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Nitrogen and phosphorus economy of a legume tree-cereal intercropping system under controlled conditions

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ABSTRACT

Considerable amounts of nitrogen (N) and phosphorus (P) fertilizers have been mis-used in agroecosystems, with profound alteration to the biogeochemical cycles of these two major nutrients. To reduce excess fertilizer use, plant-mediated nutrient supply through N_2-fixation, transfer of fixed N and mobilization of soil P may be important processes for the nutrient economy of low-input tree-based intercropping systems. In this study, we quantified plant performance, P acquisition and belowground N transfer from the N_2-fixing tree to the cereal crop under varying root contact intensity and P supplies. We cultivated *Acacia senegal* var *senegal* in pot-culture containing 90% sand and 10% vermiculite under 3 levels of exponentially supplied P. Acacia plants were then intercropped with durum wheat (*Triticum turgidum durum*) in the same pots with variable levels of adsorbed P or transplanted and intercropped with durum wheat in rhizoboxes excluding direct root contact on P-poor red Mediterranean soils. In pot-culture, wheat biomass and P content increased in relation to the P gradient. Strong isotopic evidence of belowground N transfer, based on the isotopic signature ($\delta^{15}N$) of tree foliage and wheat shoots, was systematically found under high P in pot-culture, with an average N transfer value of 14.0% of wheat total N after 21 days of contact between the two species. In the rhizoboxes, we observed limitations on growth and P uptake of intercropped wheat due to competitive effects on soil resources and minimal evidence of belowground N transfer of N from acacia to wheat. In this intercrop, specifically in pot-culture, facilitation for N transfer from the legume tree to the crop showed to be effective especially when crop N uptake was increased (or stimulated) as occurred under high P conditions and when competition was low. Understanding these processes is important to the nutrient economy and appropriate management of legume-based agroforestry systems.

1. Introduction

During the Green Revolution, from 1960 to 1995, the global production of cereals roughly doubled, almost keeping the pace of the growing world population and related food requirements, while concurrently, the consumption of nitrogen (N) and phosphorus (P) fertilizers increased 6.9- and 3.5-fold, respectively (Tilman, 1999). From an environmental perspective, considerable amounts of N and P fertilizers have been mis-used and indeed wasted in agricultural lands, with profound alteration to the biogeochemical cycles of these two major nutrients (Vitousek et al., 1997; Millennium Ecosystem Assessment, 2003; Steffen et al., 2004).

Projections for the forthcoming decades suggest that increases in N and P fertilizer use will unavoidably occur to keep pace with increasing food demand as related to the forecasted increase of world demography (Tilman et al., 2001; Vance et al., 2003). Such increase in fertilizer consumption will further threaten ecosystem services and, especially the global N and P cycles. Therefore, greater and appropriate intensification of agroecosystems is needed to meet future food demand, but we can no longer afford the considerable waste of nutrients that currently occurs at a global level, and especially in some regions of the world (Vitousek et al., 2009). The development of nutrient-conserving systems in agriculture is thus increasingly urgent, particularly with the rise of inaccessible or undesirable fertilizer use (Sanchez, 2002; Vitousek et al., 2009; Hinsinger et al., 2011). Through nutrient efficiency often found in intercropping systems, ecological intensification of agroecosystems, e.g. low-input agriculture dependent on plant-mediated nutrient supply via symbiotic N_2-fixation in legumes, is urgently needed (Cassman, 1999; Godfray et al., 2010).

