Microbiological activity and carbon mineralization in pampean soils with different agricultural use intensity

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ABSTRACT

The processes involved in the flows of matter and energy of terrestrial ecosystems depend heavily on soil biological activity, the current conventional agricultural managements could alter the biological mechanisms involved in decomposition and nutrient cycling in agroecosystems. The aim of this study was to compare the activity levels and soil microbial biomass between different agricultural pampean soil uses and its relationship to carbon mineralization. 25 years of agricultural use were compared with 25 years of ecological reserve naturalized where each agroecosystem soil were collected at 61 - 125 - 183 - 236 - 302 - 368 - 431 - 488 days for measuring their moisture, organic matter, enzymatic activity, microbial biomass carbon, soil respiration, metabolic quotient, microbial quotient and carbon mineralization rate. The distance between agroecosystems is less than 800 m, thus assuming the same soil and climatic conditions. The data were evaluated by Friedman test finding significant differences in moisture, organic matter, enzymatic activity, soil respiration y microbial quotient (p< 0.01). Difference was also found in the microbial mineralization rate of carbon (p< 0.1).

KEY WORDS

Microbiological activity; carbon mineralization; soil use.
INTRODUCTION

Organic matter decomposition in soils is the most important ecological process mediating energy flux and nutrient cycling in terrestrial ecosystems. This process is critically linked to the diversity of enzymes produced by the microbial community (Baldock, 2007). Soil microbiota is thus, one of the main component in agroecosystems, playing a key role in the processes of nutrient cycling (Carpenter et al., 2007; Murray et al., 2009; Ponge et al., 2013), soil organic compounds fragmentation, distribution, and mineralization (Labrador, 1996; Wolters, 2000), buildup and maintenance of organic matter content and physical structure (Wolters, 2000; Ponge et al., 2013), soil-plant relations (Forey et al., 2011; Ponge et al., 2013), all of which generate synergic interactions towards soil productivity (Eisenhauer et al., 2010; Ponge et al., 2013). Furthermore, signalling processes towards agroecosystem integrity have also been documented (Sanon et al., 2009; Ponge et al., 2013). Most soil microorganisms play roles yet to be adequately described, but their influences in ecosystem functioning are well recognized (Fitter et al., 2005). Even though an accurate account of the number of microorganisms in a natural soil is difficult to reach, it has been estimated that one gram of soil may contain up to one hundred thousand of bacterial species, most of them (up to 95%) still uncultured (Sait et al., 2002; Joseph et al., 2003). Abril (2003) argued that soil microbiological activity indicators are quite sensitive and they can be successfully used in soil sustainability studies.

Soil organic Carbon (C) is an important reservoir in the global C cycle. It has been estimated that there about 1200-1555 Petagrams (Pg, $10^{15}$ g) of C are stored in the first meter of soil and about 2370-2450 Pg C in the first 2 meters (Eswaran et al., 1995; Lal, 2004) compared with the total C stored in the biomass (560 Pg C), and in the atmosphere (760 Pg C) (Lal, 2004). Changes in soil use can lead to net C sequestering or emission by soils, depending on the range of
environmental conditions besides management practices (Follett, 2001; Lal, 2004; Baldock, 2007). Soil organic C contributes to a great number of biological, chemical and physical soil properties that define soil productivity (Reeves, 1997; Baldock and Skjemstad, 1999). It also supplies the energy essential for all biological processes and has a significant impact on soil physical properties such as structural stability, water retention capacity, and temperature regime, as well as cation exchange, and buffer capacity (Baldock and Skjemstad, 1999).

Based in Odum’s (1969) energy exchange theory, there are a number of efficiency indicators of soil microbiological metabolism, that relate different C soil compartments associated to the presence and activity of soil microorganisms (Anderson and Domsch, 2010).

Agricultural productivity depends both on management practices as well as soil microbiological dynamics, which in turn is affected by the former. Current agricultural production is focused on achieving the higher possible economic return, utilizing a wide range of agricultural practices and agrochemicals to increase short term productivity. However, there is an increasing body of evidence pointing to the negative effects they have on the structure and functioning of the microbial community (Wakelin et al., 2009; Zhong et al., 2010), being this particular group the most sensitive to agricultural practices in this kind of soils (Castro-Huerta et al., 2015). In one hand, and for a more integrative evaluation of agricultural practices, these negative effects or external costs should be valued in economic terms (Huguenin et al., 2006). On the other hand, grassland ecosystems with low organic matter contents have shown a remarkable capacity for C uptake and retention in soils and thus, help to mitigate the effects of global climate change (Follett, 2001; Asner et al., 2004; Morgan et al., 2010; Conant et al., 2011; Ryals et al., 2014).

