

Soil Microbial Respiration According to the Wheat Culture History

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ABSTRACT

Soil micrganisms represent one of the largest reservoirs of biodiversity in the world. Soil microorganisms are very important as almost every chemical transformation taking place in soil involves their active contributions. The study of soil dynamics in presence of phytotoxic agents is of great importance because of the role that microorganisms have in modifying the phytotoxic compounds and consequently affecting their biological properties. Possible biotransformation developed by soil microorganisms could yield compounds with modified biological properties. The aim of the present work is to study microbial respiration in soil cultivated with different varieties of *Triticum aestivum* L. by measuring the emission of carbon dioxide (CO₂), and by correlating the results to the ability of the soil to degrade allelochemicals and to release CO₂.

Keywords: Benzoxazinones, degradation, CO₂ emission, microflora, allelopathy.

Introduction

Benzoxazinoids have been described as important allelochemicals from Gramineae, Acanthaceae, Rannunculaceae and Scrophulariaceae plants (Niemeyer, 1988; Pratt *et al.*, 1995), with a biological activity including fungistatic (Bravo and Lazo, 1996; Friebe *et al.*, 1998), antifeedant (Klun *et al.*, 1967; Argandoña *et al.*, 1983) and phytotoxic (Barnes and Putnam, 1987; Perez and Ormeno-Nunez, 1991) activities. As part of the ongoing studies into allelochemicals as natural herbicide models, the description of soil dynamics in presence of phytotoxic agents is of great importance because of soil microorganisms have the ability to biotransform them in the compounds with modified biological properties, affecting their allelopathic capability.

Microorganisms in soil are one of the largest reservoirs of biodiversity in the world, where the number of species can reach 10³ and 10⁴ (Foster,

1988). One gram of soil typically contains about 10⁹ to 10¹⁰ bacteria and several hundred meters of fungal hyphae (Clegg and Murray, 2002). Given this high biological activity, it is not surprising that these microorganisms are considered to be a crucial variable in the functioning of an ecosystem and the phenomena that occur within it. As a result, in the study of allelopathy, processes that occur are continually being revised in the search for a better understanding of this phenomenon.

The root system of a plant has a high capacity to exude compounds with a wide variety of physical and chemical properties into the rhizosphere. This exudation is a response to stress or abiotic and biotic factors from the area in which the plant is located. The rhizosphere is defined as the volume of soil that is affected by the presence of the roots of growing plants (Nicholas, 2007). It has been found that the soil zone in which the most marked effects are caused by compounds released by the roots is from the root

