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Research Paper

The role of lupin shoot P and Zn status in root allocation and nutrient uptake in soil with heterogeneous P and Zn distribution

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A B S T R A C T

Many plants have been found to show preferential root growth allocation to soil patches with locally increased nutrient availability. Little is known about the influence of the plants' nutrient status on these responses. Addressing this question, we conducted a climate chamber experiment to investigate how foliar phosphorus (P) and zinc (Zn) application (alone or in combination) would affect root allocation and nutrient uptake by white lupin (*Lupinus albus* L.) seedlings growing on soil with constructed heterogeneity in P and Zn distribution. The same soil packing was used in all pots. One quarter section of each packing was enriched with Zn, one with P, one with Zn and P, while neither Zn nor P was added to the fourth section (opposite to the section with the combined Zn and P amendment). Neither foliar P nor foliar Zn application had a fertilization effect on plant growth. The allocation of root length growth showed a clear preference for P-enriched soil sections, when no foliar fertilizer was applied. With foliar P or Zn application these preferences disappeared. Cluster root allocation and root P concentration showed a similar behavior. In the treatments with foliar Zn application, root length growth was also preferentially allocated in Zn-enriched soil, while without foliar Zn there was only a tendency for such a response. Root Zn concentrations were always much higher in soil sections with than without Zn amendment. The foliar treatments had no effect on root Zn concentration. While the responses to foliar P application can be understood in terms of an optimization strategy in root allocation for P foraging, the effects of foliar Zn application on root growth allocation cannot be interpreted in this way and require further investigation.

1. Introduction

Large spatial variability even over relatively small distances is a typical feature of soils under field conditions. Also the distribution of nutrients can be very heterogeneous, even at the scale of individual plant root systems (Bauerle et al., 2008; Hinsinger et al., 2009; Lynch et al., 2012; Pierret et al., 2007; Yano and Kume, 2005). Given that the metabolic costs in terms of assimilates required for the development and maintenance of their root systems are substantial, plants have to be efficient in allocating root growth and activity to find and acquire water and all the nutrients they need to take up from soil (Lynch et al., 2012; Robinson, 1994). This explains why many plants have been found to preferentially allocate more roots to nutrient rich than to nutrient poor patches in soils with heterogeneity in the distribution of nutrients such as phosphorus (P) (Hodge, 2004; Ma et al., 2016; Robinson, 1994, 1996). Some plants also respond with the enhanced formation of special root structures. The best example is the enhanced formation of cluster roots in response to local soil P enrichment in white lupin (Shane et al., 2003; Shen et al., 2005; Shu et al., 2007). However, in other cases, little or no response to patchy nutrient distribution at all was observed in root growth allocation (Hutchings et al., 2000; Robinson, 1994).

Soil heterogeneity is generally not limited to one factor only, and plants are typically facing situations in which they have to cope with incongruent heterogeneities in the availability of water and nutrients. Optimization of root growth allocation for their acquisition then requires a compromise between the responses that would be optimal for each individual resource in order to minimize the overall metabolic costs of capture, transport and prospecting efficiency for all of them together (Eshel and Beeckman, 2013). The root growth 'strategy' that underlies and governs these responses also has to account for the fact that soil conditions are encountered during further development are characterized by large uncertainty (Ruts et al., 2012; Walter et al., 2009). There are almost no studies in which root growth responses to heterogeneous distributions of several nutrients in soil have been investigated though. Ma et al. (2016) recently showed that

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preferential root length growth into P-enriched soil patches increased the uptake of zinc (Zn) in cucumber and wheat when the latter was also enriched in these patches and that decreased Zn uptake when the patches of Zn and P enrichment were disjunct. In contrast, they found no such heterogeneity effect on shoot Zn uptake in white lupin, although cluster root formation showed a positive response to P enrichment. In addition, none of the three plants showed a root growth response to the heterogeneity in soil Zn.

In line with the idea that it reflects an innate strategy aiming at optimizing assimilate allocation for the acquisition of an essential nutrient that often is a growth limiting factor, preferential root growth allocation to nutrient-enriched soil patches was found in various studies to depend on plant nutrient status (Forde and Lorenzo, 2001; Hutchings et al., 2000; Lynch et al., 2012). This also seems to hold for cluster root formation in white lupin. After Gilbert et al. (1997) had shown that cluster root formation was reduced by foliar P fertilization, Shane et al. (2003) found that improving plant P nutrition status in addition reduced cluster root formation more in P-rich than in no-P nutrient solution in a split-root system. Somewhat different results were obtained by Ma and Rengel (2008) for wheat in a split-root soil experiment in which they modified plant P status through variation in P supply to one half of the root system and tested how this affected the growth response to soil P patchiness in the other half. In this case, a higher overall P supply increased root growth, but here roots showed a clearer root proliferation in high-P patch at low P than at high P status during initial growth stages, which disappeared over time. Despite such differences between plants in their responses to heterogeneity and total amount of P supply, these observations mean that heterogeneous soil nutrient supply can influence root growth in two ways: through local effects in response to variations in soil nutrient availability and through systemic effects governed by the internal nutrient status of the plant (Forde and Lorenzo, 2001; Robinson, 1994).

