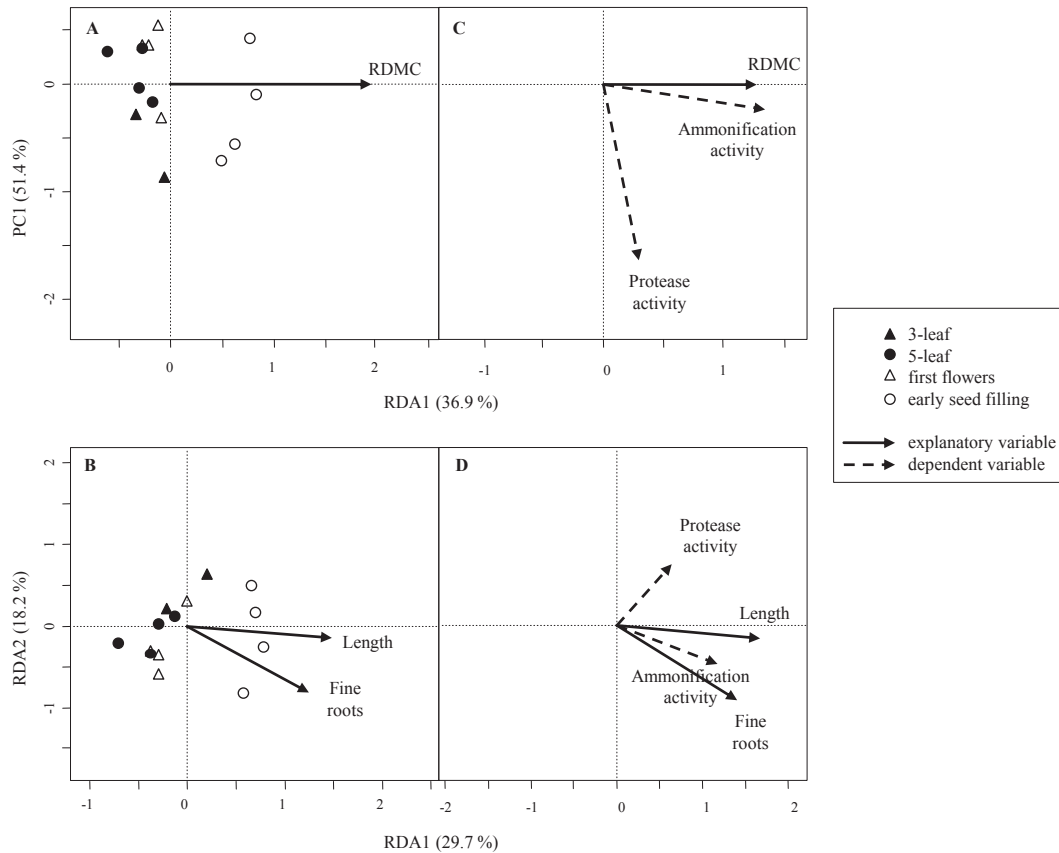
Fig. 1. Redundancy analysis of enzymatic activities in relation to the root growth related traits (A and C) and root architecture related traits (B and D). In RDA distance biplots (A and B), phenological stages are denoted for: 3-leaf with filled triangle (▲); 5-leaf with filled circle (●); first flowers with empty triangle (△); early seed filling with empty circles (○). In correlation biplots (C and D), solid lines are roots traits (explanatory variables), dashed lines are enzymatic activities (dependent variables). When only one explanatory variable was selected, RDA is constructed with only one constrained ordination axe (RDA 1), the second axe being an unconstrained residual axe (PC 1). The RDAs were significant at $P < 0.01$ and explained 32% (A and C) and 38% (B and D) of the variation.

A spring pea cultivar (*P. sativum* L., cv. Kayanne) was grown under controlled conditions (Supplementary information). Soil and roots were sampled (n = 4) at two vegetative (3-leaf and 5-leaf) and two reproductive (first flowers and early seed filling) stages. The dry masses of roots and aerial parts were measured. Root morphology was analyzed by digital scanning using WinR-HIZO (Arsenault et al., 1995). Four root architecture traits related to N interception and rhizodeposition were determined: total root length, specific root length (SRL), fine root length percentage and root mean diameter. In addition, seven root growth traits were estimated: root dry matter content (RDMC), root mass fraction (RMF), root tissue density (RTD), total root C and N contents, and water-soluble root C and N contents (Supplementary information, table 1). We determined the following soil characteristics: the total mineral N content in 2 M KCl extracts, soil N (HW-N) and C (HW-C) contents in hot water extracts, the microbial biomass C (MBC) and N (MBN), and the abundances of the total bacterial and protease communities using quantitative PCR and ammonification and protease activities (Supplementary information, table 1). Analyses were conducted using standard methods, which are outlined in the Supplementary information. Redundancy analyses (RDA) were performed to assess the relationship between protease and ammonification activities, and three sets of the most significant (after forward selection, Supplementary information, table 2, Legendre and Legendre, 1998) explanatory variables, *i.e.*, root architecture traits, root growth traits and soil variables. Then, variation partitioning was performed to quantify the relative contributions of the most significant explanatory variables to the

variations in ammonification and protease activities (Peres-Neto et al., 2006). These analyses were performed using the vegan 2.0-9 package (Oksanen et al., 2013) of R 3.0.0 (R Core Team, 2013).

The results showed potential protease activity did not change during pea development. It should be underlined that protease activities have been estimated by using the conventional method described by Ladd and Butler (1972), reflecting more potential activities rather than native activities. We cannot exclude that the use of isotope pool dilution techniques to determine native gross protein depolymerisation, such as those developed by Wanek et al. (2010), would have influenced the results. In contrast to protease activity, ammonification activity evolved as a function of pea phenology, with a significant increase at the beginning of seed filling ($H' = 9.5$, $p < 0.05$, Supplementary information, table 1). This result is consistent with a previous study where an increase of ammonification activity was observed between vegetative and reproductive stages of soybean (Yang et al., 2007). During pea reproductive stages, changes in ammonification activity may be associated with an increase in the C-to-N ratio of pea rhizodeposits that would favor C limitations for soil microorganisms (Kopáček et al., 2013). Mineralization of excess amino acids in pea rhizodeposits could then alleviate such soil C limitations (Jensen, 1996; Farrell et al., 2014). The significant decrease in soil HW-C/N ($H' = 12.90$, $p < 0.01$, Supplementary information, table 1) between the 5-leaf and flowering pea stages supports these findings. This statement is also reinforced by our observation of a significant negative correlation between ammonification activity and HW-C ($r = -0.73$, $p < 0.01$). Even if the arginine