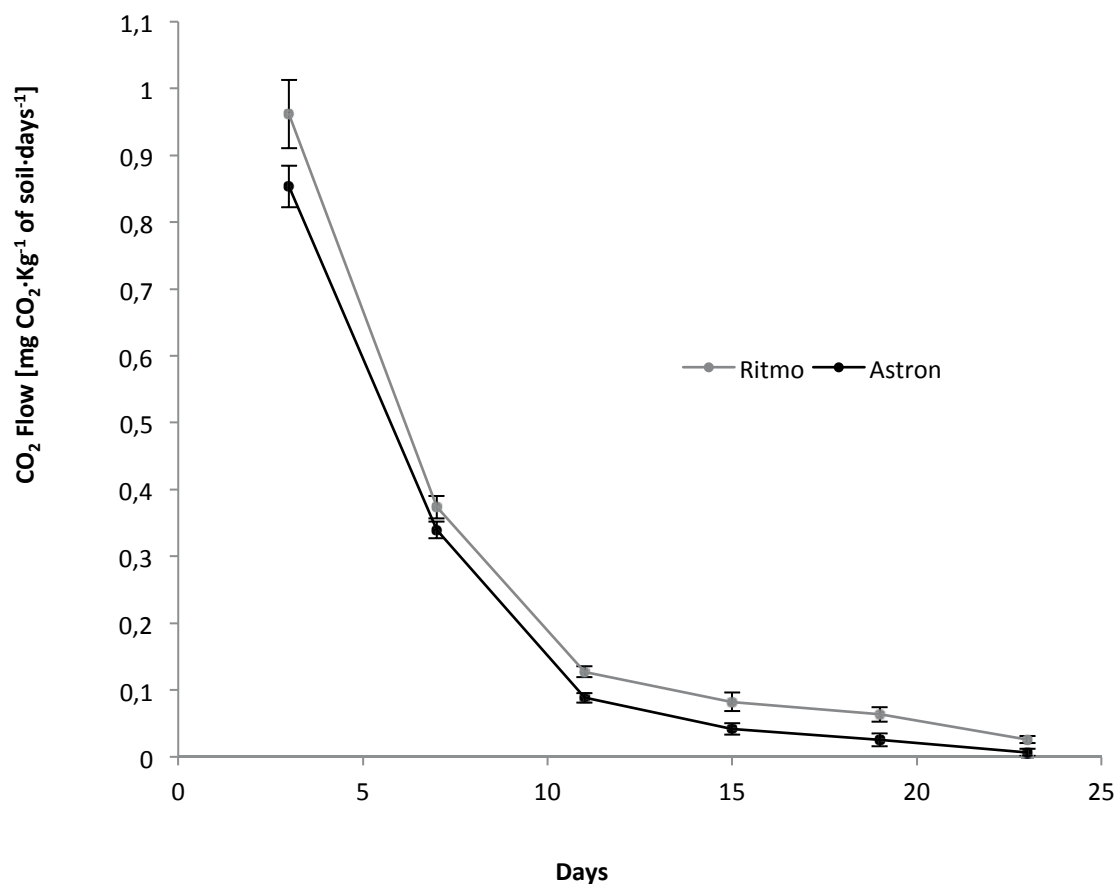


Figure 1. CO₂ flow measured for the soil extracts of two wheat varieties (Ritmo and Astron) during 23 days of incubation. Each point corresponds to the amount of CO₂ emitted by independent soil samples. Error bars represent standard deviation of the mean for n = 3.

surface to a distance of 2 mm. This distance is relative and depends on the soil properties (particle size, water content, pH buffer capacity, etc.) (Nye, 1984; Albrecht, 2002). Thus, most phytochemicals released from plants are founding the rhizosphere and this is also where one would expect the maximum activity to be shown by the compounds or reactions to occur with them, as in the case of biodegradation.

The most widely used methods are measurement of enzymatic activity (Lechevalier, 1977; Bååth *et al.*, 1998) and the emission of carbon dioxide (CO₂). The latter process is the result of the decomposition of organic substrates to obtain energy for growth and microbial functions (Wang *et al.*, 2003). In the work described here we measured the amounts of CO₂ released in order to estimate microbial activity in the soil under investigation.

It is of interest to characterize the biotic characteristics of each soil and to correlate these results with the ability of the soil to degrade allelochemicals and to release CO₂. However, the microbial characterization of soil samples (including

bacteria, fungi, actinomycetes and invertebrates) is very complex, mainly due to its heterogeneity (Macfadyen, 1970; Swift *et al.*, 1979; Lee, 1985; Binet *et al.*, 1998; Williamson *et al.*, 2005).

These work reported here concerned a study of the microbial respiration in different cultivated varieties of *Triticum aestivum* L. soils. In selecting the soils it was important to consider the microbial community in the rhizosphere, which depends on species, variety and stage of development of the growing plant. Therefore, several criteria were applied: (i) the phytotoxic activity of the seedling leachates, (ii) content of hydroxamic acids and (iii) their backgrounding the biotransformation of allelochemicals.

Materials and methods

Selection of Wheat Varieties and Soil Study of the Allelochemical Release

The soils selected were those in which plants that release the greatest quantities of compounds

into the rizosphere were grown, both on the surface (epidermis) and by root exudation. According to this hypothesis, soil microbial communities from different cultures (potentially allelopathic) can be differentiated as a result of their ability to release compounds to the environment. The contents of DIBOA and DIMBOA were determined in six wheat varieties (*Triticum aestivum* cv. Hill, Portal, Ritmo, Astron, Stakado, and Solist). Also, the wheat coleoptile bioassay was used as a protocol for the general evaluation of the bioactivity of wheat extracts (Hancock *et al.*, 1964). Under these two criteria, soils in which Astron and Ritmo varieties had been grown were selected for this study (Macias *et al.*, 2005).

Soil collection

The selected parcels for the cultivation had similar physicochemical characteristics during their cultivation (Table 1) and they did not contain any other plant species in growth. After harvest, samples of soil from the vicinities of the plants were taken to get the maximum density of the microbial associated with the plant underground parts. The samples were taken at a depth of 15–20 cm in a radius of 3 cm around the plant lines. One hundred random soil samples were collected for each crop (0.014 m³). The soil was sieved to a size of 2 mm and the samples were placed in plastic bags and stored at –20 °C prior to study. Before the degradation studies, dry plant material and calcareous stones were removed from the samples using a sieve that gave a distribution of soil particles of less than 1 mm.

