

# Factors limiting soil microbial growth and activity in wheat-based cropping systems

Andre Freire Cruz<sup>1,2</sup>, Keith Hanson<sup>1</sup> and Chantal Hamel<sup>1,2</sup>

<sup>1</sup>Agriculture and Agri-Food Canada, Semiarid Prairie Agricultural Research Centre, 1 Airport Rd, P.O. Box 1030, Swift Current, SK, S9H 3X2.

<sup>2</sup>University of Saskatchewan, Dept of Soil Science, 51 Campus Dr., Saskatoon, SK, S7N 5A8.

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## Abstract

Several soil biochemical processes related to soil fertility are microbially mediated. We know very well the factors limiting crop production but know little of the factors limiting the soil microbial community. The goal of this study is to clarify if N, P and C limit soil microbial growth and biochemical processes in the wheat and fallow phases of rotations receiving different N, P and crop residue-C inputs. Soil samples were collected July 28, 2003, and brought to the laboratory. Soils samples were treated with an N, C or P solution, with water only, or left untreated, and incubated for 48 hours. At the end of the incubation period, soil microbial biomass N was determined along with denitrification and nitrogenase activities. The results showed that the microbial activities and growth were limited by soil moisture at the date of sampling. Denitrification and nitrogenase activities were limited by N, P or C. The limiting nutrients varied with N, P and C inputs associated with the cropping system, regardless to soil moisture. The limitation of these elements to soil microbial activity was more frequently encountered under fallow.

## Introduction

Microbial activity and growth in soil is influenced by soil properties. The soil water content, a common problem in the prairies, causes large effects on microbial activity. Many reports indicate that soil microbial growth and activity are affected by crop management. For example soil microbial biomass – C and N (SMB-C, N) was shown to be reduced by the inclusion of fallow in a crop rotation. The amount of residue incorporation into soil influence soil microorganisms (Spedding et al. 2004). N and P are essential elements for microbial growth, as such; the availability of N and P in soil may influence the size and activity of soil microbial communities.

Changes in nutrient loading in ecosystems are known to affect C and N cycling (Sundareshwar et al. 2003). For example N inputs may accelerate C fixation by primary producers, whereas other nutrients like P may affect C turnover by heterotrophs. P limitation of microbial growth may impact on the transformation and availability of N, which can influence C fixation, storage and release, and this limitation has the potential to increase the loss of N and to alter ecosystems-level inputs and outputs of N.

So far many studies related to microbial activity and growth in Canadian prairie soils have focused on residue incorporation, crop rotation and soil water content. Little attention has been paid the influence of essential elements, such as C, N and P, on soil microbial activity. This

study aims to find out if different factors (C, N or P) limit the growth and activity of the soil microbial populations selected for in wheat-based crop rotations.

## Materials and Methods

### *Soil sampling*

A crop rotation experiment has been established for 36 years at the South farm of the SPARC research centre in Swift Current, Saskatchewan. The experiment had 4 rotation treatments as shown in Table 1. Sampling was done in the plots in the 2<sup>nd</sup> year of wheat and in the fallow phase. Also the N and P were applied only in the wheat phase.

Three soil cores per plot were taken and combined into one composite soil sample on July 28, 2003. The sampling depth was 0-7.5cm in both wheat and fallow plots. Composite samples from each plot were sieved (2 mm) and crop residues remaining on the sieve discarded. Approximately 50g of each sample was weighted and oven-dried for 24h to determine the soil moisture. The remaining soil was submitted to bioassays in the laboratory in attempt to identify which of N, P or C was limiting microbial activity and growth in these field plot soils.

**Table 1.** Treatments imposed to field plots for 36 years.

<b>Rotation treatments</b>	
Normal:	22.7 kg N /ha, 22.0 kg P /ha in Fallow-Wheat-Wheat (F-W-W)
-C:	49.4 kg N /ha, 22.0 kg P/ha in Fallow-Wheat (F-W)
-N:	0 kg N /ha, 22.0 kg N /ha in Fallow-Wheat-Wheat (F-W-W)
-P:	30.1 kg N/ha, 0 kg N /ha in Fallow-Wheat-Wheat (F-W-W)

N and P applied on wheat phase

### *Laboratory treatments and analysis*

100 and 200g oven dry equivalent (ODE) soil samples were treated with solutions of C, N, P or water alone. An untreated soil was prepared as control. 150mg kg<sup>-1</sup> of C (glucose), 100 mg kg<sup>-1</sup> N (NH<sub>4</sub>NO<sub>3</sub>) and 26mg kg<sup>-1</sup> P (KH<sub>2</sub>PO<sub>4</sub>) solutions were prepared on a soil dry weight basis. The amount of water used was that required to bring the soil to field capacity. The soils with the laboratory treatments were incubated in 500ml glass jars for 48h, and then analysed.

