Thermochimica Acta 526 (2011) 157–162

Contents lists available at SciVerse ScienceDirect

Thermochimica Acta

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

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

M. Eugenia Sesto Cabral[a], Fanny I. Schabes[b], E. Elizabeth Sigstad[a,b,*]

[a] *INQUINOA – CONICET, Instituto de Química Orgánica, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Ayacucho 471, CP T 4000 INI, Tucumán, Argentina*

[b] *Instituto de Química Orgánica, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Ayacucho 471, CP T 4000 INI, Tucumán, Argentina*

ARTICLE INFO

Article history:
Received 7 July 2011
Received in revised form
19 September 2011
Accepted 20 September 2011
Available online 29 September 2011

Keywords:
Calorimetry
Brachiaria platyphylla
Invasiveness
Soil microbial activity

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.*

* Corresponding author at: INQUINOA – CONICET, Instituto de Química Orgánica. Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Tucumán, Argentina. Tel.: +54 381 435 2530.
E-mail addresses: lizzie@fbqf.unt.edu.ar, esigstad@gmail.com (E.E. Sigstad).

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doi:10.1016/j.tca.2011.09.015

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

Table 1
Values of pH, organic carbon (OC), available phosphorus (P), nitrogen as $NO_3^- + NO_2^- + NH_4^+$ (N), total phenolic content (TPC), carbohydrate content (CHC), colony formation units (CFU g^{-1}) and the apparent microbial quotient (CFU g^{-1}/OC) for forest soil (FS), invaded soil (IS), not invaded soil (NIS), incubated control (CS), 100, 250 and 500 mg kg^{-1} EtOAc and MeOH extracts of *B. platyphylla*. AE, aerial parts; R, roots.

Soil	pH	OC/g kg^{-1}	P/mg kg^{-1}	N/mg kg^{-1}	TPC/μg GAE g^{-1}	CHC/μg GE g^{-1}	Colony forming units (10^{-6} CFU g^{-1})	Apparent microbial quotient (10^{-7} CFU g^{-1}/OC)
FS	6.77 ± 0.05^b	23.3 ± 0.2^c	32.0 ± 0.1^a	74.5 ± 0.5^c	ND	ND	2.27 ± 0.02^c	9.7 ± 0.2^c
NIS	6.29 ± 0.01^a	16.1 ± 0.1^a	33.3 ± 0.3^b	57.5 ± 4.3^b	144.1^a	141.7^a	0.91 ± 0.01^a	5.7 ± 0.1^a
IS	7.51 ± 0.02^c	17.9 ± 0.1^b	40.8 ± 0.7^c	51.1 ± 0.5^a	144.1^a	225.0^b	1.55 ± 0.01^b	8.7 ± 0.1^b
AE-EtOAc extract								
CS	6.28 ± 0.02^b	19.8 ± 0.2^a	36.6 ± 0.2^c	63.2 ± 2.9^b	24.8^a	17.5^a	1.87 ± 0.19^c	9.5 ± 1.1^c
100	6.61 ± 0.08^c	21.0 ± 0.6^b	26.7 ± 0.1^b	44.9 ± 2.0^a	53.6^b	35.4^b	1.48 ± 0.18^b	7.0 ± 1.1^b
250	6.57 ± 0.03^c	20.4 ± 0.1^b	26.7 ± 0.2^b	48.8 ± 4.9^a	89.4^c	63.2^c	1.39 ± 0.07^b	6.8 ± 0.4^b
500	5.90 ± 0.04^a	23.0 ± 0.5^c	20.5 ± 0.3^a	62.3 ± 0.5^b	288.9^d	119.2^d	0.99 ± 0.03^a	4.3 ± 0.2^a
AE-MeOH extract								
CS	5.95 ± 0.04^c	15.6 ± 0.1^c	41.7 ± 0.2^d	68.9 ± 3.7^c	1012.1^d	395.1^d	2.03 ± 0.03^c	13.0 ± 0.3^b
100	5.43 ± 0.01^a	14.2 ± 0.1^a	32.8 ± 0.1^c	59.6 ± 1.7^b	472.4^c	164.7^c	1.74 ± 0.04^a	12.3 ± 0.4^{ab}
250	5.86 ± 0.03^b	14.1 ± 0.1^a	28.0 ± 0.1^a	50.0 ± 2.0^a	70.1^a	41.7^a	1.94 ± 0.01^b	13.7 ± 0.2^c
500	5.91 ± 0.04^{bc}	14.7 ± 0.2^b	29.1 ± 0.2^b	57.4 ± 1.5^b	195.0^b	68.2^b	1.76 ± 0.01^a	12.0 ± 0.2^a
R-EtOAc extract								
CS	6.72 ± 0.01^d	20.9 ± 0.1^d	39.3 ± 0.6^d	55.9 ± 0.1^c	157.5^c	21.8^b	1.54 ± 0.03^b	7.4 ± 0.2^b
100	6.22 ± 0.01^b	14.3 ± 0.1^a	22.0 ± 0.6^a	54.1 ± 0.6^b	100.6^b	13.7^a	1.68 ± 0.05^c	11.7 ± 0.4^d
250	6.11 ± 0.01^a	17.3 ± 0.1^b	28.4 ± 0.5^b	52.3 ± 1.4^b	83.4^a	19.4^b	1.73 ± 0.03^c	10.0 ± 0.2^c
500	6.67 ± 0.02^c	19.9 ± 0.1^c	33.9 ± 1.2^c	49.5 ± 0.3^a	104.3^b	26.2^c	1.18 ± 0.06^a	5.9 ± 0.3^a