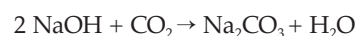
Analysis of hydroxamic acids in soil

All samples were analyzed on a Merck Hitachi HPLC system equipped with a LACHROM L-7100 Quaternary gradient pump, an L-7455 LACHROM Diode Array Detector and an L-7200 LACHROM autoinjector. Data were collected and processed using an HPLC data system (Merck Hitachi D7000). Instrumental conditions for the analysis of hydroxamic acid derivatives were Lichrospher 100 RP-18 (250×4.0 mm, 5 µm) reversed-phase column at 25 °C. Mobile phases were water:1% AcOH (a) and methanol:1% AcOH (b) at a flow rate of 1 mL min⁻¹. The injection volume was 50 µL. The following gradient was used for separation: at 0 min, 30% b; 2 min, 30% b; 19 min, 60% b; 21 min, 100% b. Under these conditions, the following retention times were obtained for each compound: DIBOA 9.01 min; DIMBOA 10.69 min; BOA 12.56 min; MBOA, 16.56 min; AMPO, 19.74 min and APO 24.03 min. The detection was carried out at the following wavelengths: 254, 253, 263, 271,

and 280 nm for DIBOA, DIMBOA, BOA, MBOA, AMPO and APO. For quantitative analysis, stock solutions (1 mg/mL) of each individual standard were prepared by dissolving accurate amounts of pure standard in acidified MeOH (1% AcOH). Working standard solutions were obtained by further dilution of stock solutions with MeOH:acidified H₂O (1% AcOH) (70:30). These solutions were used to generate the external standard response calibration curves for subsequent measurement of quality parameters and concentrations of hydroxamic acid derivatives in soils at different times.

Measurement of soil respiration

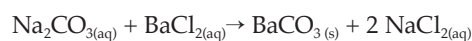
Measurements of microbial biomass in soil were performed according to the method of Jenkinson and Powlson, wherein the microbial populations is determined by recording the amount of CO₂ emitted (Jenkinson and Powlson, 1976). This method is based on the measurement of CO₂ released during the microbial activity in the soil and trapping it in a solution of NaOH, in which the following reaction takes place:



The determination of the initial and final amounts of NaOH enables the amount of NaOH that has reacted with carbon dioxide to be calculated (2 mol NaOH:1 mol CO₂). In this method, the determination of these amounts is carried out by titration with hydrochloric acid, using phenolphthalein to indicate the end point of the titration.

For these measurements, 24 g of each dry and moist soil was used with 7.16 mL of distilled water, representing 55% of imbibitions (100% point imbibition = 0.5428 mL.g⁻¹ soil). The moistened soil was placed in a 2.5 liter bottle and two 25 mL beakers, one containing 20 mL of distilled water and the other 20 mL of 0.5N NaOH, were prepared. The system was closed and the samples were incubated at 25 °C for different times.

At the end of the incubation time a 30% (w/v) solution of BaCl₂ (2.0mL) was added to each of the two beakers to precipitate the CaCO₃ formed by the reaction of NaOH with CO₂. This step is necessary because CaCO₃ may interfere in the titration of free NaOH with HCl. The reaction is:



Once the CaCl₂ had been added, the two samples were titrated with 0.5 N HCl in the presence of phenolphthalein to determine the end point. The

Table 1. Physicochemical Analysis of Wheat Crop Soil.

Exchange capacity (mequiv/100g)	33.91
Exchangeable cations (mequiv/100g)	
Ca ²⁺	28.96
Mg ²⁺	3.08
Na ⁺	0.29
K ⁺	1.58
Carbonates (%w/w)	18.25
Active limestone (%w/w)	8.81
Asimilable phosphorus (Olsen, ppm)	8.7
Total organic content (ppm)	1.35
Organic nitrogen (%w/w)	0.08
pH (H ₂ O:1/2.5)	8.33
pH (KCl)	7.31
Asimilable potassium (ppm)	635
Clay (%w/w)	52.6
Sand (%w/w)	15.4
Slime (%w/w)	32.0
Textural classification	clay

determination of free NaOH (which had not reacted with CO₂) allowed the reacted amount to be calculated (corrected to the amount given by the control, which is the amount of CO₂ in the atmosphere). With this value, the amount of CO₂ trapped was determined (along with that generated by the ground) by titration with NaOH.

Statistic

Assays for the measurement of the release of CO₂ were carried out in triplicate and calculated the corresponding standard deviations. To compare the difference between the two data series we used the Tukey’s test. These calculations were performed using the Statistica 10.0 software (Statsoft, 2012).