The jar containing 200g ODE of soil was used to determine SMB-N by chloroform fumigation-extraction using a soil to extractant ratio of 1:5 (Voroney et al. 1993). In the glass jars containing 100g ODE of soil, acetylene (C<sub>2</sub>H<sub>4</sub>) was injected (10% of internal air volume) and incubated for 12h. 20 ml of gas sample was taken from the jars and placed in evacuated exotainers. The amount of acetylene reduced to ethylene was measured by gas chromatography to estimate nitrogenase activity (Rice and Olsen 1993). Denitrification activity was determined by the gas chromatographic measurement of N<sub>2</sub>O produced in the same samples used for nitrogenase activity determination, as per the acetylene block method (Beauchamp and Bergstrom 1993). All gas analyses were done in duplicate and the average of the results was used for statistical analysis.

### Statistical analysis

The experiment had a complete randomized block design with two phases of four rotation treatments as main plots and five laboratory treatments as subplots, and tree blocks, resulting in a total of 120 experimental units.

The statistical analyses were performed using analysis of variance (ANOVA) to detect significant differences between the effects of laboratory treatments, phase and field C, N and P input. The N<sub>2</sub>O data was log transformed before analysis. The standard error was used for comparison between laboratory treatments. All statistical analyses were performed with JMP 3.2.6 and P<0.01, 0.05 and 0.1 were considered for results comparison (Table 2).

**Table 2.** Significance of C input (fallow frequency), phase, fertilizer input and laboratory treatments on denitrification, nitrogenase and SMB-N.

	Denitrification <sup>a</sup>	Nitrogenase <sup>a</sup>	SMB-N
Cinput	NS	NS	*
Phase	**	NS	**
Cinput x Phase	**	NS	NS
Fert input[Cinput]	**	NS	NS
Phase x Fert input[Cinput]	***	NS	NS
Lab Treat	***	NS	***
Cinput x Lab Treat	NS	NS	NS
Phase x Lab Treat	***	NS	NS
Cinput x Phase x Lab Treat	NS	*	NS
Fert input x Lab Treat[Cinput]	NS	**	NS
Phase x Fert input x Lab Treat[Cinput]	*	NS	NS

<sup>a</sup> Data transformed by log prior to statistical analysis.

\*\*\*, \*\* and \* significant at 1, 5 and 10% respectively

NS – non-significant

### Results and discussion

At the time of sampling, water often limited microbial activities and growth in the soils, in general view, which represents a common situation in the prairies for the plant production. Limitation by C, N and P was more frequent in the fallow phase than under wheat. In the fallow phase, tillage done twice with heavy duty cultivator might not have well incorporated the crop residues into the soil, as there was no apparent strong effect of the residue input from the previous microbial biomass-N or activity. Also under wheat, we may assume that the release of organic molecules through root exudation may provide soil microorganisms with good C supply. In addition, fertilizer was only applied to wheat, hence soil microorganisms were also supplied with N and P under wheat. Therefore, under the wheat phase the limitation of microbial activities by essential elements was lower than under fallow (Table 3).

Denitrification was limited by N and C in –C plots, and by N in –P plots, when water stress was relieved (Table 3). The combination of N fertilizer and water enhances the N<sub>2</sub>O emission rates, since NO<sub>3</sub> is the substrate for denitrification and this activity is closely related to hydrologic processes (MacKenzie et al. 1998).

Nitrogenase activity was limited by P in –C plots and by C, N and P in –N plots (Table 3). Free-living N<sub>2</sub>-fixers might have been stimulated by P because this element is part of ATP, and N<sub>2</sub> fixation requires a lot of energy for the initial process activation. The C, N and P limitation of nitrogenase in –N plots is more difficult to explain. It might be attributable to the nature of the soil microbial community selected by 36 years of low soil N conditions. It may also be attributable to the fact that under conditions of low N availability, nitrogenase activity is derepressed, and then growth factors may increase activity.

Soil microbial biomass N did not appear limited by C, N or P even though moisture stress was relieved. However, under field conditions, low fallow frequency was associated with high microbial biomass N under fallow (Table 3). It seems therefore that the variability associated with the determination of SMB-N can hide some treatment effects. The determination of SMB-C that is currently being done in our laboratory may shed light on this question.

The results of this work revealed that some microbial activities related to N cycling and microbial growth in wheat-based rotation studies sometimes are limited by C, N and P, especially under fallow, besides the effects caused by the soil moisture.