Different letters indicate significant differences among values ($p < 0.05$).

ampoule containing soil or agar. In both cases an exothermic shift occurred. The average values obtained were 4.0 ± 1.0 μW that was subtracted from P_2 to obtain the value of CO_2 evolution. The specific rate of CO_2 evolution, rCO_2, was calculated by using the expression: $rCO_2 = \{P_2 - [(P_1 + P_3)/2]\}/108.5m$. The value of −108.5 kJ mol^{-1} is the heat of reaction of CO_2 with NaOH to produce $CO_3^=$, and 'm' is the dry weight of soil.

2.8. Germination assays

Germination assays were performed in a chamber at 25 °C by placing twenty seeds on Petri dishes containing 1% agar with water, 100, 250 and 500 mg dm^{-3} AE-EtOAc or AE-MeOH extract of *B. platyphylla*. To avoid a solvent effect, 1 g agar was thoroughly mixed with the extract solution in either EtOAc or MeOH. This agar was placed 24 h at 40 °C and further placed under vacuum another 24 h to eliminate solvent. Then, aliquots were weighed and mixed with the 1% agar solution to achieve the desired extract concentration in the Petri dish. The end of germination was considered when cotyledons were differentiated (14 days) and then, number of germinated seeds, seedlings weight and length were evaluated.

Percentage of inhibition was calculated by the expression: $\%I = [(\text{control} - \text{treatment})/\text{control}] \times 100$.

2.9. Statistics

Results are reported as the mean of at least 3 measurements (±SD) on dry weight (dw) basis. One way Anova was used to determine differences between treatments by means of the computer program Origin 6.0 (Microcal, Inc.).

3. Results and discussion

Table 1 shows the chemical and microbiological characteristics of the studied soils. Soil microbial biomass (SMB) was also determined by the calorimetric method [20] for IS (636 ± 39 μg g^{-1}) and NIS (461 ± 51 μg g^{-1}).

Fig. 1 shows specific thermal power (p)–time (t) curves of microbial growth for the studied soils. It is worth to note here the similar p–t curves for FS (Fig. 1A, curve a) and for IS (Fig. 1A, curve b). On the other hand, major modifications were observed in the kinetics of glucose degradation for soils treated with 500 mg kg^{-1} EtOAc extract of roots (Fig. 1C, curve d) and with the MeOH extract of aerial parts (Fig. 1D, curves b–d) of *B. platyphylla*.

Table 2 shows the thermodynamic parameters calculated from the p–t curves of Fig. 1. It was interesting to note that the values of peak time (t_p) specific thermal power when no glucose was added (p_0) and rate of microbial growth (μ) for the IS were not significantly different from those of the FS indicating that the microbiota of both soils showed a similar basal metabolism (given by p_0) and degraded glucose at a similar rate (given by t_p and μ). However, values of apparent increased biomass (ΔCFU g^{-1}) and of heat yield ($Y_{q/\Delta CFU/g}$) indicated higher and more efficient biomass production in the FS probably, due to a higher Nitrogen content of the latter. In turn, the IS was more efficient to convert glucose into microbial biomass than the NIS. This was clearer when the values of SMB were used for these calculations. The IS produced 19.2 mg g^{-1} with