Results and discussion

The study of microorganisms by conventional methods includes culture processes, isolation and *in vitro* classification techniques. These techniques can be very laborious and, in some cases, the use of culture media can only promote the growth of certain groups of microorganisms (Bakken, 1985). Moreover, the separation of microorganisms from the soil particles is unreliable (Schallenberg *et al.*, 1989).

Thus, indirect measures of microbial activity are used to estimate the microbial community in soils.

The results for the two types of soil are shown in Figure 1, in which the flows of CO₂ for different incubation times for the two soil types are indicated. The behavior is similar to that usually described for these measurements, i.e., a high initial CO₂ evolution that gradually decreases to almost zero after 23 days of incubation.

As the nature of the microorganisms in the soil is unknown, CO₂ measurements were recorded for different incubation times. This ensures that there is sufficient time for the growth of the various colonies of microorganisms that may exist in the soil samples.

As can be observed in the figure, on the third day of incubation there is a high release of CO₂ from both soils, with values of 2.885 mg CO₂.kg⁻¹ for the Ritmo variety soil and 2.560 mg CO₂.kg⁻¹ for the Astron variety soil. A marked decrease then occurred to levels of 1.398 and 0.973 mg CO₂.kg⁻¹ for the respective soils on the eleventh day of incubation. Thereafter, the CO₂ emission continued to decrease smoothly to values of 0.595 and 0.151 mg CO₂.kg⁻¹, respectively.

For all of the times studied, the soil associated with the growth of *T. aestivum* cv. Ritmo showed significant differences from the soil planted with the variety Astron (Tukey’s test, α=0.01). The first

Table 1. DIBOA and DIMBOA production in root exudates of wheat varieties Astron and Ritmo. Conversion of BOA and MBOA in their associated soils and CO₂ levels generated by the soil.

Soil*	Root exudates** (mmol kg ⁻¹ Fresh Weight)		Respiration [‡] [mg CO ₂ ·kg ⁻¹ of soil·days ⁻¹]	% Conversion [§]	
	DIMBOA	DIBOA	CO ₂	BOA	MBOA
Astron	58.89 ± 1.06	18.21 ± 0.41	0.853 ± 0.031	84 ± 3	34 ± 2
Ritmo	51.33 ± 2.31	13.90 ± 0.87	0.962 ± 0.051	99 ± 2	39 ± 1

* Soil according to variety of cultivated wheat. ** Root exudate values measured in a hydroponic medium for 50 seedlings and six days of growth after germination. [‡]CO₂flow [mg CO₂·kg⁻¹ of soil·days⁻¹] during two days. [§]Maximum values for the conversion in soil after 10 days of incubation. Dose: BOA: 2 mg·g⁻¹ of soil. MBOA: 0.25 mg·g⁻¹ of soil.

soil showed higher emission of CO₂, which indicates larger populations of microorganisms in this soil. This finding can be correlated with the ability of these microorganisms to convert the allelochemicals into BOA and MBOA. Previously, it has been reported that DIBOA and DIMBOA degrades to BOA and MBOA, followed by conversion of BOA to APO, and MBOA to AMPO (Macias *et al.*, 2004;2005). BOA and MBOA degraded rapidly in both soils, with a loss of 70-99% after 10 days. However, the soil cultivated with wheat variety Ritmo had higher activity at some initial doses of BOA and MBOA. Thus, the Ritmo variety soil showed a greater degradation capacity (Table 2). In other words, the higher populations levels of microorganisms, the greater the amount of chemicals degraded.

The soil that contains greater quantities of allelochemicals has lower microbial populations. Astron soil, which has the highest amounts of DIMBOA and DIBOA in root exudates, had lower microbial populations in the soil. This situation reflects the possible antibiotic effect of these compounds in the environment close to the plant, which causes a decrease in the biotransformation processes of the allelochemicals released.

These results show that the exudation of antibiotic compounds could be crucial in the allelopathic activity. It is important that allelochemicals and their degradation products have two properties: phytotoxic activity and toxicity to microorganisms.

The results described above are consistent with those published previously, where a plant influences the populations type of the microorganisms present in soil and, consequently, its ability to transform allelochemicals (Hashimoto and Shudo, 1996; Grayston *et al.*, 1998; Glenn *et al.*, 2003). Thus, the microflora associated with the root system of plants depends on the variety of wheat and the compounds released into

the soil. The populations can be increased for those microorganisms that are able to metabolize the toxins released by the plant or use them as a carbon source (Voloshchuk *et al.*, 2007).

Conclusions

The rhizosphere of the Astron wheat variety showed inhibitory effects and reduced the emission of CO₂ may be due to the higher amounts of DIMBOA and DIBOA in the root exudates.

Acknowledgments

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