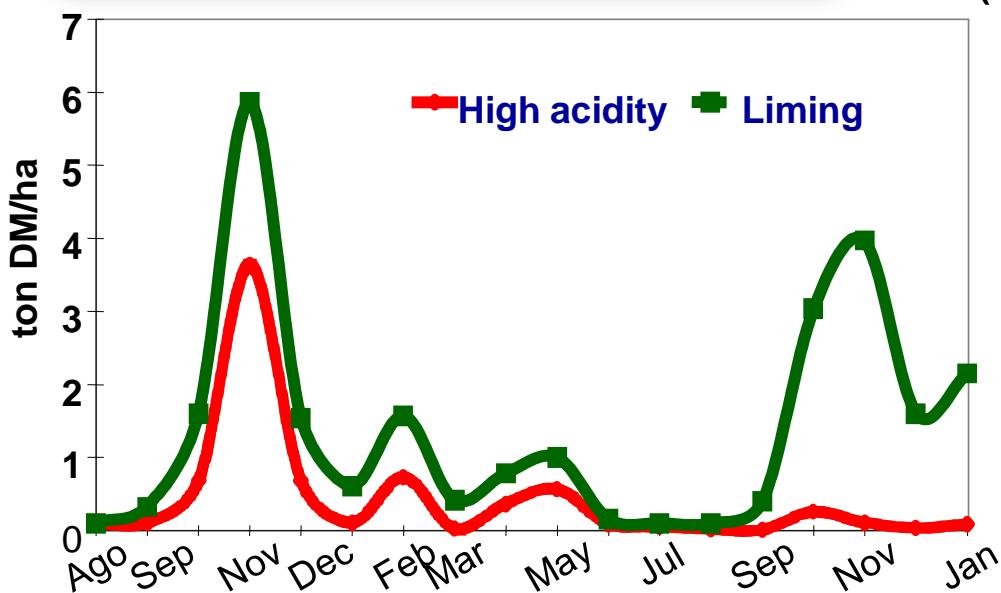
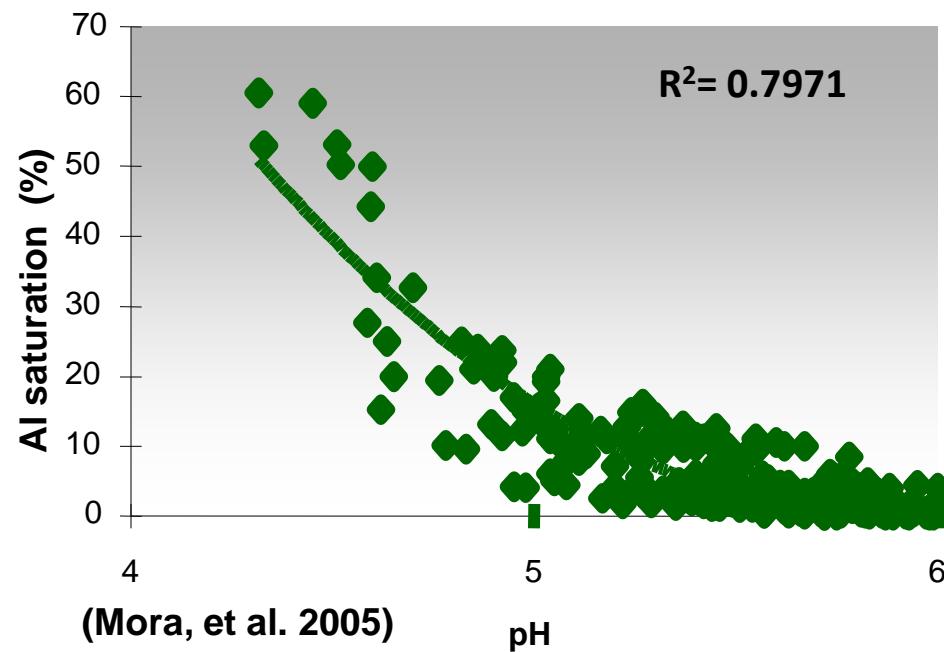




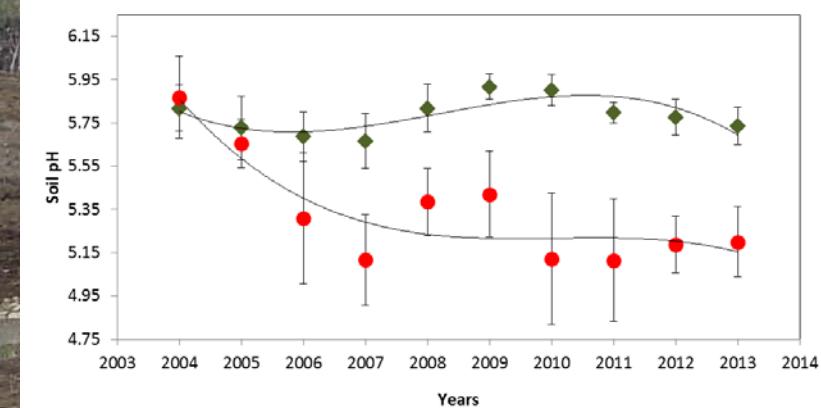
Manejo de suelos para una producción sostenible en el Sur de Chile frente al cambio climático

Dra. María de la Luz Mora Gil
Universidad de La Frontera

GENERAL BACKGROUND



(Mora et al., 2002)





Phosphorus concentration in different soils order in topsoil in temperate soils

Soil order	Total P (mg P kg ⁻¹)	Organic P (mg P kg ⁻¹)
Mollisol	201 - 708	52 - 212
Andisol	1150-4011	650-3000
Aridisol	271 - 908	30 - 77
Alfisol	196 - 820	41 - 136
Ultisol	620-2040	92-1528
Inceptisol	480 – 1947	47 – 176