In this context, we hypothesized that soils under low impact agricultural practices would show higher microbiological abundance and activity when compared to soils under high impact
agricultural practices and that would affect soil C mineralization rate.

Objectives

- To evaluate differences in microbiological abundance and activity of the same argiudoll soil under different intensities of agricultural practices.
- To relate differences in microbiological abundance and activity with C mineralization rate.
MATERIALS AND METHODS

Two different agroecosystems under different anthropic disturbance regimes were evaluated in the Argentina’s rolling pampas, one of the largest and most productive agricultural regions in the world. One system is an ecological reserve with no anthropic activity during the last thirty years, and the other system were agricultural fields, with well over fifty years of continuous intensive agricultural activity. Both systems shared the same type of argiudoll soil, Order Mollisols (Soil Survey Staff, 2014) and same climate. All work was performed in experimental fields belonging to the Universidad Nacional de Luján (Luján, Buenos Aires Province, Argentina (34º34’ S; 59º05’ O).

In each agroecosystem five sampling sites were selected as replicates. The sites were selected for a litterbag experiment with standardized litter material. From each replicate (5) and system (2), soil samples were collected from the top 5 cm at the same time litterbags were retrieved for the decomposition experiment (data not shown), at approximately 60 day intervals for 16 months (61, 125,183, 236, 302, 368, 431, 488 days). Soil samples were taken to the laboratory to measure the moisture, organic matter (OM) (Walkey and Black, 1934), enzymatic activity by means of the fluorescein diacetate hydrolysis method (Schnürer and Rosswall, 1982; Adam and Duncan, 2001) (µg Fluorescein*g soil⁻¹* hour⁻¹), microbial biomass carbon (SMBC) (µg MBC*g soil⁻¹) following Vance et al. (1987), microbial respiration (MR) (mg CO₂-C*g soil⁻¹* hour⁻¹) (Anderson, 1982), Metabolic coefficient (qCO2) C respired by unit of microbial biomass C following Anderson and Domsch (1978), microbial coefficient (qBio) as a measure of how much of the total soil carbon corresponds to microbial biomass carbon (Anderson and Domsch, 1989), and microbial carbon mineralization rate according to Pinzari et al. (1999).

All the results were analyzed using the non-parametric Friedman test (1937).
RESULTS

A summary of the results can be seen in Table 1. They show highly significant differences between systems (p< 0.01) for soil organic matter, soil moisture, enzymatic activity, soil respiration, and microbial quotient. A significant difference (p< 0.1) was found for mineralization rate, while microbial biomass carbon and metabolic quotient were not different between systems.

<table>
<thead>
<tr>
<th>Friedman's Test</th>
<th>p-value</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic Matter (%)</td>
<td>&lt; 0.0001</td>
<td>yes***</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>0.004</td>
<td>yes***</td>
</tr>
<tr>
<td>Enzymatic Activity (µg Fluorescein* g soil⁻¹* hr⁻¹)</td>
<td>&lt; 0.0001</td>
<td>yes***</td>
</tr>
<tr>
<td>Microbial Biomass C (µg SMBC* g soil⁻¹)</td>
<td>0.114</td>
<td>no</td>
</tr>
<tr>
<td>Respiration C (µg CO₂-C* g soil⁻¹* hr⁻¹)</td>
<td>0.001</td>
<td>yes***</td>
</tr>
<tr>
<td>Metabolic Quotient (qCO₂)</td>
<td>1.000</td>
<td>no</td>
</tr>
<tr>
<td>Microbial Quotient (qBio)</td>
<td>0.004</td>
<td>yes***</td>
</tr>
<tr>
<td>Mineralization Rate (%C* hr⁻¹)</td>
<td>0.058</td>
<td>yes*</td>
</tr>
</tbody>
</table>

Table 1.- Results for the Friedman’s test for the variables measured.

