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A calorimetric study of the interaction between *Brachiaria platyphylla* and soil microbial activity

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ABSTRACT

Recently, lemon tree plantations in Tucumán province, Argentina were invaded with the weed *Brachiaria platyphylla* (broadleaf signalgrass). In order to understand the mechanisms involved with its propagation, soils collected from the invaded (IS), not yet invaded (NIS) and from an adjacent forest soil (FS) were comparatively studied by chemical and calorimetric techniques. Also the ethyl acetate (EtOAc) extract of aerial parts and roots as well as the methanol (MeOH) extract of aerial parts were studied in their interaction with soil. Two probable factors involved with *B. platyphylla* invasibility were found. The first deals with the quality of the IS that resembled more the indigenous FS than the NIS. The microbial quotient (C_{mic}/C_{org}) was higher for IS than for NIS. Also, the calorespirometric quotient (p/rCO_2) was lower for the former soil indicating a greater microbial mineralizing activity. The second factor deals with the effect of the organic extracts on soil. The EtOAc extract of aerial parts of *B. platyphylla* showed antimicrobial and allelopathic effects that could be attributed to its phenolic content. However, the MeOH extract of aerial parts and EtOAc extract of roots seemed to induce the activity of phenol degrading bacteria. Therefore, the phenolic content of this species would not be a problem for itself although they seem to act as allelochemicals for native species.

1. Introduction

Invasive species can change the diversity and relative abundance of native species and can alter the successional dynamics of the community over time [1]. The mechanisms facilitating the invasion of exotic plants, resulting in the displacement of indigenous flora are often cited as direct or indirect resource and interference competition [2]. Plant interference can be defined as any physical or chemical mechanism that induces the reduction of plant growth over time due to the presence of other plant. On the other hand, competition is a process where plants interfere with the growth of neighbouring plants by utilization or competition for resources including light, space, nutrients and water [3]. Most of these investigations have been conducted on plants and fauna but soil microbial communities can also respond to and mediate exotic plant invasion as was demonstrated with the invasion of *Mikania micranta* [4]. An increase of the microbial quotient, C_{mic}/C_{org} (microbial biomass carbon/organic carbon) and of basal respiration and a decrease of the respiratory quotient, qCO_2, with the increase of *M. micranta* cover was reported.

Recently, fields with lemon tree plantations in the Province of Tucumán, Argentina, were invaded by the Poaceae species *Brachiaria platyphylla* (Munro ex C. Wright) Nash (broadleaf signalgrass). This species is highly invasive and very resistant to the most common herbicides causing great expenses to the producers in their fight against it. Broad leaf signalgrass or *B. platyphylla* is a warm season annual grass that is more difficult to control than other weeds [5]. In studies where the interference of broadleaf signalgrass with corn was investigated, it was shown that the yield reduction of corn occurred due to weed density (>150 plants m^{-2}) and when both species emerged together [6]. Studies were also conducted to evaluate environmental conditions on *B. platyphylla* seed germination [7]. However, to our knowledge, nothing has yet been investigated about the interaction of this weed with soil and soil microbial activity. The effect of organic extracts of *Ixorhea tschudiana*, an endemic species to north western Argentina, on soil microbial activity was studied by calorimetry [8]. Results indicated that 83 and 250 mg kg^{-1} of the MeOH and $CHCl_3$ extract seemed to selectively inhibit the growth of certain microorganisms and to enhance the activity of soil actinomycetes. Thus, calorimetry proved to be an excellent tool to study these types of interactions and therefore, it was used to understand the complex mechanisms involved with the invasion of *B. platyphylla.*

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In this sense, soils collected from the invaded area were studied and compared with studies of soil collected from a yet not invaded area of the same field as well as with soil collected from an adjacent forest that contains the indigenous soil. Also, the interaction of soil with the EtOAc and MeOH extracts of aerial parts and of the EtOAc extract of roots of *B. platyphylla* was investigated by using calorimetric techniques.

2. Experimental

2.1. Plant and soil material

Brachiaria platyphylla (Munro ex C. Wright) Nash (whole plant) was collected in a lemon trees plantation located in Los Pizarros, La Cocha, Tucumán province, Argentina (27° 45′ S, 65° 39′ W) in February 2007. Simultaneously, soil was sampled by choosing seven points at random and up to a depth of 10 cm, after removing the top layer, from the invaded area by *B. platyphylla* (IS) and from areas still not invaded (NIS) from the same field. Soil samples from the adjacent forest were also collected (FS). In the laboratory, plant material was air-dried and roots were excised from aerial parts. Soil material was sieved (2 mm × 2 mm) and stored at 5 °C in polyethylene bags until used. Identification of the plant species was done by botanists of Botanic, Pharmacy Institute, Faculty of Biochemistry, Chemistry and Pharmacy, National University of Tucumán, Argentina. The soil used for treatment with extracts of aerial parts of *B. platyphylla* were collected previously from the same area and were already stored in the laboratory whereas the FS was used to test the EtOAc extract of roots. Seeds of tomato (*Solanum lycopersicum*) and onion (*Allium cepa*) were acquired from the market with 88% germinability.

