Soil Biology & Biochemistry 79 (2014) 117–124

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Cropping enhances mycorrhizal benefits to maize in a tropical soil

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ARTICLE INFO

Article history:
Received 26 June 2014
Received in revised form
11 September 2014
Accepted 13 September 2014
Available online 29 September 2014

Keywords:
Arbuscular mycorrhiza
Bioassay
Chromolaena odorata
Fallow
Maize
Phosphorus
Quantitative real-time PCR
Southern Cameroon

ABSTRACT

Crop production in subsistence agriculture in tropical Africa is still sustained mainly by short-to medium-term fallows to recuperate natural fertility of the soils. Microbes play a pivotal role both in the process of soil fertility restoration and in nutrient acquisition by the crops. Here we ask the question how the duration of fallow affects the composition of indigenous arbuscular mycorrhizal fungal (AMF) communities and their contribution to maize nutrition and growth, in acidic, low P soils of southern Cameroon. This question has been addressed in a bioassay where soils collected from continuously cropped fields, short-term fallows dominated by *Chromolaena odorata* and long-term fallows (secondary forests) have been sterilized and back- and cross inoculated with living soils from the different land-use systems. Particular microbes larger than the pore size of the filter paper (mainly the fungi including the AMF) contained in the cropped and short-fallowed soils caused greater growth and P uptake stimulations to the maize as compared to the forest soil. By using molecular profiling, we demonstrated a shift in the composition of AMF communities along a gradient of fallow duration, changing from dominance by *Rhizophagus* in the forest fallow soil, to dominance by *Claroideoglomus* under cropland. Our results contradict the hypothesis that deterioration of quality of root symbiotic communities would be responsible for a rapid yield decline following deforestation, and indicate a positive feedback of cropping on mycorrhizal functioning under conditions of shifting agriculture in tropical Africa.

1. Introduction

Crops in tropical Africa often experience far too little inputs to ensure sustainable production over extensive periods of time. Therefore the soils are commonly exposed to fallow periods of different duration aiming at restoration of their fertility (Manlay et al., 2002). This strategy may work, but both duration of the fallow period as well as the identity of the plant cover seem critical for the performance of the subsequent crop (Koutika et al., 2004). Traditionally, the land has been abandoned for decades before it was brought again under cultivation. Fallow duration is continuously shortening owing to increasing population pressure and exploding demand for food and cash by the local populations (Ajayi et al., 2003; Waithaka et al., 2006).

Due to widespread lack of sufficient external inputs such as mineral fertilizers, the growth and nutrition of crop plants on the African soils are more than elsewhere dependent on beneficial soil organisms such as arbuscular mycorrhizal fungi (AMF). The extent of benefits to the plants is dependent on AMF abundance, the AMF community structure, crop plant species/genotype, and soil and climate context (Merryweather and Fitter, 1996; Burrows and Pfleger, 2002). Importantly, the AMF community structure is in turn greatly affected by the composition of associated plant communities. This mutual interaction could result in different outcomes: On one hand, most beneficial fungi could be supported by a specific crop plant, leading to a positive soil feedback. On the other hand, pathogen accumulation theory would predict that parasitic organisms or symbiotic cheaters would proliferate in situations where plant diversity would be low, resulting in a negative soil feedback on crop growth (Hodge and Fitter, 2013; Revilla et al., 2013).

For a long time, scientists have searched for reasons of rapid yield decline of crops planted on previously fallowed lands (Juo et al., 1995; Kimetu et al., 2008). Decline of available nutrient pools, changes in physical soil properties, building up specialized pathogen populations, and deterioration of symbiotic soil microbial

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http://dx.doi.org/10.1016/j.soilbio.2014.09.014
0038-0717/

communities could potentially explain this effect (Bever et al., 2012; Miki, 2012; Huang et al., 2013), but relative importance of these above reasons is very likely affected by the crop species, soil and environmental context and by intensity of land management.

The objective of this work was to test whether symbiotic quality for maize of the native AMF communities present in a tropical soil under long-term fallow (i.e., secondary tropical forest) deteriorates due to cropping. Such worsening of mycorrhizal benefits could then be made responsible for rapid maize yield declines observed during the first few years following deforestation (Kimetu et al., 2008; Moebius-Clune et al., 2011) and targeted counter-measures could be enforced to conserve this important component of soil fertility in tropical regions. To achieve this goal, we conducted an experiment, where indigenous AMF communities were eradicated from the different soil, and these soils were then (back-)inoculated by their original AMF communities or (cross-)inoculated by the AMF from the different land-use systems. This was necessary to separate the biotic (mycorrhizal) effect from the possible confounding (physico-chemical) factors of the different soils.

2. Material and methods

2.1. Experimental soil

The soil was collected in Southern Cameroon, at the village of Metet (3° 25′ N, 11°45′ E, 336 m a.s.l), from three land use systems representing the main components of the shifting cultivation practiced in the area: (1) long-term fallow (secondary forest) aged 27 years, (2) continuously cropped land with over four cycles of crop cultivation after forest clearance, and (3) short-term (5 year old) fallow dominated by *Chromolaena odorata*. The dominant food crops grown in the cropland were groundnut and maize followed by cassava, yams and banana. The soil in the area was classified as a Typic Kandiudult (Hauser et al., 2005) according to USDA soil taxonomy, long-term average of the annual rainfall being 1380 mm. Three kilograms of topsoil (0–20 cm depth) was collected with a soil corer (5 cm diameter) from each of sixteen independent 8 × 4 m plots (fields) of each of the three land use system within 3 km from the village, bulked for each land use system, air-dried for two weeks, sieved to pass 5 mm sieve and twenty five kilograms of each sample shipped to Switzerland for soil analyses (Table 1) and the bioassay.