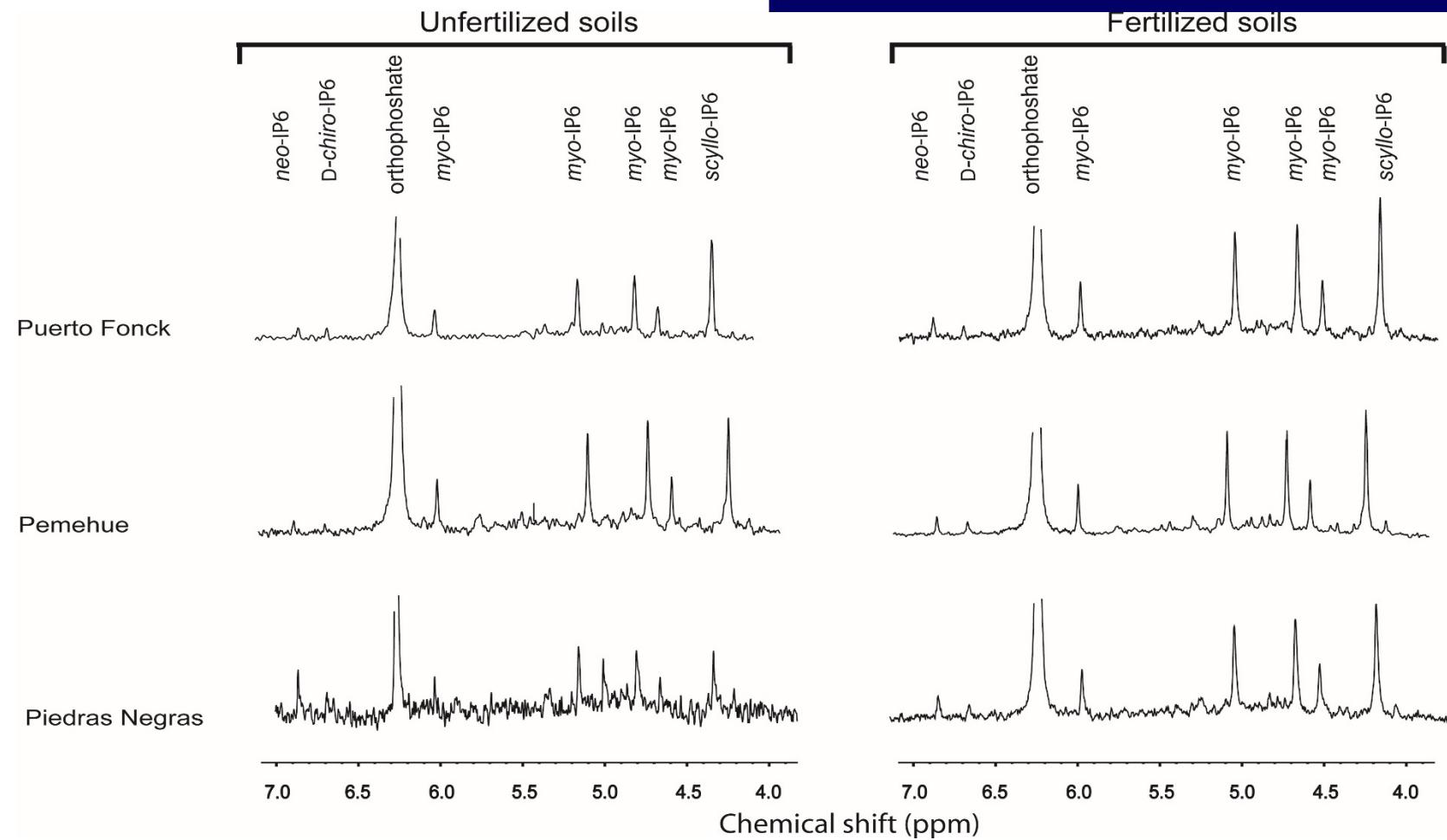
**6-16 ton dry matter/ha
5 to 25 ppm Olsen P
Total P 2000-4000 ppm
P extraction around 400 kg ha⁻¹**





Concentrations of different forms of P determined in NaOH-EDTA extracts of residual soils by ^{31}P NMR.
unfertilized soils.

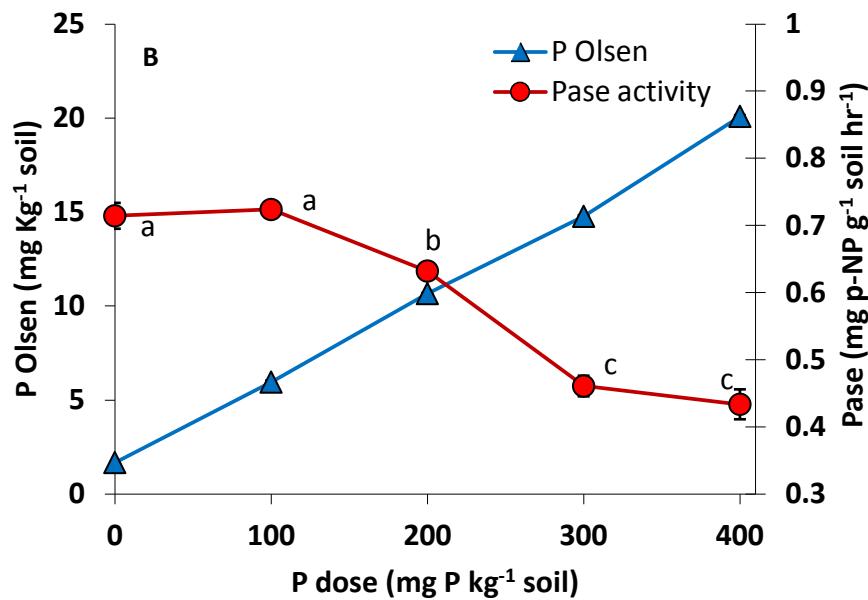
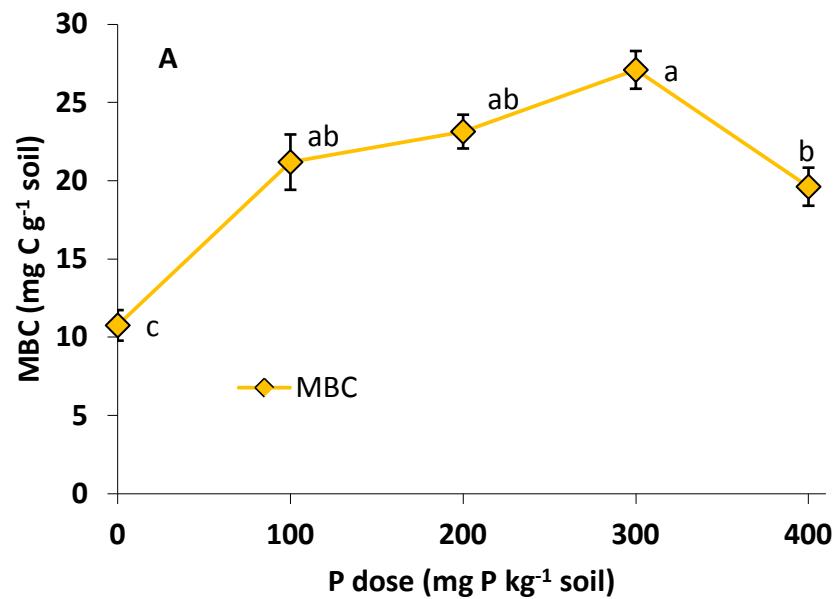
		Inorganic phosphorus		Organic phosphorus			
Soil		orthophosphate	pyrophosphate	<i>myo</i> -IP ₆	<i>scylio</i> -IP ₆	D- <i>chiro</i> -IP ₆	<i>neo</i> -IP ₆
		mg P kg^{-1}				mg P kg^{-1}	
PF	F	500.4	30.7	173.3	120.1	29.3	18.6
	UF	439.0	39.9	156.4	139.0	33.1	24.3
PEH	F	593.5	17.2	106.0	72	54.3	29.1
	UF	1115.2	27.2	167.6	107.8	16.1	12.4
PN	F	727.6	31.6	177.1	101.7	28.2	23.7
	UF	125.1	21.7	37.2	10.6	36.0	10.2

