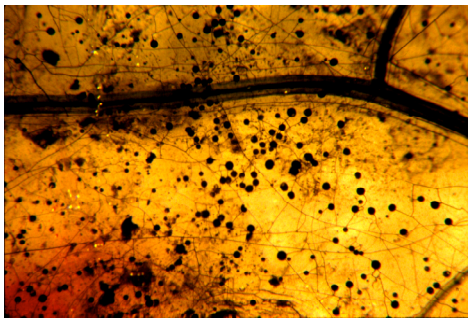
Seasonal variation in the soil microbial community in wheat-growing soil and influence of C, N and P inputs



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Key words: soil microbial community structure, PLFA, NLFA, FAME, mycorrhizal fungi, saprobes, phosphorus, nitrogen fertilization, carbon input

Abstract

It has long been know that N and P fertilization increases plant growth and yield, but the impact of fertilization on soil microorganisms has rarely been considered. Long-term plots (36-year old) under fallow-wheat-wheat (F-W-W) rotations with no P or no N fertilization, or normally fertilized, and plots receiving low C inputs due to frequent fallow (F-W rotation) were used to define the impact of C, N and P on the seasonal variation of the soil microbial communities in the fallow-after-wheat or the wheat-after-fallow phases of the rotations. The soil was sampled on June 8, July 4, August 5 and September 16, in 2003. There was no significant ($P \leq 0.05$) time by treatment interactions. Populations of bacteria, arbuscular mycorrhizal (AM) fungi and saprophytic fungi, as estimated by phospholipids fatty acid (PLFA) indicators, were strongly reduced on July 4th, a date corresponding to rapid plant growth. Sporulation of fungal saprobes was enhanced at that date, as indicated by the neutral lipid fatty acid (NLFA) to PLFA fraction ratio of the fatty acid C18:2. It appears that a competition for resources exists between soil microorganisms and wheat, at least in July at the time of active crop growth. While P availability had little effect on soil microorganisms, absence of N fertilization increased sporulation in AM and saprophytic fungi. In spite of the biotrophic[1] nature of AM fungi, C input in the form of infrequent fallow or presence of living wheat plant favoured the partitioning of fatty acids into reserve lipid i.e., NLFA.

Introduction

Soil microorganisms are abundant in agricultural soils. Their biomass C oscillates between 400 kg and 1.4 t ha^{-1} under wheat during a growing season, in our long-term experiment[1]. Bacteria decompose soil organic matter (SOM) and conduct several redox reactions involved in soil fertility. Fungal saprobes decompose SOM. Arbuscular mycorrhizal (AM) fungi maintain soil physical quality and increase plant nutrient uptake capacity, within a symbiotic relationship with plants. Although microorganisms are important components of soil quality, little is known of the impact of cropping on

[1] AM fungi needs a living host plant to exist.

soil microorganisms. Our objective was to assess the impact of C, N and P inputs on soil microorganisms, in wheat and fallow phases of wheat-based cropping systems.

Methods

Different N, P and C input treatments (Table 1) have been applied for the last 36 years in a complete block design with three blocks. Wheat, cv. AC Eatonia, was seeded at 71 kg ha^{-1}, on May 14, 2003, in designated plots. Soil cores (0-7.5 cm) and sets of three plants, when present, were taken at three locations along a diagonal transect laid across each plot, on June 8, July 4, August 5 and September 16, 2003. Both the fallow-after-wheat and the wheat-after-fallow phases of the rotations were sampled. Soil samples were frozen at -12 ºC up to analysis. Fallow plots were tilled on May 21, June 30 and July 21 with a heavy duty cultivator (16 cm sweep and 0-7.5 cm deep).