***Highly significant differences (p< 0.01)
*Significant differences (p< 0.1)

Organic matter (Fig. 1) was significantly higher in the ecological reserve (p< 0.01) throughout the entire sampling period. It was found to be within the expected values for argiudoll soils, below 8%. Soil moisture (Fig. 2) was highly variable, but it also remained significantly higher in the ecological reserve during the sampling period.
Figure 1.- Soil organic matter (%) during the sampling period for the Ecological reserve and the Agricultural use. Organic matter content was significantly higher in the ecological reserve. Curves are a non-parametric regression (LOESS). Different capital letters indicate significant differences between agroecosystems (Friedman’s test, p< 0.01).
Figure 2.- Soil moisture (%) for the two agroecosystems tested, the Ecological reserve and the Agricultural use. Soil moisture was significantly higher in the ecological reserve. Curves are a non-parametric regression (LOESS). Different capital letters indicate significant differences between agroecosystems (Friedman’s test, p<0.01).

Enzymatic activity (Fig. 3) was also highly variable, with values ranging from 1 to 6 µg Fluorescein*g soil⁻¹*hour⁻¹ but it also remained significantly higher in the ecological reserve during the whole sampling period. Soil microbial biomass carbon (Fig. 4) shows very variable values without statistically significant differences.
Figure 3. Soil enzymatic activity as measured by the fluorescein diacetate hydrolysis method. Friedman’s test showed a significantly higher enzymatic activity in the soils from the Ecological reserve when compared to the soils from the Agricultural use. Curves are a non-parametric regression (LOESS). Different capital letters indicate significant differences between agroecosystems (p<0.01).

Figure 4. Soil microbial biomass carbon found in the two tested agroecosystems. Friedman’s test show no
significant differences between systems during the sampling period. Curves are a non-parametric regression (LOESS).

Soil microbial respiration (Fig. 5) ranged from 0.4 to 3 μg C-CO₂·g soil⁻¹·hr⁻¹. Results show consistently higher respiration values in the ecological reserve. The metabolic quotient (qCO₂) (Fig. 6) was not significantly different between agroecosystems.

Figure 5.- Soil microbial respiration for the two agroecosystems tested during the entire sampling period. Respiration was significantly higher in the Ecological reserve when compared to the Agricultural use. Curves are a non-parametric regression (LOESS). Different capital letters indicate significant differences between agroecosystems. Friedman’s test (p< 0.01).
Figure 6.- Metabolic quotient (qCO₂) measured in the two agroecosystems tested. No significant differences were found over the entire sampling period (Friedman’s test). Curves are a not-parametric regression (LOESS).

The microbial quotient (qBio) was significantly different between the two agroecosystems (Fig. 7), being higher in the Agricultural use (p< 0.01). Soil C mineralization rate (Fig. 8) was marginally higher in the Agricultural use (Friedman´s test, p< 0.1).
Figure 7.- Microbial quotient measured in the two agroecosystems tested was higher in the Agricultural use when compared to the Ecological reserve. Curves are a not-parametric regression (LOESS). Different capital letters indicate significant differences between agroecosystems. Friedman’s test (p< 0.01).

Figure 8.- Soil C mineralization rate measured in the two agroecosystems tested was higher in the Agricultural use when compared to the Ecological reserve. Curves are a not-parametric regression (LOESS). Different capital letters indicate significant differences between agroecosystems. Friedman’s test (p< 0.1).
Figure 8.- Soil C mineralization rate (qM) for the two agroecosystems. Curves are a not-parametric regression (LOESS). Different capital letters indicate significant differences between agroecosystems. Carbon mineralization rate was higher in the Agricultural use according the Friedman's test (p < 0.1).
DISCUSSION

The results presented show a high temporal variability in all the studied biological variables. This was an expected outcome, and agrees with previous studies on this issue (Anriquez et al. 2005; Paz-Ferreiro et al., 2013; Benintende et al., 2015). Soil moisture is known to have a strong effect on soil microbiological activity (Lavelle et al., 2006). Although it varied during the whole sampling period, the significant differences found could be the variable affecting the other variables measured the most (Lavelle et al., 2006). Soil organic matter on the other hand, was shown to be the variable measured that varied the less during the study. It is important to notice that soil microbial biomass not only varied over the period of the study, but it also showed changes in its physiological functioning, in particular soil respiration and metabolic quotient which are also strongly affected (Anderson and Domsch, 2010).