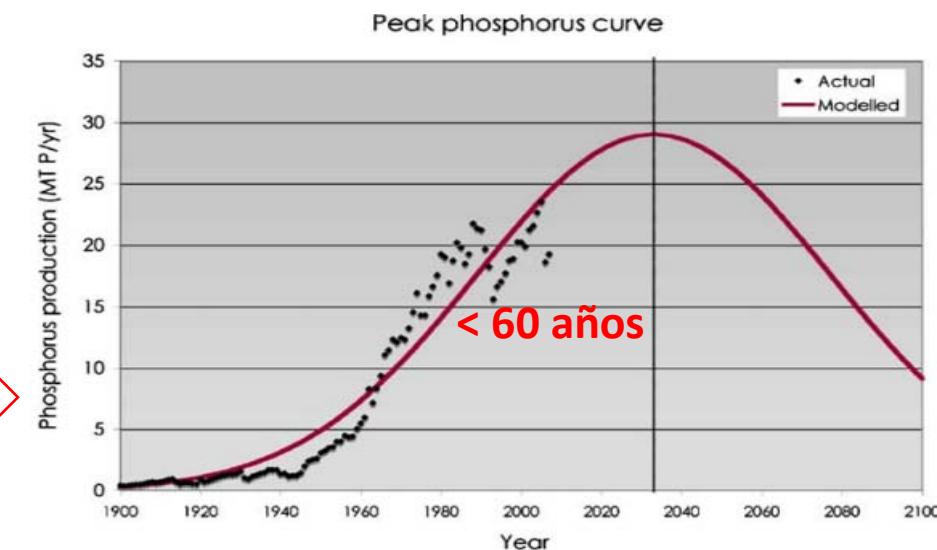
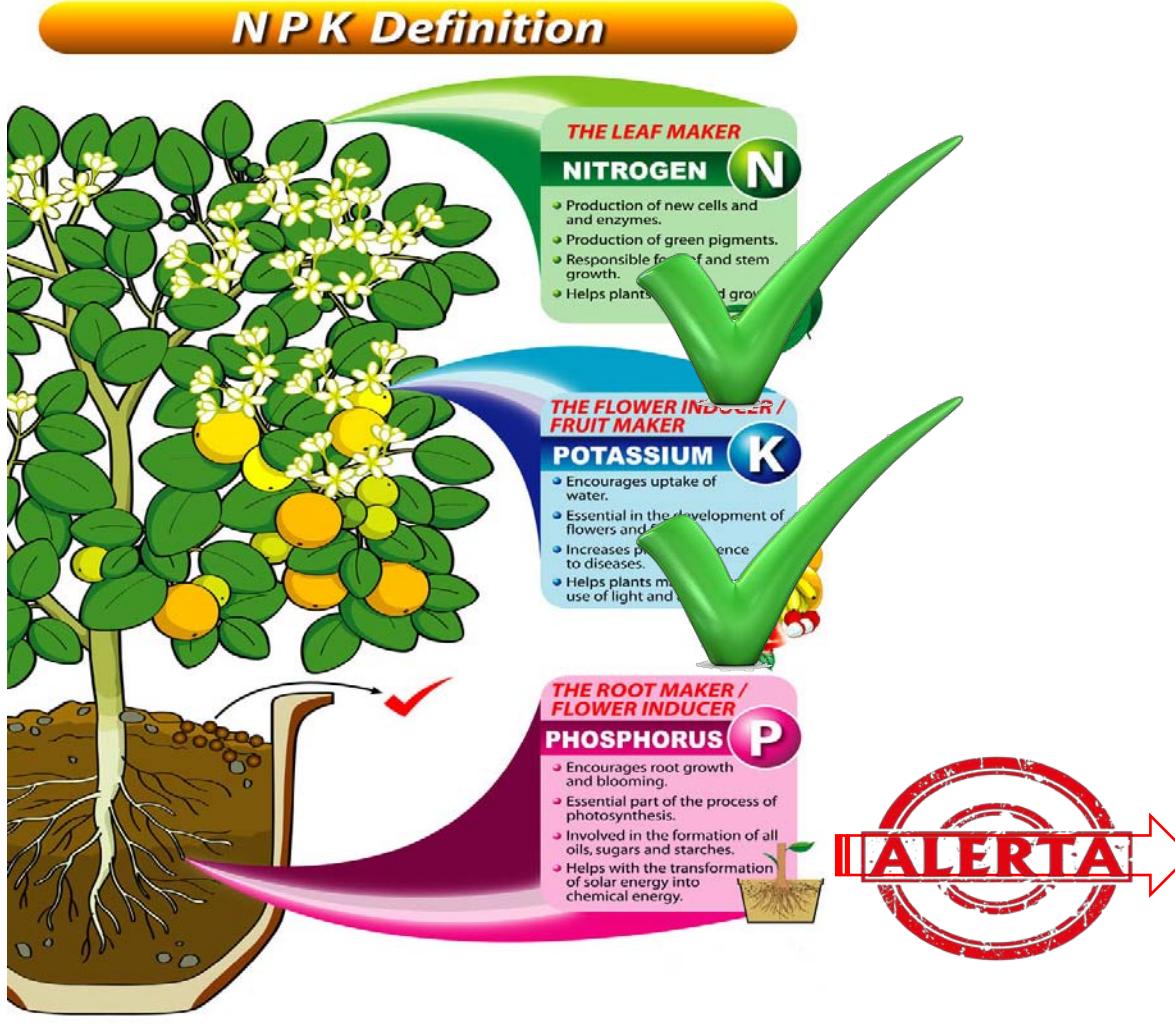


³¹P-NMR spectra of NaOH-EDTA extracts of residual P fraction derived from Hedley fractionation in Andisol (Velasquez et al., 2016)



- ❖ Addition of P at dosages from 100 to 400 mg P kg⁻¹ soil increased MBC by at least 2-fold, but a decrease of about 27% was observed at a rate of application above 300 mg P kg⁻¹.
- ❖ The positive effect of P fertilization on MBC was attributed to the linear increase of P availability from 2 to 20 mg P kg⁻¹ soil.





