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Effects of corn stover management on soil quality

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ABSTRACT

The incorporation of stover into soil can bring beneficial effects in terms of soil fertility, stabilization of soil structure, maintenance of soil organic carbon, etc. We evaluated the effects, after 6 years of consecutive treatment, of corn stover incorporation *versus* corn stover removal on soil quality, using physicochemical and biological parameters as indicators of soil quality. Throughout the experimental period, soil organic carbon decreased as a result of stover removal (from 20.1 to 14.7 g kg^{-1}). Substrate-induced respiration and bacterial gene abundance decreased by stover removal over the same period (24.0 and 47.6%, respectively). Biolog EcoPlates™ data showed faster rates of D-xylose and D-mannitol utilization by the soil bacterial communities under stover incorporation. 16 S and 18 S rRNA Illumina sequencing data did not show significant differences in terms of microbial diversity and composition between stover incorporation and stover removal treatments. Finally, the incorporation of stover resulted in higher values (27.2% higher) of soil quality, as reflected by the value of a Soil Quality Index, which integrates the values of a variety of microbial indicators of soil quality. In conclusion, incorporation of stover after corn harvest is a beneficial agronomic practice which enhances soil N and C pools and stimulates microbial communities, leading to an increase in soil quality.

1. Introduction

Corn (*Zea mays* L.) stover has been identified as potential feedstock for cellulosic ethanol production because of its high cellulosic content, large volume of biomass production and wide availability around the world [54]. However, the removal of stover can lead to a decline in soil quality and, hence, agricultural productivity by decreasing the content of soil organic carbon (SOC) and increasing the risk of soil erosion [3,17,30]. Conversely, incorporation of stover into agricultural soil can improve soil quality through a variety of processes such as stabilization of soil structure, prevention of soil erosion, maintenance of SOC, nutrient recycling, provision of energy for soil microbial communities, etc.

Soil microorganisms play an essential role in soil functioning and the delivery of soil ecosystem services. Thus, soil microbial parameters related to the activity, biomass and diversity of soil microbial communities are frequently used as indicators of soil quality [10,40], owing to their sensitivity, fast response, integrative character and ecological relevance. Nonetheless, there are limited reports on the effects of stover incorporation *versus* stover removal on soil microbial communities. Lehman et al. [26] reported a reduction in the fungi-to-bacteria ratio as a result of the removal of stover. Johnson et al. [17] found a decrease in soil enzyme activities after three consecutive cycles of stover removal. Moebius-Clune et al. [35] observed a reduction in decomposition activity and glomalin concentration in soil after 32 years of stover removal. In contrast, the long-term incorporation of stover has been found to increase soil microbial biomass [12,48].

The aim of this work was to evaluate the effects of six years of corn stover incorporation *versus* corn stover removal on agricultural soil quality, with special emphasis on the changes induced in soil microbial parameters that provide information on the activity, biomass and diversity of soil microorganisms. In particular, there is limited information on the changes induced by stover management on soil microbial diversity and composition. Recent developments in sequencing technologies have facilitated the sequencing of the genomes of soil microbial communities. Therefore, 16 S and 18 S rRNA gene-based Illumina sequencing were used to study differences between soil microbial communities subject to stover incorporation *versus* stover removal. We hypothesized that, after six years, the incorporation of corn stover from agricultural fields would positively impact soil quality by increasing soil organic carbon and stimulating soil microbial communities.

2. Materials and methods

2.1. Experimental design

A field experiment was conducted in Almacelles (NE Spain, 41º43′

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N, 0º26′ E) at an altitude of 324 m above sea level. The climate in this region is semiarid with low precipitation (192 mm) and high temperature (daily average temperature of 19.1 °C) during the corn-growing period. The soil is a Typic Calcixerept with loamy texture, well drained and without salinity problems.

Experimental plots of 18 × 17 m were arranged following a completely randomized design with three replications. Treatments consisted of: (1) corn stover incorporation (SI), carried out annually after crop harvest by disc plowing to a depth of approximately 30 cm, and (2) corn stover removal (SR) carried out mechanically.

Corn hybrids belonging to the 600 to 700 FAO cycle were planted annually (continuous corn cropping sequence) in early spring at a density of 80,000 plants ha^{-1} with a distance of 71 cm between rows. Plots were irrigated by sprinkler 2–3 times per week depending on the weather conditions, resulting in approximately 1000 mm of water per season. Nitrogen, phosphorus and potassium fertilizers were applied annually at a rate of 300 kg NH_4NO_3 (33.5% N) ha^{-1}, 150 kg P_2O_2 ha^{-1} and 250 kg K_2O ha^{-1}, respectively. Plots were treated with 3.3 L ha^{-1} of the pre-emergence herbicide Trophy (40% Acetochlor + 6% Dichlormid) and 1 L ha^{-1} of the post-emergence herbicide Fluoxypyr 20% plus 1.5 L ha^{-1} of Nicosulfuron in order to control *Abutilon theophrasti* M. and *Sorghum halepense* L., respectively.

Aboveground corn biomass was determined at physiological maturity, by harvesting 4 m of the central row in each plot.

2.2. Soil parameters

After crop harvest, composite soil samples (from six cores taken at a 0–30 cm soil depth) were randomly collected from each plot. Immediately after collection, soil was sieved to less than 2 mm and subjected to physicochemical characterization. Soil pH, the content of carbonate, limestone, nitrate, ammonium, total nitrogen and organic carbon were measured according to standard methods [31]. Heavy metals and minerals were quantified by inductively-coupled plasma atomic emission spectrophotometry (ICP-AES).