Deciphering nutrient acquisition strategies and nutrient transfer processes is necessary to encourage positive nutrient interactions in intercropped legumes and non-legume systems for improved low-input agriculture (Hinsinger et al., 2011; Isaac and Kimaro, 2011). Depending on species functional and morphological traits, such

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intercropping systems may yield positive plant–soil and plant–plant interactions by exploiting various nutrient sources or soil fractions (Vandermeer, 1989; Haggar and Ewel, 1997; Altieri, 2002; Li et al., 2007; Hinsinger et al., 2011). Accurate estimates of soil nutrient sources, rhizosphere acquisition and mineral transfer processes under varying abiotic gradients are necessary to minimize interspecific competition in intercropped production systems (Vance et al., 2003; Isaac et al., 2007; Maestre et al., 2009). To increase yield response in multi-species systems, precision in nutrient supply, particularly of P and N, is of the utmost importance.

Effects of P additions on N acquisition for N_2-fixing species have been well documented (Ribet and Drevon, 1996; Hogh-Jensen et al., 2002; Almeida et al., 2000; Li et al., 2008; Isaac et al., 2011a, 2011b). However, plants intercropped under P limitations may not experience N limitation due to 1) overall suppressed growth or 2) direct pathways of N acquisition, such as through root exudates (Sierra and Nygren, 2006; Rasmussen et al., 2007; Carlsson et al., 2009). More work is needed on pathways of N transfer in multi-species systems and direct and indirect consequences of increased P availability for the tree component and associated crop growth. Secondly, by focusing on early growth dynamics of intercropping and by focusing on the soil–root interface, greater attention is given to initial root modifications to soil parameters and belowground N transfer. Accordingly, we are interested in the effects of P and N sources on plant uptake and transfer under an early growth agroforestry system under controlled conditions employing a N_2-fixing tree species and a cereal crop. The species selected for this study are *Acacia senegal*, an understudied multi-purpose leguminous tree species, and durum wheat (*Triticum turgidum durum* L.) as the intercropped cereal.

Our research objective was to evaluate crop performance in response to associated tree nutrition and to elucidate the underlying plant–plant interactive processes and mechanisms (competition/facilitation) under controlled conditions. We used N_2-fixing trees supplied with a P gradient to achieve a range of P conditions for tree seedlings. Consequently, this provided a pre-established gradient of tree P status and variable substrate P levels for associated wheat. We quantified differences in cereal crop (wheat) growth, N and P acquisition under intercropping with legume trees on a) variable substrate P levels with interspecific root contact in pot-culture with sand and vermiculite and b) uniform soil P level with no interspecific root contact in rhizoboxes with a low P soil. Our hypotheses are 1) a facilitative effect of tree under high P availability on crop N and P uptake, and 2) a competitive effect of tree under P limitation.

2. Material and methods

2.1. Pre-intercropping phase

A. senegal var. *senegal* (Willd.) seeds were sourced from the Sahelian zone (provenance Korofane, Niger), germinated and cultivated in growth chamber and greenhouse conditions. Pre-contact N_2-fixing acacia plants were inoculated by soaking roots in liquid inoculum (/*Rhizobium*/sp. strain CIRAD300-303). Individual plants were then transplanted into 1-dm^3 pots containing 90% sand (sterilized quartz) and 10% vermiculite. A second addition of inoculum was provided to each pot (0.5 cm^3 inoculum per individual) during transplanting. Plants were grown in a growth chamber with climatic conditions of 14/10 h light/dark cycle, 400 μmol photons m^{-2} s^{-1} photon flux density, 27/22 °C day/night temperature and relative humidity at 75%. At four weeks, plants were transferred to a glasshouse for the remainder of the growth period.

A broad nutrient solution, provided as 50 cm^3 every 3 days during the 12 week growth period, consisted of (mM): 1.0 $Ca(NO_3)_2$, 1.0 KNO_3, 1.0 $MgSO_4$, 0.002 H_3BO_3, 0.0005 $ZnSO_4$, 0.003 $MnSO_4$, 0.0006 $CuSO_4$, 0.00001 Na_2MoO_4, 1.0 urea after one week and 1.0 FeNaEDTA. KH_2PO_4 was supplied exponentially based on three target levels of P supply: low additions (equaling 200 μmol $seedling^{-1}$ under exponential condition over 12 weeks), mid range additions (400 μmol $seedling^{-1}$ under exponential conditions over 12 weeks), and high additions (600 μmol $seedling^{-1}$ under exponential conditions over 12 weeks). Exponential additions were employed to avoid early growth toxicity and to match nutrient supply with exponential growth. The percentage of N derived from atmosphere (%Ndfa) in these nodulated acacia was determined with the natural abundance method.