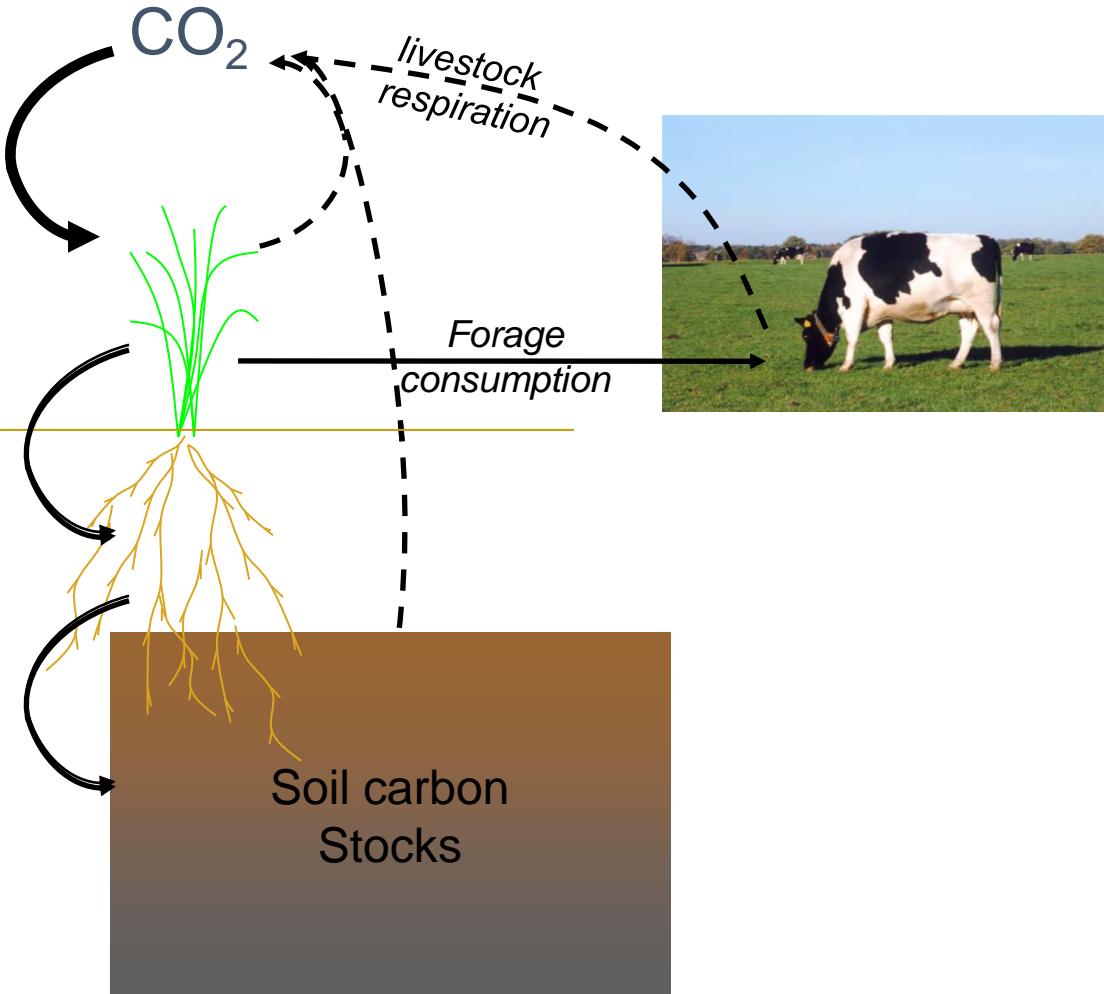


- ❑ Globally P input use efficiency is < 20%
- ❑ 5 million ha of acidic volcanic soils currently produce >50% primary production, but is capable to produce more for global food security
- ❑ Acidic volcanic soil immobilize P fertilizer and can accumulated up to $3000 \text{ kg P ha}^{-1}$





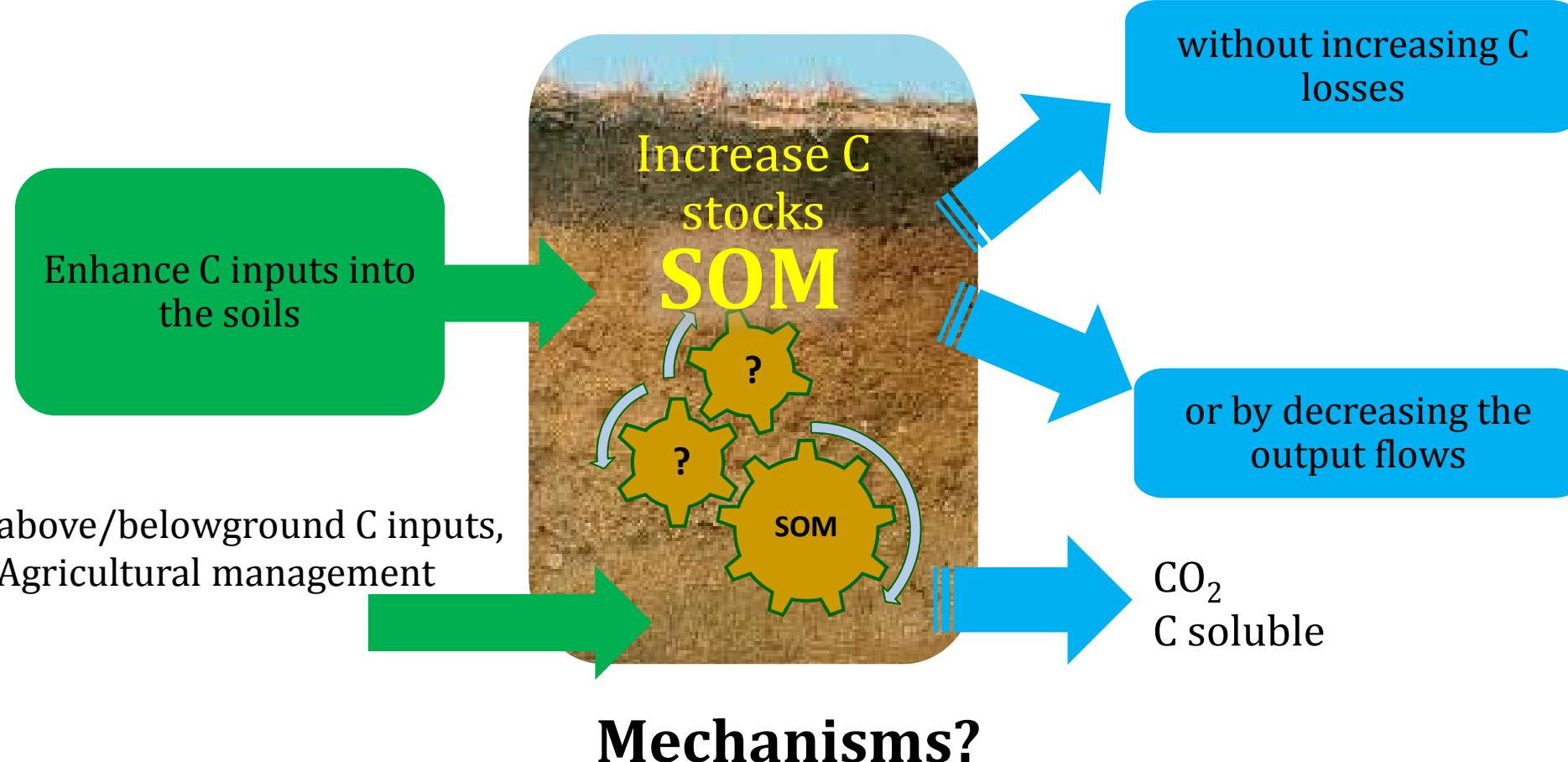
C fluxes in grassland ecosystems



1. Our practices have a carbon impact on carbon stocks
 - Grazing intensity / seasonality
 - Composition of species
 - Soil fertility



Challenges to increase C stocks



Producción agrícola



20% a las emisiones de gases de efecto invernadero provienen del sector agrícola





Crecimiento poblacional



2050 → 9,3 billones de personas

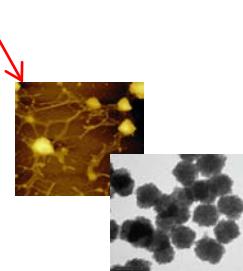
La producción de alimentos se debe doblar entre el 2005 al 2050 para poder proporcionar **seguridad alimentaria**



La fertilización actual y la superficie cultivable de la tierra no son suficientes para producir los alimentos requeridos



Ante este escenario resulta imperativo desarrollar **una producción sostenible frente al cambio climático**





INTRODUCTION



- ⦿ Globally P input use efficiency is < 20%
- ⦿ 5 million ha of acidic volcanic soils currently produce >50% primary production, but is capable to produce more for global food security
- ⦿ Acidic volcanic soil immobilize P fertilizer and can accumulated up to $3000 \text{ kg P ha}^{-1}$
- ⦿ OM in Andisols is stable against chemical attack. Borie and Zunino, (1989) P associated in stable organic macromolecules
- ⦿ 41% C and 35% P not extracted by fractionation (Velasquez et al., 2016)



INTRODUCTION

- The P fertilizer increases organic P (Velasquez et al., 2016)
- Cattle manure treated with phytase–nanoclay complexes are promising tool to increase P availability in Andisols (Calabi et al., 2012)
- Andisols and cattle manure treated with synthetic nanoclay immobilized phytase resulted in a significant inorganic P increase positively affecting plant growth and P nutrition of wheat plants. (Menezes-Blackburn et al. 2014)
- Calabi et al., 2012 shown that immobilization of AP increased the specific enzyme activity nanoclays were used as support materials.