For the determination of soil microbial parameters, soils were stored fresh at 4 °C for a maximum of one month until analysis. Sub-samples for molecular analyses were stored at −20 °C. Soil enzyme activities were determined at optimal conditions of temperature, pH and substrate concentration, so as to get an assessment of maximum potential enzyme activity in soil. β-glucosidase, arylsulphatase and alkaline phosphatase were determined according to Dick et al. [8] and Taylor et al. [50]. Urease was measured following Kandeler and Gerber [19].

Potentially mineralizable N, an indicator of biologically active soil N, was measured as described by Powers [41]. Respiration and substrate-induced respiration (SIR) were measured following ISO 16072 Norm [14] and ISO 17155 Norm [15], respectively. Microbial biomass carbon (MBC) was determined following Vance et al. [51]. Community-level physiological profiles (CLPPs) of soil cultivable heterotrophic bacteria were determined with Biolog EcoPlates™ following Epelde et al. [9].

For the molecular analyses, DNA extraction was performed as soon as samples were processed and carried out from three aliquots (each of them corresponding to 0.25 g dry weight soil) of each sample using the Power Soil DNA Isolation Kit (MO Bio Laboratories, Carlsbad, CA). Prior to DNA extraction, soil samples were washed twice in 120 mM K_2PO_4 (pH 8.0) to wash away extracellular DNA.

The amount of DNA in the samples was measured on a ND-1000 spectrophotometer (Thermo-Scientific, Wilmington, DE). For the estimation of the abundance of 16 S rRNA gene fragments for total bacteria and 18 S rRNA gene fragments for total fungi, qPCR measurements were carried out using the primers, reaction mixtures and PCR conditions described by Epelde et al. [11] (Table 1).

Table 1
Primers and PCR conditions.

Primers	PCR conditions	References
qPCR for total fungi: Fung5F and FF390R	95 °C for 30 s, 94 °C for 30 s, 52 °C for 30 s, 72 °C for 1 min (40 cycles); 95 °C for 15 s, 60 °C for 1 min, 95 °C for 30 s for the melt curve, with a final extension of 60 °C for 15 s	Lueders et al. [28]
qPCR for total bacteria: Ba519 F and Ba907 R	95 °C for 30 s, 94 °C for 30 s, 50 °C for 30 s, 72 °C for 1 min (40 cycles); 95 °C for 15 s, 60 °C for 1 min, 95 °C for 30 s for the melt curve, with a final extension of 60 °C for 15 s	Lueders et al. [28]

2.3. Gene amplification, sequencing and data processing

16 S rRNA gene amplicon libraries were prepared using primers 519 F (CAGCMGCCGCGGTAA) adapted from Øvreås et al. [39] and 806 R (GGACTACHVGGGTWTCTAAT) modified from Caporaso et al. [6], targeting the V4 hypervariable region. For the 18 S rRNA, primers 1183 F (AATTTGACTCAACRCGGG) and 1443 R (GRGCATCACAGACCTG) [44] were used. Amplification was carried out using a dual indexing approach modified from Lanzén et al. [25]. Briefly, adapter-linked forward and reverse primers were used in the first amplification step using the following reaction, to a total of 20 µl volume: 1 µl template community DNA; 1 µM each of forward and reverse primers; and 1 × KAPA3G Plant PCR mix (Kapa Biosystems, Wilmington MA). The following PCR parameters were used: initial denaturation at 95 °C for 15 min, followed by 25 cycles of 95 °C for 20 s, 55 °C for 30 s, 72 °C for 30 s with a final extension at 72 °C for 7 min. Amplicon libraries were cleaned using AMPure XP (Beckman Coulter Genomics). Barcoded primers were used in the second amplification step (10 cycles) to a total of 50 µl volume as described in Lanzén et al. [25]. Sequencing was carried out using an Illumina MiSeq with the V2 kit and pair-ended 2 × 250 nt at Tecnalia, Spain.

Read-pairs from 16 S rRNA amplicons were overlapped using *vsearch* ([46]; options *fastq_maxdiff = 5, fastq_allowmergestagger*). Overlapped reads were trimmed from primer sequences from both ends using *cutadapt* [32] and then truncated using *vsearch–fastq_filter* to a length of 252 nt (discarding shorter sequences and those with > 0.5 expected errors). 18 S rRNA gene reads were treated in the same manner except for truncation due to their length polymorphism in the targeted region. Reads shorter than 200 nt were discarded.

All quality-filtered overlapped sequences from 16 S rRNA and 18 S rRNA amplicons, respectively, were first clustered into fine-scale OTUs using SWARM v2 [29], then subjected to reference-based (with the *rdp_gold dataset for 16S and SilvaMod106 for 18S)* and thereafter *de novo* chimera filtering, using UCHIME as implemented in *vsearch* [46]. Resulting representative (centroid) sequences from chimera-filtered OTUs were thereafter subjected to a second clustering using *vsearch* with 97 and 98% similarity thresholds for 16 S and 18 S rRNA sequences, respectively [45].

Representative OTU sequences were aligned to the SilvaMod v106 (16 S and 18 S rRNA) reference databases using *blastn* (v.2.2.25 + task megablast) and taxonomically classified with CREST using default parameters [24]. Resulting taxon distributions were studied at order rank as determined by CREST.

2.4. Statistical analysis

The effects of corn stover incorporation (SI) *versus* corn stover removal (SR) on soil physicochemical and microbial properties were