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Reaction of microorganisms to rewetting in continuous cereal and legume rotation soils of semi-arid Sub-Saharan Africa

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Abstract

A 20-day incubation experiment with continuous cereal (CC) versus cereal legume (CL) rotation soils of two semi-arid Sub-Saharan sites (Fada-Kouaré in Burkina Faso, F, and Koukombo in Togo, K) were carried out to investigate the effects of rewetting on soil microbial properties. Site- and system-specific reactions of soil microorganisms were observed on cumulative CO_2 production, adenylates (ATP, ADP, and AMP), microbial biomass C and N, ergosterol, muramic acid and glucosamine. Higher values of all parameters were found in the CL rotation soils and in both soils from Fada-Kouaré. While the inorganic N concentration showed only a system-specific response to rewetting, the adenylate energy charge (AEC) showed only a site-specific response. ATP recovered within 6 h after rewetting from ADP and AMP due to rehydration of microorganisms and not due to microbial growth. Consequently, no N seemed to be immobilized by microorganisms and all NO_3 in the soil was immediately available to the plants. The fungal cell-membrane component ergosterol was three (CC) and five (CL) times larger at Fada than in the respective soils at Koukombo. The concentrations of the bacterial cell-wall component muramic acid were by 20% and of mainly fungal glucosamine by 10% larger in the CL rotation soils than in the CC soils. This indicates long-shifts in the microbial community structure.

Keywords: Adenylates; AEC; Ergosterol; Glucosamine; Muramic acid; Microbial biomass; CO_2 evolution rate

1. Introduction

In West Africa, where rainfall is irregular and often scarce, legume rotations with groundnut and cowpea have been reported to cause several changes in soil chemical and biological properties (Bagayoko et al., 2000a, b; Alvey et al., 2001). Legume rotations gradually increased soil pH, early mycorrhizal infection and N availability and decreased the number of phytoparasitic nematodes, thereby leading to an increase in total dry matter production of the following cereal crop (Buerkert et al., 2000; Bagayoko et al., 2000a). Under controlled conditions, differences in bacterial community structure, higher microbial biomass N and a higher fungal biomass in the rhizosphere of rotation soils are additional, site-specific microbiological rotation effects (Marschner et al., 2004). This indicates that soil microorganisms are an important component for improving plant growth by legume rotations.

A key-event in the biochemistry of soils from semi-arid regions is the mineralisation pulse after seasonal rewetting. The responsible mechanisms of this effect have not been conclusively identified, especially for low organic matter soils from semi-arid Sub-Saharan Africa, although numerous experiments have been carried out to analyse the effects of drying–rewetting cycles (Fierer and Schimel, 2003; Wichern et al., 2004; Wu and Brookes, 2005). The aim of the present experiment therefore was to analyse the effects of the onset of the rainy season after a long period of drought on the microbial community of soils from semi-arid Sub-Saharan sites under continuous cereals and legume rotation cultivation. In these soils, microorganisms are adapted to repeated and sometimes long periods of drought (Marschner et al., 2004), and may react differently

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than microorganisms in soils from temperate humid climate (van Gestel et al., 1993; Fierer et al., 2003). The rewetting effects were monitored by measuring CO_2 evolution, inorganic N, adenylates (ATP + ADP + AMP), the fungal biomarker ergosterol, microbial biomass C and biomass N. Bacterial muramic acid and fungal glucosamine give also information on the long-effects of cropping systems on the microbial community structure due to their persistence as microbial cell-wall components.