Table 1. Field treatments applied for 36 years

CNP: Recommanded N and P fertilization, on a fallow-wheat-wheat (FWW) rotation
noN: No N, P fertilization as recommended, on a FWW rotation
noP: N fertilizer as recommended, no P, on a FWW rotation
Low C: N and P fertilization as recommended, on a FW rotation

Plant biomass was determined after drying at 40 ºC. Lipids were extracted from 4 g of soil with dichloromethane (DMC) : methanol (MeOH): citrate buffer (1:2:0.8 v/v); phospholipids and neutral lipids were eluted with MeOH and DMC, respectively, on silica gel columns; fatty acids from the phospholipid (PLFA) and the neutral (NLFA) fractions were transmethylated, and analysed by gas chromatography[2]. Supelco® BAME standards' mixture was used for bacterial peaks identification. C18:2 was used as indicator for fungal saprobes and C16:1_5 as indicator for AM fungi. PLFA make up cell membranes; they indicate amount of active biomass. NLFA are from reserve lipids; they are used as indicator of resting structures (spores).

Shannon-Weaver biodiversity index was computed. Data were transformed if necessary to meet the requirement of the tests, and analyzed using ANOVA with Network Jump v. 3.2.6, or using the backward stepwise option of Systat v. 10, for discriminant analysis.

Results and discussion

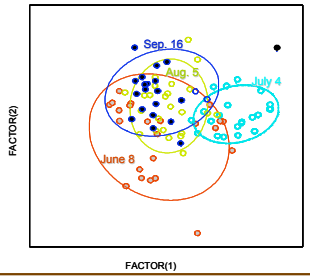

Figure 1. Microbial community structure on July 4th is different from other sampling date (Wilks' lambda $P < 0.0001$).

None of the interactions between input treatments and sampling time were significant ($P \leq 0.05$), thus main effects are presented. There was a strong seasonal variation in soil microbial communities and populations. Variation in seven of the bacteria-related PLFA and in the fungal saprobes PLFA indicator (not shown) were identified as the significant factors driving a seasonal shift in soil microbial community structure (Fig. 1). Biodiversity was lowest ($P < 0.0001$) (Fig. 2B), bacteria ($P = 0.009$), fungal saprobe ($P < 0.0001$) and AM ($P = 0.03$) fungi biomasses were lowest (Fig. 2 C, D, E), and sporulation in fungal saprobes was favoured ($P < 0.0001$) (Fig. 2F) on July 4th. The

season of 2003 was dry and competition for water with fast growing plants at that date is the likely cause of the variation in soil microbial population observed[3].

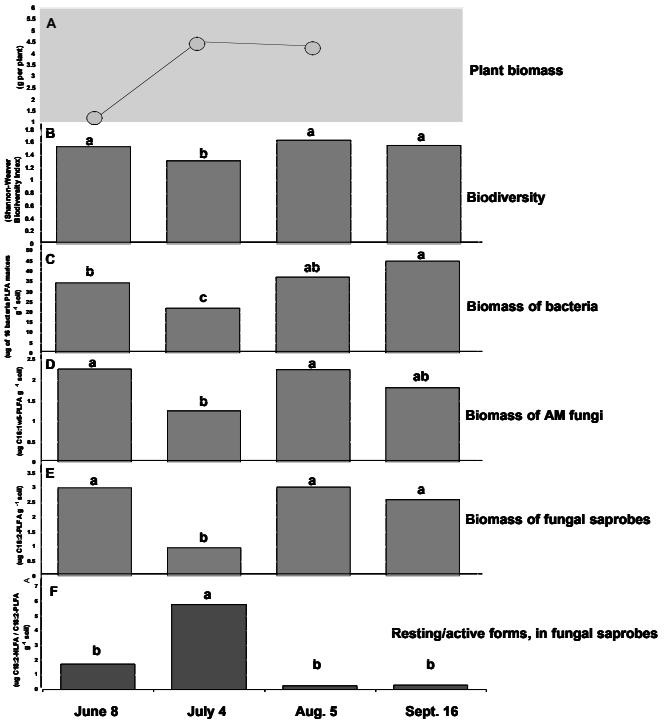

Figure 2. Microbial diversity (B) and microbial biomasses (C, D, E) decrease while in competition with the wheat crop. Competition was strongest on July 4 when wheat plants are large and actively growing (A). Different letters indicate different means, according to a protected LSD test at *P*=0.05.