Activation of microbial soil community by glucose and N addition to increase P availability and dry matter yield: «P Priming»

ANDISOL

High P fixation capacity.

High organic matter content

High ion exchange capacity.

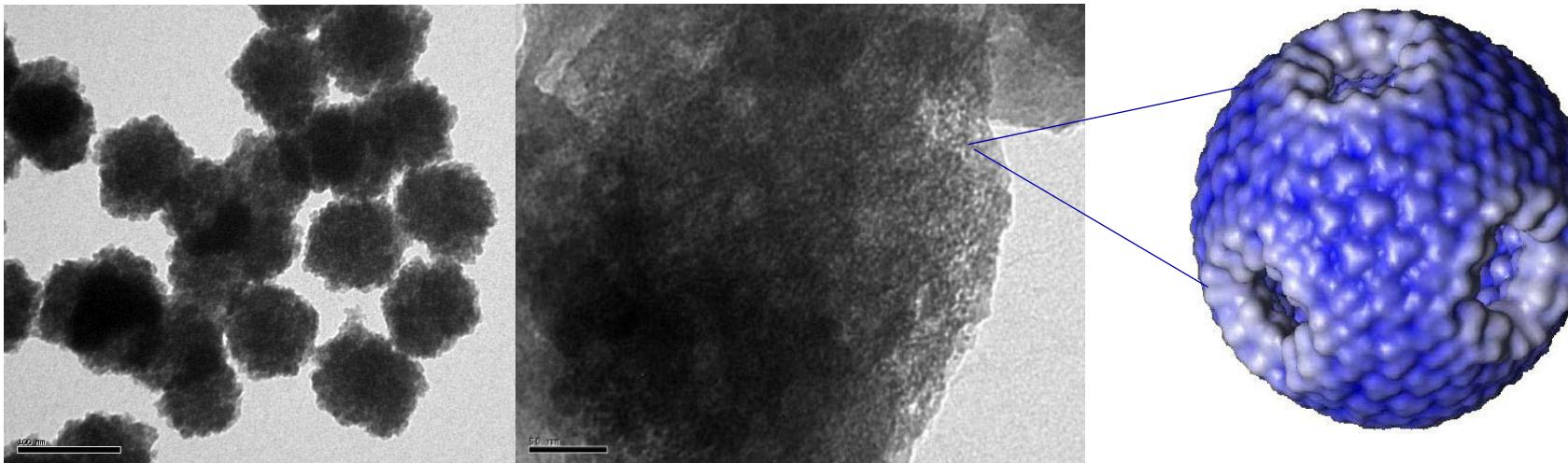
Aggregate stability

High water retention

☒ We are working for near to twenty years in Chilean ash derived soils (Andisols)



Nanoclays



- Stable microaggregates with pores within the nanoscale range
- Physical characteristics similar to silica nanomaterial like:
 - ✓ High surface area
 - ✓ Pore size
 - ✓ Pore volume

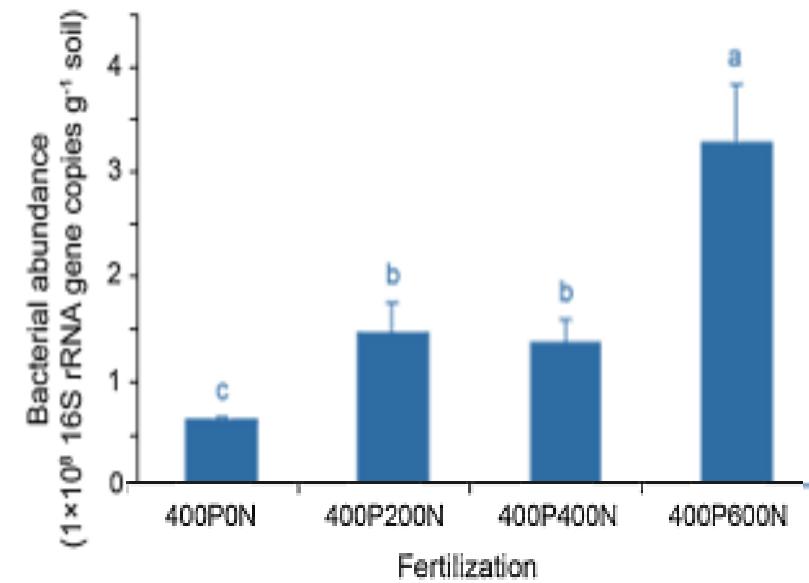


Effect of nitrogen and phosphorus fertilization on the composition of rhizobacterial communities of two Chilean Andisol pastures

Milko A. Jorquera · Oscar A. Martínez ·
Luis G. Marileo · Jacquelinne J. Acuña ·
Surinder Saggar · María L. Mora

- In this study demonstrates that the long-term urea fertilization induce changes in the composition of rhizobacterial communities of two Chilean Andisol pastures, particularly in the absence of P fertilization.

Phosphorus fertilization did not produce significant changes in the composition of pasture rhizobacterial communities.



Bacterial abundance (16S rRNA gene copies g^{-1} soil) estimated by quantitative PCR from rhizobacterial communities of pastures fertilized with phosphorus ($400 \text{ kg ha}^{-1} \text{ year}^{-1}$) and different doses of nitrogen (0, 200, 400 and $600 \text{ kg ha}^{-1} \text{ year}^{-1}$). Same letter denotes no statistical difference ($P \leq 0.05$; Tukey test)



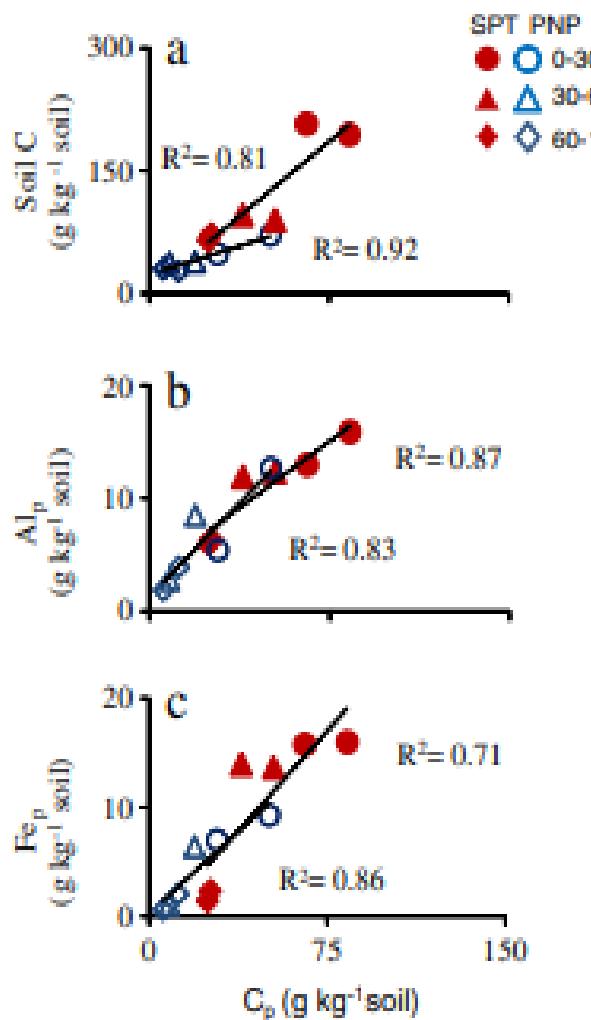


Fig. 3 Relationship between C_p and (a) soil C, (b) Al_p , and (c) Fe_p . Data represent the means and standard deviation of two replicate field samples of two pristine rain forest soils (SPT San Pablo de Tregua and PNP Puyehue National Park). Each data point is the average of duplicates