Soil organic decomposition of organic residues is mediated mainly by soil microorganisms that consume energy with the net effect of C and nutrients´ release, available then to other organisms (Guggenberger, 2005). Soil biological ability to process organic matter is related to the presence and diversity of enzymes produced by the soil microbiota (Baldock, 2007). Therefore, its measurement is a widely accepted method for evaluating microbial activity in a wide range of substrates (Solaiman, 2007). Fluorescein diacetate (FDA), as used in Adam and Duncan (2001), is hydrolyzed by a great deal of microbial enzymes, such as proteases, lipases, and esterases, leading to the release of a fluorescent byproduct which can be detected with by either a spectrophotometer or a fluorometer. Most of these enzymes, are released by a wide range of decomposers, among them soil bacteria and fungi (Dick et al., 1996). Even though these enzymes do not have a direct relationship with soil C dynamics, enzyme activity is a reliable indicator of the microbial community ecological state. The results presented in this work clearly show that this microbial community is significantly more active in the ecological reserve
with a low anthropic impact, when compared to the agricultural use sites, under higher anthropic
disturbance

Soil microbial biomass C (SMB$_C$) measurement, originally introduced by Jenkinson and
Powlson (1976) later modified by Vance et al. (1987) is intended to evaluate C content in the
microbial biomass. This C content is the part of the soil organic matter linked to the part of the
soil biota that responds the fastest to soil conditions. Our study shows that it is highly sensitive to
changing conditions, leading to the high temporal variability observed, with no significant
differences between the agroecosystems tested.

Soil microbial respiration, known also as basal respiration, is a very reliable indicator of
microbial activity (Paul and Clark, 1996; Frioni, 1999; 2006). It is also directly linked to other
metabolic and cyclic C processes, and in this experiment, it was clearly shown to be significantly
higher in the low anthropic impact ecological reserve, compared to the highly impacted
agricultural use sites. Not surprisingly, enzymatic activity was also higher in the low anthropic
impact sites over the entire sampling period.

The metabolic quotient (q$_{CO_2}$) is a microbiology indicator of the amount of CO$_2$ respired
per unit of microbial biomass over a fixed amount of time C (Frioni, 2006; Benintende et al.,
2008). For high quality or low perturbation substrates it has lower values when compared to
stressed soils. According to Odum´s (1969) bioenergy theory, the increase in plant, animal and
microbial diversity goes along with a higher energy use efficiency, until a dynamic equilibrium is
reached. At this point, there should be a low respiration rate per unit of microbial biomass, and
any extra C input in some compartment of the soil biota should be equal to the outputs. In this
context, and regarding soil disturbance, microbial biomass dynamics should change depending on
perturbation intensity. Low perturbation systems should then have low microbial respiration per
unit of microbial biomass (q$_{CO_2}$) (Anderson and Domsch, 2010) and a high microbial biomass
per unit of energy input (qBio) (Frioni, 2006; Anderson and Domsch, 2010). Anderson’s (2009) C unit respired by unit of microbial, was significantly lower in multispecific rather than in monospecific forests. It can be therefore assumed that, in ecosystems with highly diverse organic inputs into the soil, microbial community should be energetically more efficient, than less diverse organic inputs systems (Anderson and Domsch, 2010).

In this study however, the qCO₂ values found do not show significant differences between the two studied agroecosystems, while the qBio values do. These results were not expected because, even though SMB₇ values were the same the agricultural system does show lower microbiological activity as shown by the results for enzymatic activity and respiration. This can be due to the similar SMB₇ values between ecosystems and a significantly lower SOM₇ in the agricultural use system. It is worth to notice that a high qCO₂ is often explained as a high microbiological activity and interpreted as a positive attribute. However, in ecological terms, a high qCO₂ is a reflection of a high maintenance energy demand (C) under microbial community stress. If the soil system is not resilient, C can be lost through respiration followed by a loss of microbial biomass (Anderson and Domsch, 2010). This can be the situation in the Agricultural use under a high perturbation regime for the C mineralization quotient (qM). Since qM estimates the amount of CO₂ lost to microbial respiration in relation to total C (Frioni, 2006; Anderson and Domsch, 2010), the observed difference between agroecosystems can be a reflection of the higher perturbation regime in the agricultural use, along with its lower organic matter content (SOM₇). The results suggest that the high perturbation soils from the agricultural use system are mineralizing faster and losing more C than the low perturbation soils from the ecological reserve. The results presented here also suggest that the long term viability of the microbial community in high perturbation soil systems is in jeopardy when compared to soils under lower perturbation regimes casting a shadow over the long term sustainability of the high anthropic impact related to...
conventional agriculture.

CONCLUSION

The results presented in this study underscore some of the negative effects of the intensive agricultural practices associated to conventional agriculture over the soil microbiota. Significantly lower levels of enzymatic activity and soil respiration, along with a higher C mineralization rate and lower organic matter contents, all results point in the direction of a lower long term sustainability of the microbiological communities, associated to soils under high anthropic impact.

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