Following up on our previous study (Ma et al., 2016), we were interested here in how plant P and Zn status would affect root growth responses to simultaneous heterogeneity in soil P and Zn. Instead of using the experimental approach of Ma and Rengel (2008) with a split root system to separate systemic and local effects on root growth, we used foliar nutrient application to change plant nutrient status. The shortcoming of the experimental design used by Ma and Rengel (2008) was that plant P status was manipulated by a treatment which was itself heterogeneous. Thus, it is not really clear how much of the observed change in responsiveness to local P patchiness was due to the changed ratio between the P exposure levels of the two halves of the root system and how much to the change in overall P supply. Furthermore, instead of the essentially 2-dimensional (2D) rhizoboxes used in our previous experiments (Ma et al., 2016) to study the influence of patchiness in soil P and Zn distribution on root system development we used cylindrical pots in the present study. We had used the 2D rhizoboxes because they allowed us to monitor root development over time using neutron radiography (NR) imaging. The disadvantage of the 2D rhizoboxes is that they constrain root growth in one horizontal dimension. In addition, they do not allow a design in which developing root systems have an equal choice for all four combinations of exposure to two levels (low vs. high) of two nutrients. Thus, we used cylindrical pots in the present study.

In pursuit of the above research question, we performed an experiment in which we investigated how the root growth of white lupin (*Lupinus albus* L.) seedlings responds to soil heterogeneity in the horizontal distribution of P and Zn around the developing root system under different levels of foliar P and Zn application. Lupin was selected as experimental plant because previous studies, as presented above, had shown preference of cluster root formation for P-enriched soil patches and had indicated a dependence of this response on plant P status. In addition, we also like to point out the potentially growing importance of lupin as agricultural crop plants in a drier and warmer climate. The world's growing demand for food and feed is likely to convert marginal lands to agricultural crop production that is suited to lupin cultivation but not for most other crops (Gladstones, 1998), and while lupin is primarily cultivated for animal feed in current agriculture, it also has a high but still underutilized potential as a human food crop, as it is rich in protein, dietary fibers, vitamins, and antioxidants, and low in available carbohydrates (Villarino et al., 2016).

2. Materials and methods

2.1. Soil

The soil used in this study has been described previously in Ma et al. (2016). It is a sandy (34 g kg^{-1} clay, 17 g kg^{-1} silt and 949 g kg^{-1} sand), slightly alkaline (pH 7.7 in 0.01 M $CaCl_2$) soil with low contents in $CaCO_3$ (13.4 g kg^{-1}) and organic carbon (0.99 g kg^{-1}) and low concentrations in total Zn (10.2 mg kg^{-1}), total P (210 mg kg^{-1}), DTPA-extractable Zn (0.14 mg kg^{-1}), and Olsen-extractable P ($\leq$2 mg kg^{-1}). The soil was sterilized by means of X-ray irradiation (Synergy Health, Switzerland) and fertilized adding 50 mg N (as $Ca(NO_3)_2{\cdot}4H_2O$), 50 mg K (as K_2SO_4), 7.5 mg Mg (as $MgSO_4{\cdot}7H_2O$), 0.05 mg Mo (as $Na_2MoO_4{\cdot}2H_2O$), 0.5 mg B (as H_3BO_3), 1 mg Mn (as $MnSO_4{\cdot}H_2O$), 1 mg Cu (as $CuSO_4{\cdot}5H_2O$), 0.05 mg Co (as $CoSO_4{\cdot}7H_2O$) per kg dry soil as solutions. Thereafter, it was wetted to field water-holding capacity with nanopure water (high-purity deionized water with electrical conductivity of 5.5×10^{-6} S m^{-1}) produced by a Nanopure water system (Thermo Scientific, USA), thoroughly mixed, incubated for 1 week and dried at 30 °C before it was used to prepare the experimental soil packings.

2.2. Preparing the culture system and experimental design

Cylindrical pots of 9.4 cm inner diameter and 10.3 cm height were used for the experiment. Soil packings were constructed using four batches of differently amended soil. These batches prepared by mixing either (a) 30 mg kg^{-1} P, (b) 20 mg kg^{-1} Zn, (c) 30 mg kg^{-1} P and 20 mg kg^{-1} Zn, or (d) neither P nor Zn as nutrient solutions into the fertilized soil. Phosphorus was added as $Ca(H_2PO_4)_2{\cdot}H_2O$ and Zn as $ZnSO_4{\cdot}7H_2O$. After adding the respective amendment, each batch was wetted to field water-holding capacity with nanopure water, thoroughly mixed, incubated for 1 week and dried at 30 °C.

All pots were filled in the same way. Each pot was subdivided into four quarter sections, and each section was filled with 200 g soil of a different batch, with opposite sections contrasting in their amendments according to the scheme shown in Fig. 1. During the filling thin dividers were installed in order to prevent mixing between the sections. The packing density was 1.4 g cm^{-3} (800 g dry soil per pot in total). In total, 16 pots were prepared.

After filling, the packings were wetted to approximately 0.1 g g^{-1} gravimetric soil water content through very slow application of nanopure water using a 5 mL syringe. The water was applied at approximately constant rate in concentric circles (concentric-circles syringe irrigation method) in order to make its distribution as homogeneous as possible. One day later, a soil core with a diameter of 1.5 cm from the center of the cylinder was taken out, and replaced with unamended soil to ensure that the primary roots of the seedlings planted into the center would all encounter the same homogeneous initial conditions after transplanting. On the day before planting, the packings were wetted again to approximately 0.1 g g^{-1} gravimetric soil water content.