Neculman et al. (2013). Biol Fertil Soils

Isolation of culturable phosphobacteria with both phytate-mineralization and phosphate-solubilization activity from the rhizosphere of plants grown in a volcanic soil

Milko A. Jorquera · Marcela T. Hernández ·
Zed Rengel · Petra Marschner · María de la Luz Mora

Ocurrence of phosphobacteria in the rhizosphere of ryegrass, white clover, wheat, oat and yellow lupin

	Rizosphere										
	Perennial ryegrass		White clover		Wheat		Oat		Yellow lupin		
	a	b	a	b	a	b	a	b	a	b	
PMB	33.8	73.3	38.0	73.8	4.1	7.7	9.8	22.1	0.9	5.3	
PSB	5.6	12.2	3.0	5.8	34.0	63.4	26.2	59.3	8.9	52.0	
PMPSB	6.7	14.5	10.5	20.4	15.5	28.9	8.2	18.6	7.3	42.7	
Total phosphobacteria (TP)	46.1		51.5		53.6		44.2		17.1		

PMB: Culturable bacteria that mineralize phytate only

PSB: culturable bacteria that solubilize phosphate only.

PMPSB: culturable bacteria that mineralize phytate and solubilize phosphate.

TP: (Σ PSM+PSB+PSPSB)

a: Percent in relation to 300 colonies randomly chosen from LB agar

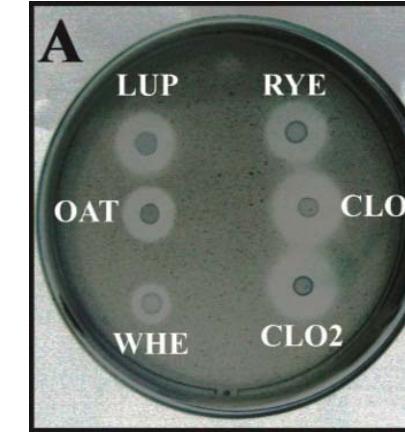
b: Percent in relation to TP



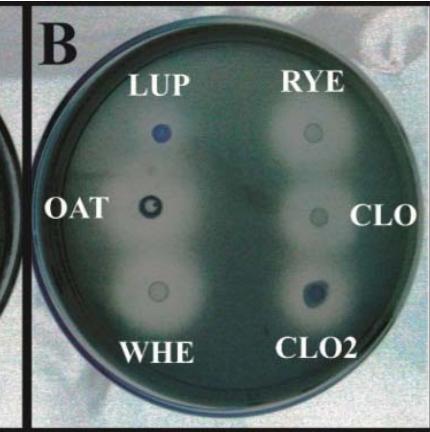
Genetic Characterization of selected phosphobacteria based on their capacity to utilize both Na-phytate and Ca-phosphate on agar media (PMPSB) to genus level

Rhizosphere	Genus ^a
Perennial ryegrass	<i>Pseudomonas</i>
White clover	<i>Enterobacter</i>
White clover	<i>Pseudomonas</i>
Wheat	<i>Pseudomonas</i>
Oat	<i>Pseudomonas</i>
Yellow lupin	<i>Pantoea</i>

Phytate



Phosphate



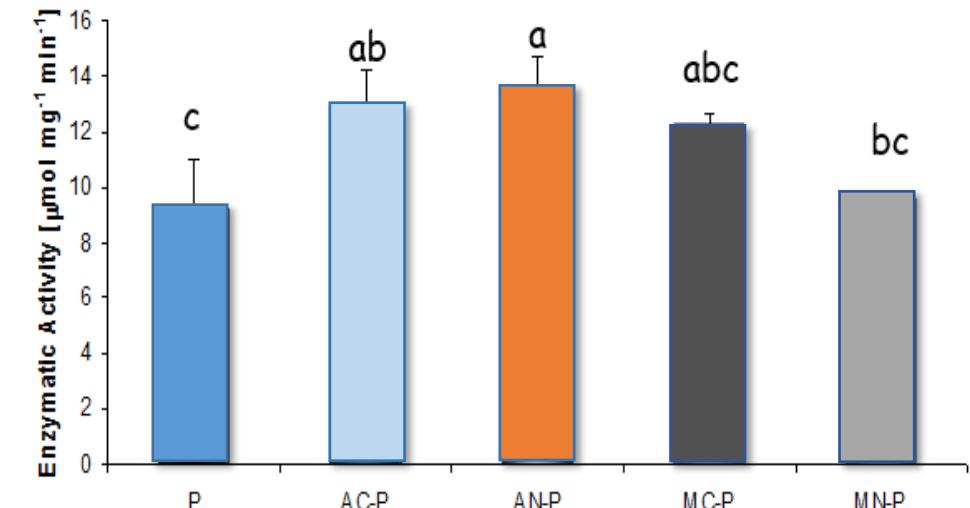
^a Based on partial sequencing of 16S rDNA gene and comparison with the National Center for Biotechnology Information database



Improving bioavailability of phosphorous from cattle dung by using phosphatase immobilized on natural clay and nanoclay

Marcela Calabi-Floody^a, Gabriela Velásquez^a, Liliana Gianfreda^b, Surinder Saggar^c, Nanthi Bolan^d,
Cornelia Rumpel^e, María Luz Mora^{a,*}

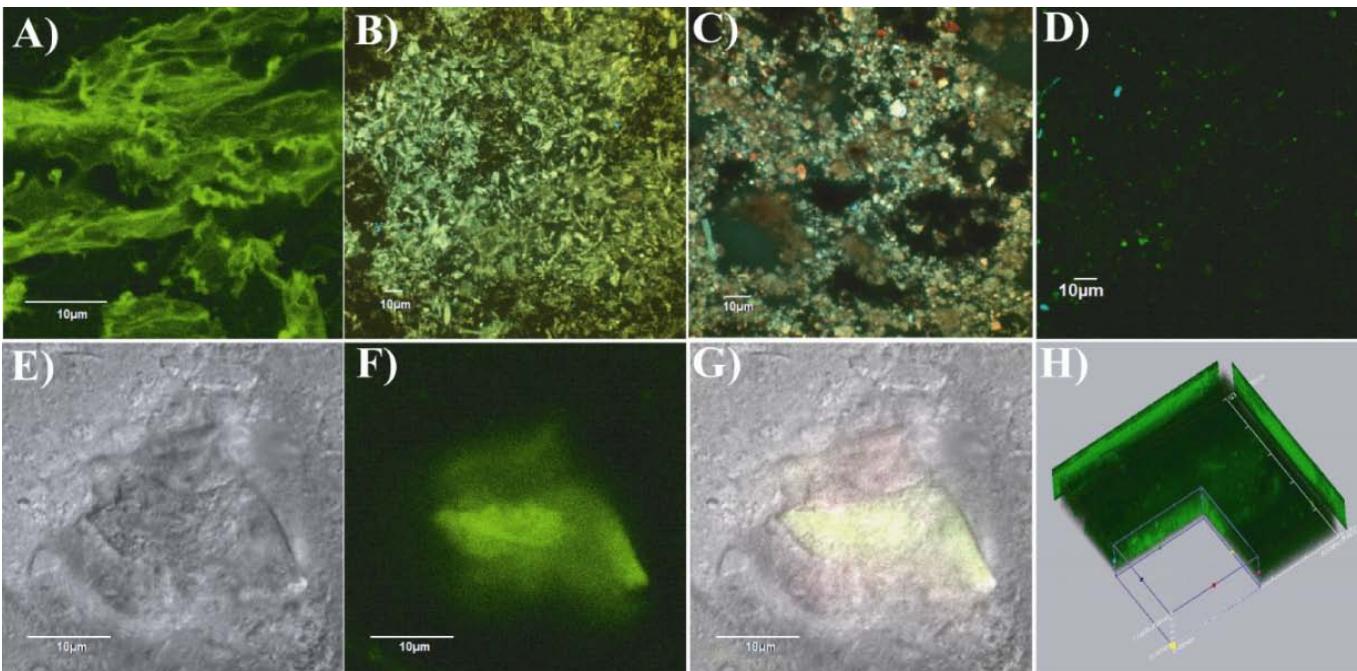
Enzyme activity was higher for immobilized enzyme than free enzyme and the support materials (allophanic and montmorillonite clays and nanoclays) increased enzyme activity from 4% to 48%, with nanoclays complexes having the highest specific activity.