2.2. Plant extracts

Roots (after grinding) or aerial parts of *B. platyphylla* were successively extracted with EtOAc and MeOH (2×) during several days. Extracts were taken to dryness in a vacuum evaporator and were used for germination assays (2.8) and to evaluate their effect on soil.

2.3. Soil amendment with extracts

An aliquot of soil (10 g dry weight, dw) was amended and thoroughly mixed with 595 mg extract dissolved in the minimum amount of the solvent of extraction. Then, soil was dried during 24 h at 40 °C and further placed 24 h in desiccator under vacuum to eliminate the solvent. Aliquots of this extract containing soil were used to amend soil (700 g dry weight, bulk density: 1.03 g cm^{-3}) as to obtain final extract concentrations of 500, 250 and 100 mg kg^{-1}. These amended soils were placed in pots (105 cm^3, 9 cm deep) of a 25 pots garden plug at field capacity humidity (FCH = 25.5% and 24.0% for EtOAc and MeOH extracts of aerial parts, respectively). The plugs with treated soil samples (6 replicates per treatment, each one used as replicate for chemical, microbiological and calorimetric experiments) were wrapped with polyethylene to avoid humidity losses and left at room temperature from April to June 2007 (20–30 °C). EtOAc extract of roots was incorporated to FS (bulk density: 0.90 g cm^{-3}, FCH: 30.5%) in the same way as those of aerial parts except that the final water content (WC) of soil was 60% of FCH. After incubation, the six replicates per treatment were air dried to a WC close to 50% of FCH and stored individually in polyethylene bags at 5 °C until analysed.

2.4. Chemical and microbiological soil analysis

The WC was determined by drying an aliquot (2×) until constant weight at 105 °C [9]. Bulk density and FCH were determined by the graduated cylinder method [10]. The pH was measured with a glass electrode on a suspension of soil in deionised water (1:1) [10]. Organic carbon (OC) was determined by wet oxidation with $K_2Cr_2O_7/H_2SO_4$ [11]. Extractable phosphorus (P) was photometrically determined by the Olsen extraction method [12]. The Folin Ciocalteu's method was used to determine total phenol content (TPC) [13] in soil extracts and the results are expressed as μg of gallic acid equivalent (GAE) per gram of dry soil. Total carbohydrate content (CHC) in soil extracts was determined by the phenol–sulphuric acid method [14] and results are expressed as μg of glucose equivalent (GE) per gram of dry soil. Extractable nitrogen, as the sum of NO_3^-, NO_2^- and NH_4^+ was determined by the diffusion method [15]. Colony forming units (CFU g^{-1}) were determined by the serial dilution method by using trypteine soy agar (TSA) as culture media.

2.5. Soil extraction

Soil extracts were produced by sonicating soil suspended in MeOH (1:1.5, w/v) during 10 min. Then, soil extracts were filtered through Whatmann N° 1 filter paper and evaporated to dryness in rotary evaporator. An aliquot (about 4 mg) extract was re suspended in 1 ml MeOH for TPC and CHC determinations.

2.6. Calorimetric analysis

A twin heat conduction type calorimeter (Lund University, Sweden) was used [8,16,17]. Soil sample (3.0–4.0 g, dw) was stabilized during 24 h at 25 °C in a polyethylene bag. Then, an appropriate amount of water containing glucose as to get FCH and 1.5 mg glucose per g of dry soil was added. The soil was thoroughly mixed by hand and then, the equivalent to 1.0–1.5 g (dw) was weighed in the calorimeter ampoule (8.0 cm^3). The ampoule was hermetically closed and after the 30 min needed to equilibrate the calorimetric system, thermal power (P)–time (t) curves of microbial growth were recorded at 25 °C. An ampoule containing 1.0 g agar was used as reference. Blank experiments were performed with soil at FCH to correct the p–t curves of microbial growth for other thermal effects than those of glucose. By using Microsoft Excel 2002 (Microsoft corporation) and the Origin 6.0 program (Microcal, Inc.) the curves obtained were converted into mass specific thermal power (P)–time (t) curves and integrated to obtain the specific heat (q) associated with the glucose degradation. From the semilogarithmic conversion of the portion of the curve that indicates exponential microbial growth ($\log p = \log p_0 + \mu t$) the rate of microbial growth constant, μ, was calculated as well as the value of p at $t = 0$, p_0. Replicate curves were then averaged and the average curve of blank experiments was subtracted to obtain the average p–t curve of microbial growth due to glucose degradation. The value of μ together with the value of peak time (t_p) allows quantification of the increment in CFU g^{-1} (ΔCFU g^{-1}) by applying the equation of microbial growth [8,18]. Results are reported as an average of three replicates ± SD. This SD was determined from the curves without correction and recalculated for the corrected average curve.

2.7. Calorespirometry

Calorespirometric assays were performed by using soil (1–1.5 g, dw) at FCH. Once the system was equilibrated and the values of thermal power, P were constant (P_1), a vial containing a solution of 0.4 N NaOH (trap of CO_2) was introduced and values of P were collected again (P_2). After collecting data for 2–3 h, the vial was removed, and metabolism was measured again (P_3) [8,19]. As our calorimeter does not allow a simultaneous manipulation of both ampoules (reference and sample), several blank experiments were run by introducing a vial with water instead of NaOH into the