During the establishment phase of the acacia seedlings, we have formerly shown that increasing the P supply level resulted in increasing biomass, P and N content of the acacia (Isaac et al., 2011a). Regardless of P addition level, all acacia seedlings nodulated and were fixing atmospheric nitrogen. Fixed N varied from 50% to 72% of total plant N without any significant difference between P supply levels. Both the number of nodules and the N derived from atmosphere, determined by the ^{15}N natural abundance method, did not increase along the P gradient. Phosphorus stimulated growth and increased mineral N uptake from solution without affecting the amount of N derived from the atmosphere under non-limiting N conditions with nitrate supply. See Isaac et al., 2011a for full description of treatment additions and results of pre-contact acacia plants.

Durum wheat (*T. turgidum durum* L. cv. Acalou) was grown from seed in hydroponics in the same growth chamber as above. Climatic conditions were set at a 14/10 h light/dark cycle, 400 μmol photons m^{-2} s^{-1} photo flux density, 27/22 °C day/night temperature and relative humidity at 75%. Wheat grains were surface-sterilized by 6% H_2O_2 for 10 min and transferred to covered 5-dm^3 buckets with deionized water. After 7 days, buckets were uncovered and filled with a broad nutrient solution for 3 weeks. This broad nutrient solution was similar to that used in the cultivation of the acacia plants but adapted slightly to include, among other changes, higher N. It consisted of (mM): 2.0 KNO_3, 0.7 K_2SO_4, 1.0 $MgSO_4$, 1.65 $CaCl_2$, 0.005 KH_2PO_4, 0.1 FeNaEDTA, 0.004 H_3BO_3, 0.001 $ZnSO_4$, 0.006 $MnSO_4$, 0.001 $CuSO_4$, and 0.0001 Na_2MoO_4. Continuous aeration of the nutrient solution was supplied with air pumps in each bucket. Solutions were changed every 3 days during the 3 week growth period.

2.2. Experimental design

To investigate plant interactions, we used two designs: 1) direct transplanting of wheat into sand and vermiculite filled pots with pre-established acacia trees and 2) a device based on the rhizobox design (Hinsinger and Gilkes, 1996; Li et al., 2008; Fig. 1). Two different designs were used to represent varying conditions: resource limitation (competition) and resource sufficiency. The former conditions were achieved in sand and vermiculite pot-culture with an established bioavailable P gradient with possible root contact (intermingling) between the two intercropped species. The latter conditions were achieved with a low P soil in a rhizobox with no direct root contact between the two intercropped species. These trials were in an additive design, employing monocrops of wheat and acacia and intercropping of both species. Acacia plants were 12 weeks old at the beginning of the experiment and wheat, which was transplanted in both designs, was three weeks old. Subplot treatments were the three pre-contact P supply levels to acacia. The whole experiment unit was repeated in three blocks.

In pot-culture, one representative wheat plant from hydroponics was transferred to each pot containing one pre-established acacia plant (Fig. 1). For monocropped wheat treatments, wheat plants were also transferred to pots with no acacia and no previous P supply. The post-contact pot-culture (monocropped and intercropped wheat) was provided with 50 cm^3 of a modified nutrient solution every 3 days (mM): 1.0 KNO_3, 0.7 K_2SO_4, 1.0 $MgSO_4$, 1.65 $CaCl_2$. Deionized water was used to flush the sand substrate before application of solution. The advantages of the pot-culture system are reduced stress of tree seedlings and the use of intact undisturbed substrate.