2. Materials and methods

2.1. Soil and experimental layout

In December 1998, soil samples were taken from a sorghum/groundnut experiment in the Sudanian zone of Burkina Faso on a Haplustalf at Fada-Kouaré (11°59′N, 0°19′E; 850 mm mean annual rainfall and 28.3 °C mean annual temperature) and in December 1999 from a maize/groundnut experiment in the Guinean zone of Togo on an isohyperthermic Plinthic Kanhaplustult at Koukombo (10°17′N, 0°23′E; 1100 mm, mean annual rainfall and 27.8 °C mean annual temperature). The soil of each site was taken at 0–20 cm depth, air-dried and sieved to 2 mm, packed in plastic bags, shipped to Germany and stored in the dark at about 15 °C. Clay content, cation exchange capacity and organic C were 15%, 28 $\mu mol_c\,g^{-1}$ soil and 5.2 $mg\,g^{-1}$ soil in the Fada soil and 5%, 19 $\mu mol_c\,g^{-1}$ soil and 3.7 $mg\,g^{-1}$ soil in the Koukombo soil, respectively (Buerkert et al., 2000). Mean soil pH in water was 6.4 in the two Fada soils and 6.2 in both Koukombo soils. Eight replicates of the air-dried continuous cereal (CC) and cereal legume rotation (CL) soil samples from Fada-Kouaré (CC-F and CL-F) and Koukombo (CC-K and CL-K) were rewetted to 45% water holding capacity with tap water and incubated at 25 °C in the dark. Soil biological indices were measured 0, 0.5, 6, 12, 24, 48, 72, 100, 240 and 480 h after rewetting soil. CO_2 evolution was determined daily over a period of 20 days.

2.2. Analytical procedures

CO_2 evolution was determined in 50 g air-dried soil, placed in 80 ml incubation cylinders made of stainless steel nets, and transferred into 1000 ml incubation vessels containing NaOH solution at the bottom, rewetted with 5 ml of bidistilled water and incubated for 20 days at 25 °C in the dark with eight replicates. Evolved CO_2 was absorbed in 10 ml of 0.1, 0.05 or 0.03 M NaOH, which was titrated daily with HCl of the same molarity. At 7 and 13 days after rewetting each sample was wetted again with 1 ml of bi-distilled H_2O to prevent the samples from drying.

Adenine nucleotides (ATP, ADP, and AMP) were extracted with an alkaline DMSO buffer according to Bai et al. (1988) as described by Dyckmans and Raubuch (1997) using a moist sample equivalent to 3 g oven-dry soil.

Microbial biomass C and biomass N were determined by fumigation extraction (Brookes et al., 1985) using pre-extraction to remove high background levels of organic C and inorganic N. A sample of 25 g soil (on an oven-dry basis) was pre-extracted with 100 ml of 0.05 M K_2SO_4 on a horizontal shaker at 200 rev min^{-1} for 30 min and centrifuged. Fumigated and non-fumigated portions of 10 g moist soil were taken from the remaining soil and extracted with 40 ml 0.5 M K_2SO_4 as described above. Microbial biomass C and N was calculated according Wu et al. (1990) and Brookes et al. (1985). In the 0.05 M K_2SO_4 pre-extracts and in the 0.5 M K_2SO_4 extracts of the non-fumigated samples, NO_3-N and NH_4-N were determined using segmented flow analysis.

The fungal cell-membrane component ergosterol was extracted from 2 g soil with 100 ml ethanol by oscillated shaking at 250 rev min^{-1} for 30 min according to Djajakirana et al. (1996). Ergosterol was determined by reversed-phase HPLC with 100% methanol as the mobile phase and detected at a wavelength of 282 nm.

The fungal and bacterial cell-wall components glucosamine and muramic acid were determined according to Appuhn and Joergensen (2006) in 500 mg air-dried soil after hydrolysis with 6 M HCl. Fluorometric emission of amino sugar ortho-phthaldialdehyde (OPA) derivatives was measured at a wavelength of 445 nm with 340 nm as the excitation wavelength (Agilent 1100, Palo Alto, USA). Fungal glucosamine was recalculated into fungal C and muramic acid into bacterial C using the procedure and conversion values proposed by Appuhn and Joergensen (2006).

2.3. Statistical analysis

All results were tested for normal distribution of residuals using the Kolmogorov–Smirnov test. Soils were compared using a GLM-repeated measures ANOVA with site and system as between-subject factors. Extracted variables at the different times were taken as inner-subject factors, and means were separated using Tukey's HSD (honestly significant difference). All statistical analyses were performed with SPSS 11.5 (Backhaus et al., 2003).

3. Results

In the two Fada soils, cumulative CO_2 production was more than twice as high than in the Koukombo soils. This difference was especially strong in the two CL rotation soils. Throughout the incubation period the CO_2 evolution rate of two Fada soils was always larger than that of the two Koukombo soils, but this difference was largest immediately after rewetting (Fig. 1). The CO_2 evolution rate showed a roughly 90% decrease in all treatments during the 20-day incubation period with intermediate maxima around day 10 and day 17. In contrast to the cumulative CO_2 production, the amount of inorganic N after 20 days of incubation was similar in both soils,