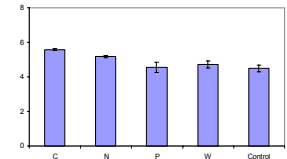
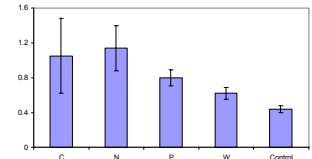
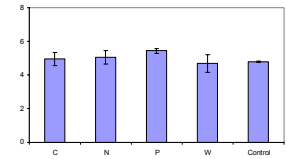
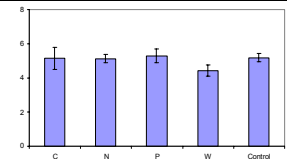
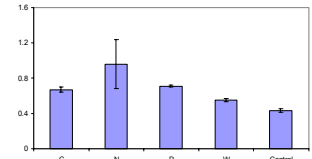
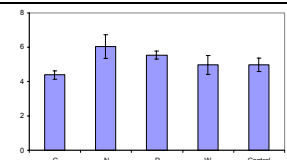
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**Table 3.** Laboratory treatments (C, N, P, water or control) limiting microbial growth and activities in soils from field with normal conditions (+C, +N and +P), low carbon input (-C), no nitrogen (-N) and no phosphorus (-P) at fallow and wheat phase.

	Treat	Soil characterization	Denitrification N <sub>2</sub> O (ppm)	Nitrogenase C <sub>2</sub> H <sub>2</sub> (ppm)	SMB-N
<b>Fallow phase</b>	<b>Norm</b>	PO <sub>4</sub> <sup>-</sup> (mg/kg soil): 26.92 NO <sub>3</sub> <sup>-</sup> (mg/kg soil): 19.13 PO <sub>4</sub> flux (μgPO <sub>4</sub> /cm <sup>2</sup> /d): 0.008 NO <sub>3</sub> flux (μgNO <sub>3</sub> /cm <sup>2</sup> /d): 0.86 SMB-N (μg/g soil): 171.35			
	<b>-C</b>	PO <sub>4</sub> <sup>-</sup> (mg/kg soil): 28.92 NO <sub>3</sub> <sup>-</sup> (mg/kg soil): 16.23 PO <sub>4</sub> flux (μgPO <sub>4</sub> /cm <sup>2</sup> /d): 0.005 NO <sub>3</sub> flux (μgNO <sub>3</sub> /cm <sup>2</sup> /d): 1.07 SMB-N (μg/g soil): 117.34			
	<b>-N</b>	PO <sub>4</sub> <sup>-</sup> (mg/kg soil): 29.14 NO <sub>3</sub> <sup>-</sup> (mg/kg soil): 14.37 PO <sub>4</sub> flux (μgPO <sub>4</sub> /cm <sup>2</sup> /d): 0.010 NO <sub>3</sub> flux (μgNO <sub>3</sub> /cm <sup>2</sup> /d): 1.06 SMB-N (μg/g soil): 152.12			
	<b>-P</b>	PO <sub>4</sub> <sup>-</sup> (mg/kg soil): 12.32 NO <sub>3</sub> <sup>-</sup> (mg/kg soil): 17.27 PO <sub>4</sub> flux (μgPO <sub>4</sub> /cm <sup>2</sup> /d): 0.002 NO <sub>3</sub> flux (μgNO <sub>3</sub> /cm <sup>2</sup> /d): 1.28 SMB-N (μg/g soil): 131.67			
<b>Wheat phase</b>	<b>Norm</b>	PO <sub>4</sub> <sup>-</sup> (mg/kg soil): 33.47 NO <sub>3</sub> <sup>-</sup> (mg/kg soil): 12.63 PO <sub>4</sub> flux (μgPO <sub>4</sub> /cm <sup>2</sup> /d): 0.013 NO <sub>3</sub> flux (μgNO <sub>3</sub> /cm <sup>2</sup> /d): 1.04 SMB-N (μg/g soil): 121.32			
	<b>-C</b>	PO <sub>4</sub> <sup>-</sup> (mg/kg soil): 27.57 NO <sub>3</sub> <sup>-</sup> (mg/kg soil): 10.84 PO <sub>4</sub> flux (μgPO <sub>4</sub> /cm <sup>2</sup> /d): 0.011 NO <sub>3</sub> flux (μgNO <sub>3</sub> /cm <sup>2</sup> /d): 0.98 SMB-N (μg/g soil): 100.26			
	<b>-N</b>	PO <sub>4</sub> <sup>-</sup> (mg/kg soil): 34.73 NO <sub>3</sub> <sup>-</sup> (mg/kg soil): 4.39 PO <sub>4</sub> flux (μgPO <sub>4</sub> /cm <sup>2</sup> /d): 0.019 NO <sub>3</sub> flux (μgNO <sub>3</sub> /cm <sup>2</sup> /d): 0.43 SMB-N (μg/g soil): 108.82			
	<b>-P</b>	PO <sub>4</sub> <sup>-</sup> (mg/kg soil): 15.42 NO <sub>3</sub> <sup>-</sup> (mg/kg soil): 23.13 PO <sub>4</sub> flux (μgPO <sub>4</sub> /cm <sup>2</sup> /d): 0.003 NO <sub>3</sub> flux (μgNO <sub>3</sub> /cm <sup>2</sup> /d): 1.37 SMB-N (μg/g soil): 106.77			

C, N, P didn't limit in empty squares