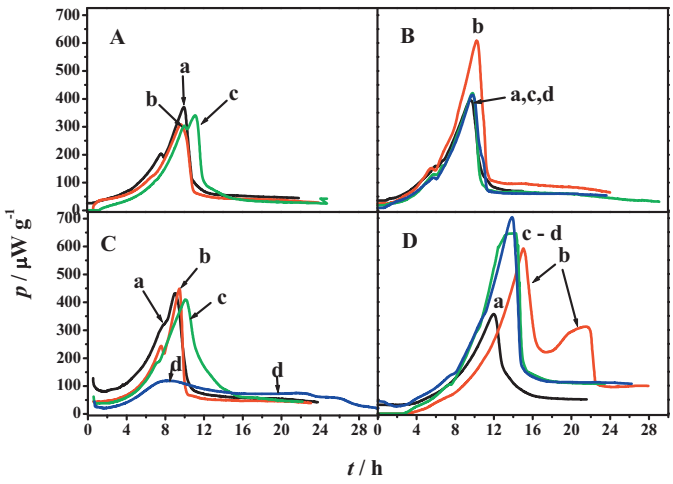

Fig. 1. Specific thermal power (p)–time (t) curves for the degradation of 1.5 mg g^{-1} glucose by the microorganisms of (A) soils collected from (a) forest, FS; (b) invaded, IS and (c) not invaded, NIS with *B. platyphylla*. Soil samples incubated with (B) EtOAc extract of aerial parts; (C) EtOAc extract of roots and (D) MeOH extract of aerial parts of *B. platyphylla* (a) control, (b) 100, (c) 250 and (d) 500 mg kg^{-1} extract.

Table 2
Values of peak time (t_p), specific thermal power (p_0) when no glucose was added, specific thermal power (p_{tp}) at t_p, heat (q_R) due to the degradation of 1.5 mg g^{-1} glucose, the growth rate constant (μ), the increment of CFU g^{-1} during microbial growth (ΔCFU g^{-1}) and the heat yield ($Y_{q/\Delta CFU/g}$) for soil samples collected from: forest (FS), not invaded (NIS) and invaded soil (IS) and those for control (CS) and incubated with: 100, 250 and 500 mg kg^{-1} of EtOAc and MeOH extracts of *B. platyphylla*. AE, aerial parts; R, roots.

Soil	t_p (h)	p_0/μW g^{-1}	p_{tp}/μW g^{-1}	$-q$/J g^{-1}	μ (h^{-1})	Increment of CFU g^{-1} (10^{-7} ΔCFU g^{-1})	Heat yield, $Y_{q/\Delta CFU/g}$ (nJ CFU^{-1})
FS	10.0 ± 0.2^a	10.8 ± 4.1^a	370.0 ± 16.5^b	6.17 ± 0.37^b	0.157 ± 0.019^b	10.7	57.7
NIS	11.1 ± 0.0^b	17.8 ± 4.1^b	340.8 ± 21.5^a	6.20 ± 0.39^b	0.117 ± 0.014^a	5.7	108.8
IS	9.7 ± 0.2^a	10.8 ± 2.2^a	308.3 ± 6.9^a	5.21 ± 0.16^a	0.154 ± 0.005^b	5.8	89.8
AE-EtOAc extract							
CS	9.6 ± 0.3^a	23.9 ± 3.4^b	394.8 ± 46.3^a	6.95 ± 0.25^b	0.135 ± 0.007^a	3.5	198.6
100	10.3 ± 0.1^b	21.6 ± 2.3^b	608.1 ± 20.2b	9.07 ± 0.36^c	0.145 ± 0.003^{ab}	4.5	201.5
250	9.8 ± 0.2^a	15.4 ± 0.5^a	420.4 ± 10.9^a	6.01 ± 0.15^a	0.150 ± 0.005^b	4.0	150.3
500	9.8 ± 0.4^{ab}	19.9 ± 3.6^b	414.3 ± 43.1^a	6.12 ± 0.46^a	0.138 ± 0.005^a	2.1	291.4
AE-MeOH extract							
CS	12.0 ± 0.2^a	13.3 ± 1.5^b	356.4 ± 13.9^a	7.28 ± 0.62^a	0.122 ± 0.005^b	5.7	127.7
100	15.0 ± 0.1^c	7.6 ± 0.2^a	591.8 ± 33.3^b	15.74 ± 0.66^c	0.128 ± 0.003^b	14.3	110.1
250	14.2 ± 0.5^b	9.9 ± 2.5^{ab}	645.6 ± 10.6^c	13.06 ± 1.14^b	0.143 ± 0.010^c	20.6	63.4
500	13.9 ± 0.0^b	20.3 ± 3.5^c	703.4 ± 107.3^{bc}	13.05 ± 1.91^b	0.113 ± 0.002^a	6.4	203.9
R-EtOAc extract							
CS	9.0 ± 0.3^b	13.4 ± 1.1^b	432.2 ± 0.7^c	8.16 ± 0.11^b	0.170 ± 0.000^b	6.9	118.3
100	9.5 ± 0.1^b	3.6 ± 0.5^a	448.6 ± 24.0^c	5.89 ± 0.21^a	0.225 ± 0.007^c	26.7	22.1
250	10.1 ± 0.1^c	16.9 ± 1.9^c	409.4 ± 6.9^b	8.27 ± 0.76^b	0.143 ± 0.004^a	4.5	183.8
500	8.2 ± 0.2^a	9.6 ± 2.7^b	119.5 ± 9.9^a	7.57 ± 1.04^b	0.158 ± 0.006^a	2.2	344.1