Specific activity of acid phosphatase free (AP) and immobilized on allophanic clays (AC-AP) and nanoclays (AN-AP), and montmorillonite clays (MC-AP) and nanoclays (MN-AP).



- ⦿ Our research group has shown that APase immobilized on allophanic clays can increase the enzyme activity by 33% compared to free enzyme (Rosas et al., 2008).
- ⦿ Phytase activity after immobilization on synthetic allophane showed a significant increase in thermo and photolytic stability (Menezes-Blackburn et al., 2011)

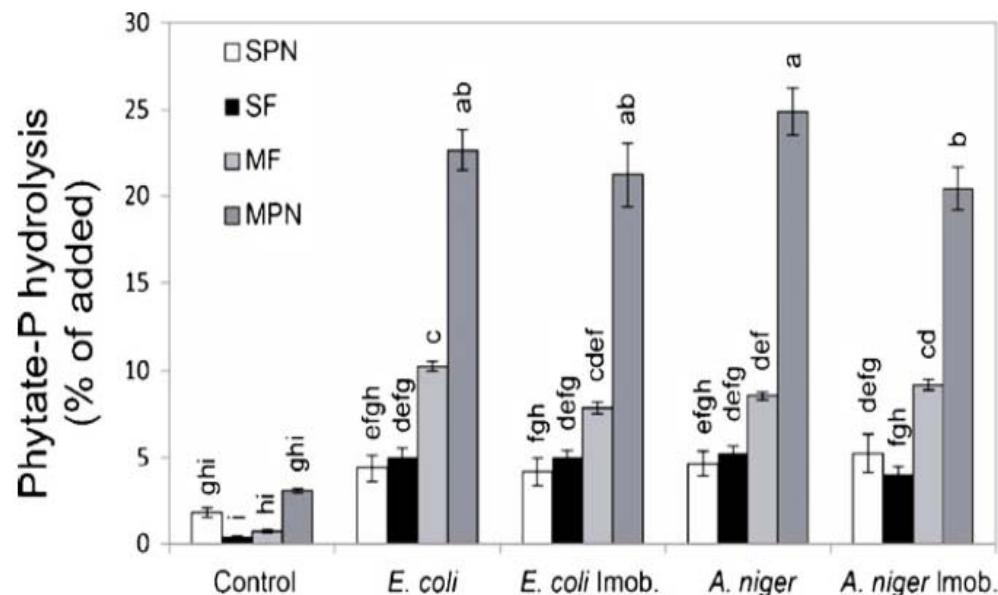


Fluorescence images by confocal microscopy of (A) free acid phosphatase, (B) allophanic nanoclays phosphatase complex, (C) allophanic nanoclays with organic matter (D) allophanic nanoclays treated with H_2O_2 , and immobilization studies (E) differential interference contrast (Nomarski) of the allophane synthetic complexes (F) fluorescence image of acid phosphatase immobilized on synthetic allophane, (G) overlap of the images E and F, (H) 3D projection of montmorillonite nanoclays-phosphatase complexes



A novel phosphorus biofertilization strategy using cattle manure treated with phytase–nanoclay complexes

Daniel Menezes-Blackburn · Milko A. Jorquera ·
Liliana Gianfreda · Ralf Greiner · María de la Luz Mora