The proportion of NLFA to PLFA in fungal saprobes (*P*= 0.009)and AM (*P*= 0.03)fungi was increased in absence of N fertilization (Fig. 3). N is an essential element also in fungi. It appears that N shortage induced sporulation in the two fungal groups. An impact on the metabolic state of bacteria was not revealed by the ratio of NLFA to PLFA, as reserves in bacteria occur in forms other than NLFA.

High soil P availability is known to reduce AM fungi development[4]. No significant (*P*≤ 0.05) impact of P fertilization could be detected in this study, but in another study conducted in the same field experiment, in September 2003, AM fungi were more abundant in the top 0-15 cm layer of soil receiving no P fertilizer than in soil fertilized as recommended, according to PLFA C16:1_5[5]. In this case, the wheat-after-wheat phase of the rotation was sampled, unlike in the study presently reported in which the wheat-after-fallow phase was considered. Low levels of AM fungi in soil after fallow may have reduced P level in normaly fertilized plants as well as in plants receiving no P fertilizer, reducing in this way the effect of the fertilization treatments.

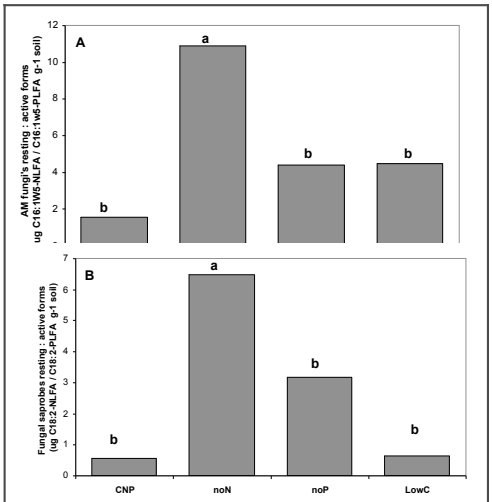

Figure 3. Absence of N fertilization favoured the sporulation of both AM fungi (A) and fungal saprobes (B). Different letters indicate different means, according to a protected LSD test at *P*=0.05.

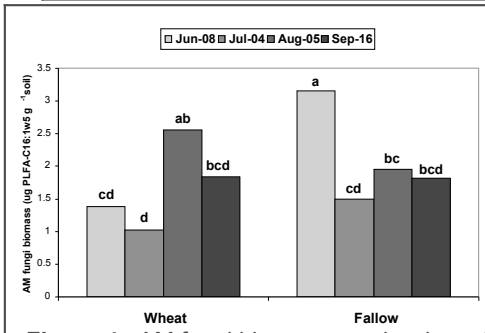

Figure 4. AM fungi biomass was low in spring after a year of fallow and increased under a wheat crop. Different letters indicate different means, according to a protected LSD test at *P*=0.05.

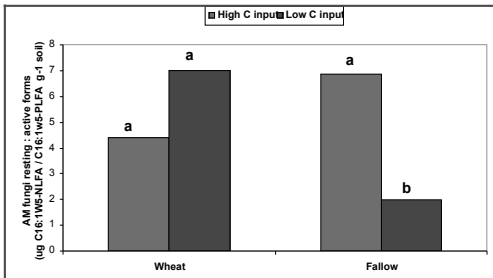

Figure 5. Sporulation in AM fungi is favoured by high crop frequency or presence of wheat. Different letters indicate different means, according to a protected LSD test at *P*=0.05.

AM fungi are known as obligate biotrophs i.e., living plant roots are their only source of C. This explains why their biomass was low after a year of fallow and high after a year of wheat cropping (P= 0.06) (Fig. 4). Unexpectedly, high C inputs in the form of infrequent fallow somehow influenced AM fungi physiology, as the sporulation of AM fungi was favoured by high C input, in the fallow phase of the rotation (P= 0.03) (Fig. 5).

Conclusions

- Seasonal variations in soil microbial communities are marked.
- Suppression of soil microbial biomasses and biodiversity corresponded to the time of maximum plant nutrient and water demand.
- N limitation seems to favour sporulation in AM and saprophytic fungi.
- A wheat crop favours AM fungi proliferation, fallow reduces it.
- Sporulation in AM fungi was stimulated by the presence of a host, but also by high C input to soil in the form of infrequent fallow.

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