Different letters indicate significant difference among values ($p < 0.05$).

a heat yield of 0.27 kJ g^{-1} whereas NIS produced 8.7 mg g^{-1} with 0.71 kJ g^{-1}. These results were in coincidence with the higher values of OC, P, CHC and CFU g^{-1} for the IS than for NIS as shown in Table 1 indicating that *B. platyphylla* improved soil quality. An index used to monitor soil quality is the ratio between the SMB and OC content which is called microbial quotient [4,21,22]. This ratio reflects the contribution of SMB to OC and can be used as indicator of net carbon loss or accumulation. It gives an insight of soil capability to support growth [23]. As we did not determine SMB except for IS and NIS, we used the value of UFC g^{-1}/OC ratio for the other soils and called it apparent microbial quotient. The calculated microbial quotient (C_{mic}/C_{org}) for IS and NIS were 35.5 ± 2.4 and 28.6 ± 3.3 mg g^{-1}, respectively indicating that the microorganisms of the IS had slightly greater capability to take part in the recycling of carbon than those of NIS. Besides, calorespirometric experiments confirmed these findings. Fig. 2 shows the results obtained. The calorespirometric constant (p/rCO_2) is the ratio between the basal metabolism or basal specific thermal power and the specific rate of CO_2 evolution of soil without glucose. This parameter gives a measure of the mineralizing activity of the microorganisms and

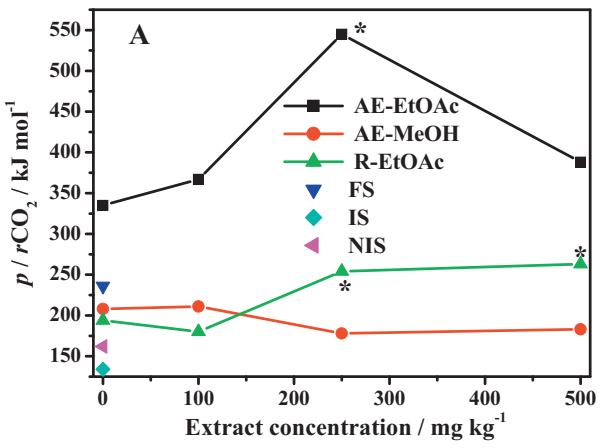

Fig. 2. Values of the calorespirometric quotient (p/rCO_2) as a function of extract concentration. *Significantly different from control ($p < 0.05$).

it is equivalent to the metabolic quotient, qCO_2, determined by conventional methods [21]. The lower the value the higher is the mineralizing activity. The decreasing trend of the values of p/rCO_2 was FS, NIS and IS. It is well documented that improvement of the conditions of the microbial community promotes the permanence of a plant species over others [24–26]. This might be a key factor on *B. platyphylla* invasion as was also reported for *Mikania micranta* invasion [4].

In order to understand the factors involved with the improvement of soil edaphic properties, the interaction of *B. platyphylla* organic extracts with soil were investigated.

The EtOAc extract of aerial parts of *B. platyphylla* (AE-EtOAc) seems to contain antimicrobial substances. Note in Table 1 that values of CFU g^{-1} decreased as extract concentration increased. Furthermore, values of CFU g^{-1} determined for the three soils containing extract were negatively correlated with the corresponding total phenolic content (TPC) values ($R^2 = 1$) indicating that these compounds might be responsible for the antimicrobial activity as it was previously reported [27]. Moreover, when the values of ΔCFU g^{-1} (see Table 2) were plotted as a function of TPC a negative correlation was also obtained ($R^2 = 0.99$) probably indicating the incidence of these compounds on the degradation of glucose. Note in Table 2 that the microbiota of the soil containing 250 mg kg^{-1} AE-EtOAc was the most efficient to degrade glucose (see $Y_{q/\Delta CFU/g}$) as compared with the other soils of the same treatment. However, p/rCO_2 gave the highest value for this soil (Fig. 2). It was reported that soil microbial biomass always keeps the ATP pool and adenilate energy charge (AEC) values higher or similar to those measured during exponential microbial growth [28]. It was hypothesized that this occurs as a survival strategy so when the microorganisms receive a readily available carbon source they are ready to metabolize. Thus, microorganisms of soil treated with 250 mg kg^{-1} AE-EtOAc might be the most affected and therefore show poor mineralizing activity in comparison to the other soils of the same treatment. But as soon as they got glucose they were the most efficient to grow.