2.2. Soil analyses

Soil properties were analyzed on 4 analytical replicates per each living soil sample. In addition, four analytical replicates of the γ-irradiated forest and cropland soils were analyzed to assess the influence of γ-irradiation of soil physico-chemical properties. Soil pH was measured in aqueous soil suspension (1:2.5, v:v) using the Corning 125 pH meter (Corning B.V. Life Sciences, Amsterdam, Netherlands) after shaking the samples for 16 h. Total soil organic carbon (C) and total nitrogen (N) concentrations were measured by elemental analyser (Flash EA 1112, Thermo Electron, Ecublens, Switzerland) on dried and pulverized samples (using ball mill MM200, Retsch, Haan, Germany). Available soil phosphorus concentration was assessed using the anion resin extraction according to Kouno et al. (1995), with BDH anion resin strips (product # 55164 2S, BDH Laboratory Supplies, Poole, England) and 16 h extraction time. Phosphorus concentration in the extracts was measured by the Malachite Blue method (Ohno and Zibilske, 1991). Total phosphorus concentration in soil was measured by inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7500c; Agilent, Basel, Switzerland) in HNO_3 extracts of incinerated (550 °C, 12 h) soil samples.

Table 1
Results of two-way ANOVA testing the effect of mycorrhizal inoculation (4 levels), soil provenance (3 levels), and the interaction of the two factors, on maize biomass production and mineral nutrition. Four replicates per treatment combination are included. *F*-values are accompanied by indicators of statistical significance: ns, $p \geq 0.05$; *, $0.05 > p \geq 0.01$; **, $0.01 > p \geq 0.001$; ***, $0.001 > p$.

	Inoculation (*I*)	Soil provenance (*S*)	Interaction $I \times S$
Plant (shoot + roots) biomass (g)	123.3***	5.3**	2.3 ns
Plant (shoot + roots) phosphorus content (mg)	202.1***	13.9***	4.6**
Shoot phosphorus concentration (mg g^{-1})	15.8***	1.4 ns	0.9 ns
Root phosphorus concentrations (mg g^{-1})	4.5**	4.4*	0.9 ns
Plant (shoot + roots) nitrogen content (mg)	164.0***	4.9*	4.3**
Shoot nitrogen concentration (mg g^{-1})	19.3***	0.9 ns	0.8 ns
Root nitrogen concentrations (mg g^{-1})	9.7***	1.9 ns	0.7 ns

2.3. Bioassay

About 4 kg of the soil from each land-use system was kept at 4 °C for 1 month before starting the bioassay and the rest was sterilized by γ-radiation at Studer Hard AG, Däniken, Switzerland by applying a dose of 25–75 kGy with ^{60}Co source. This irradiated soil was then back- or cross-inoculated with 20% (v:v) of the unsterile soil from the same or the other land use systems, respectively, or left non-inoculated. The inoculum was homogeneously mixed with the sterilized soil. These differently treated soils were then filled in 1 l pots (1 kg of soil each, 4 replicate pots per soil treatment) and planted with two pre-germinated seeds of maize. Before planting, all pots received 50 ml of unsterile soil filtrate (100 g of each of the three soils mixed with 5 l distilled water and filtered twice through a Whatman No 4 paper filter). For planting, we used moderately acid-tolerant maize cultivar CMS 8704 obtained from the Cereal Breeding Program of the Institute of Agricultural Research for Development (IRAD), Yaoundé, Cameroon. The seeds were first surface sterilized in 95% ethanol for 5 min, subsequently washed with distilled water and then pre-germinated on a wet filter paper for five days at 25 °C. The pots were incubated in a growth chamber PGC20 (Conviron, Winnipeg, Canada) under following conditions: 12 h photoperiod with a photosynthetic photon flux density of 231 μmol s^{-1} m^{-2} during the daytime, 28/18 °C (day/night), and 75/85% relative air humidity (day/night). Pots were arranged randomly in the chamber and their positions re-arranged weekly. One week after planting, the plants were thinned to one plant per pot. Plants were watered daily with deionized water to maintain 60% water holding capacity of the soils. Each pot received a total of 430 mg N pot^{-1} in a form of $NaNO_3$ (applied weekly as a solution) to reduce severe N deficiency.

2.4. Harvest

At 42 days after planting, shoots were cut at the substrate level and fresh weights recorded. The shoots were then oven-dried at 105 °C for 4 h and then at 65 °C for 72 h and the dry weights recorded. Roots were washed from the soil, blotted dry and the fresh weights recorded. Fresh roots were then cut to fragments 1–2 cm long, mixed and divided in three parts: A portion of approximately two grams (fresh weight) were preserved in 50% ethanol and stored at 4 °C for microscopy. A second subsample (about 5 g fresh weight) was frozen at −80 °C for molecular analyses. Remaining roots were weighed fresh, dried at 105 °C for 4 h