Apparently, substances included in the EtOAc extract of roots (R-EtOAc) and MeOH extract of aerial parts (AE-MeOH) of *B. platyphylla* are involved with the higher mineralizing activity of the microbiota contained in the IS. Note in Table 1 the higher apparent microbial

Table 3
Percentage of inhibition with respect to control of tomato and onion seedlings for: germination (G), seedlings weight (m_s), seedlings length (l_s) and roots length (l_r). Only inhibition parameters significantly different from control are reported ($p < 0.05$).

Sppextract – mg dm^{-3}	G	m_s	l_s	l_r
Tomato				
EtOAc – 100	−36		−34	−15
EtOAc – 250		−55		
EtOAc – 500		−56	−51	−22
MeOH – 250			−26	−18
MeOH – 500	−23		−24	−23
Onion				
EtOAc – 100			−24	
EtOAc – 250		−33	−41	−55
EtOAc – 500	−33	−33	−43	−69
MeOH – 100		+30	+44	
MeOH – 500	−27			

quotient for soils treated with 250 mg kg^{-1} AE-MeOH and with 100 and 250 mg kg^{-1} of R-EtOAc extracts. However, calorimetric results indicated that optimum extract concentrations where soil microbiota was efficient to degrade glucose were 250 mg kg^{-1} AE-MeOH and 100 mg kg^{-1} R-EtOAc (Fig. 2). The mineralizing activity of the microbiota contained in both soils seemed to be the same. Something interesting to remark is that the apparent biomass formed (ΔCFU g^{-1}) by degradation of glucose by the microorganisms of soils of AE-MeOH were also negatively correlated with TPC of soils ($R^2 = 1$). Moreover, TPC in these soils decreased as extract concentration increased (see Table 1) indicating probably that the MeOH extract induces the activity of phenols degrading bacteria.

These results were really interesting and made us think in a hypothesis for the mechanism of invasion. What about if phenols of *B. platyphylla* acted as allelochemicals against the native plant community? And once this community was weakened soil microflora was modified to its convenience? Where phenol degrading bacteria were involved?

To prove this hypothesis firstly we had to prove that AE-EtOAc and AE-MeOH extracts had allelopathic activity. Table 3 shows the results obtained. Note that 100 mg kg^{-1} AE-EtOAc inhibited germination of the dicot tomato in 36% whereas a much lower inhibition was obtained with 500 mg kg^{-1} MeOH extract. A concentration of 500 mg kg^{-1} of both extracts inhibited germination of the monocot onion. However, 100 mg kg^{-1} AE-MeOH extract increases seedlings weight and length whereas the AE-EtOAc extract reduces them. These results prove that the EtOAc extract has allelopathic activity.

4. Conclusions

This work shows that calorimetry in combination with chemical and microbiological analysis can facilitate the understanding of plant invasion. Soil collected from the invaded area (IS) with *B. platyphylla* as well as soil collected from a no yet invaded (NIS) area from the same field and soil collected from the adjacent forest (FS) were comparatively studied. It was found that the soil invaded by this weed showed higher quality than the no invaded one and that it approached the quality of the adjacent forest soil. The calculated microbial quotient was higher for the IS than for NIS indicating greater mineralizing activity for the microorganisms contained in the IS. This was confirmed by a lower p/rCO_2 ratio for the latter soil. This might be related to the high invasibility of this species. As it changes the characteristics of the soil such as an increased pH and P content, probably for its own survival strategy, other native species are exterminated.

Another probable factor involved with the invasibility of *B. platyphylla* was found when organic extracts of aerial parts and roots were investigated in their interaction with soil. The EtOAc extract of aerial parts of *B. platyphylla* showed antimicrobial activity which was attributed to its phenolic content. On the other hand, the MeOH extract of aerial parts of the weed and the EtOAc extract of roots seemed to induce the activity of phenol degrading bacteria. Therefore, the phenolic compounds of this species would not be a problem for the plant itself although they seem to act as allelochemicals for native species; this could be another key factor in the species invasibility.

Acknowledgments

The authors thank CONICET, UNT and FONCyT for providing founds to perform this research as well as to UNT–FONCYT and to CONICET for the scholarships of M.E. Sesto Cabral.

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