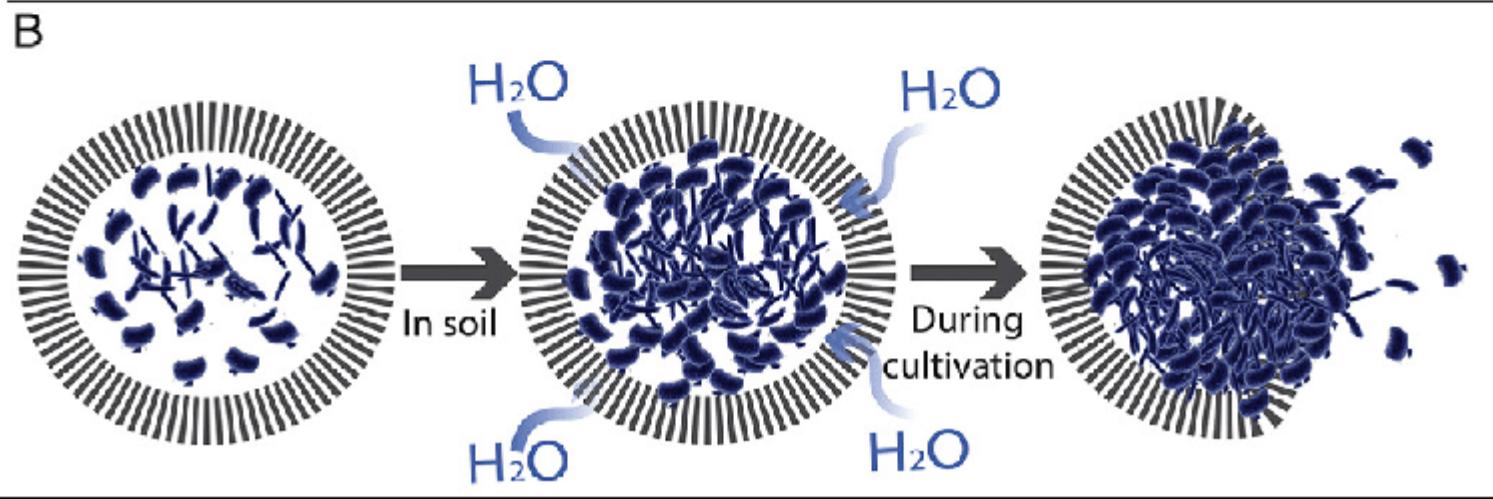
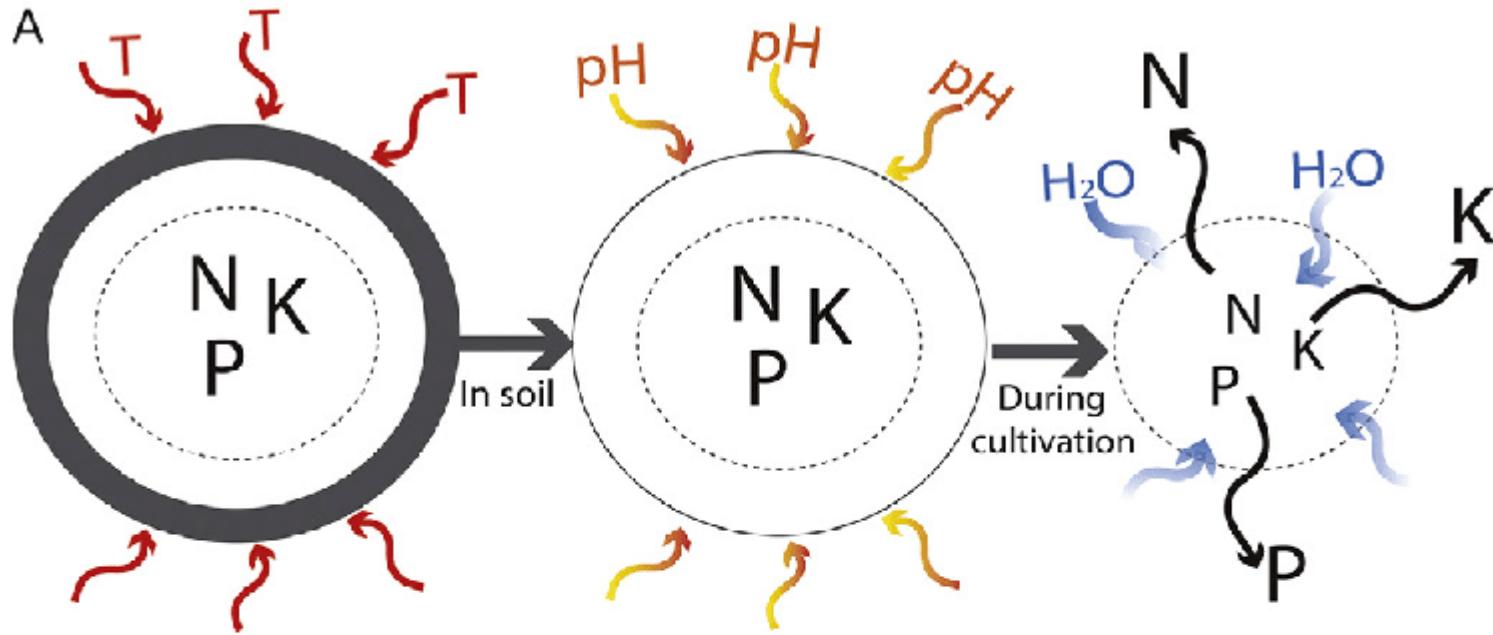
- ▣ Differences in the hydrolysis of freshly added phytate between the type and immobilization of enzymes were not significant
- ▣ Hydrolysis of additional phytate was affected by the substrate type, being highest (approximately 25 %) for MPN, followed by MF (~10 %) and approximately 5 % for both soils.
- ▣ Control samples presented some native phytase activity, which was responsible for the hydrolysis of 1–3 % of the added phytate.



Smart Fertilizer and Sustainable Agriculture

“P Priming”

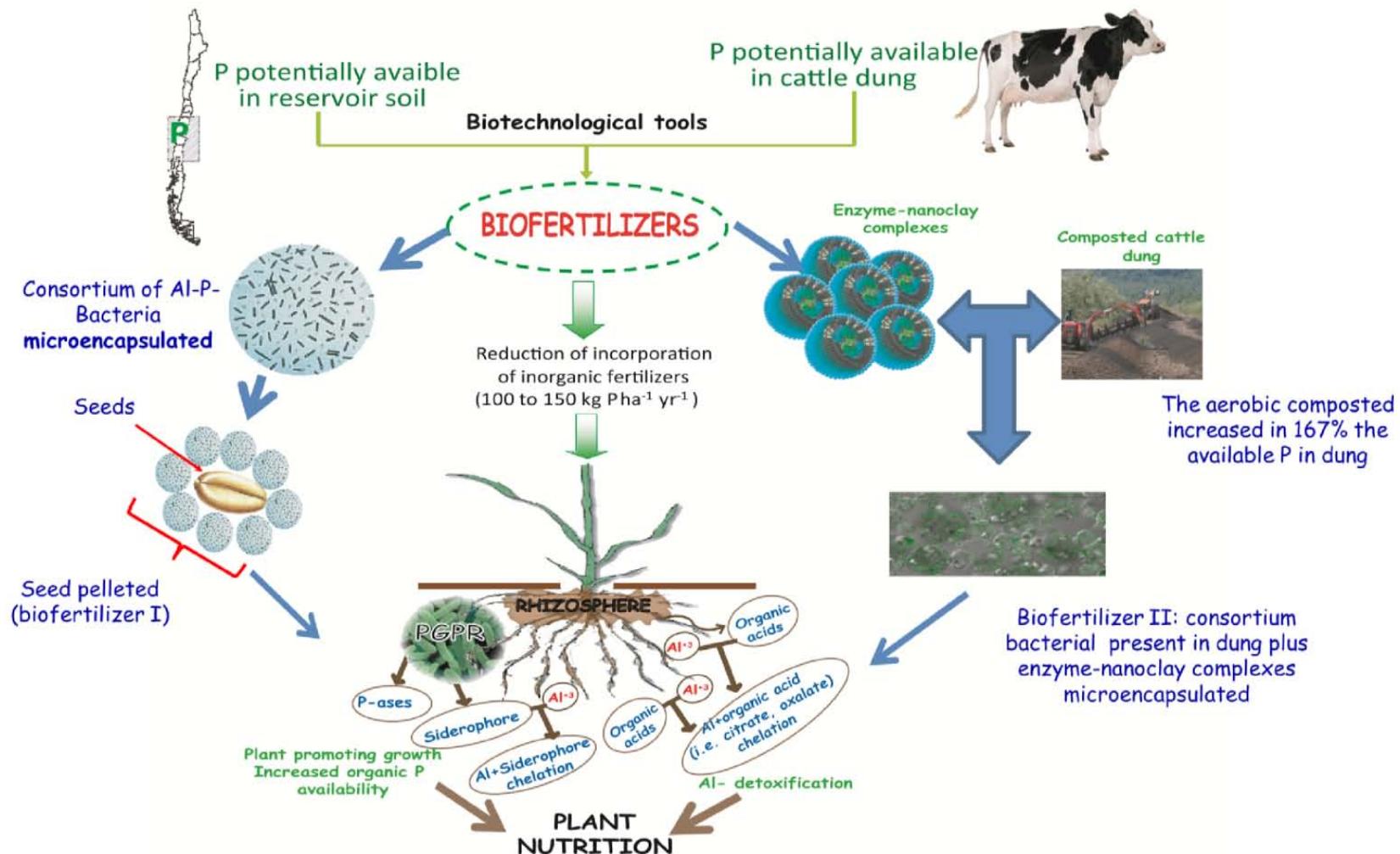
María de la Luz Mora
Universidad de La Frontera-Chile



Schematic representation of smart delivery systems: (A) advanced polymeric materials, degraded under external stimulus such as temperature, pH, and with water permeability to achieve a slow nutrient release; (B) microorganism encapsulation. Calabi-Floody et al./ Advances in Agronomy, 147 (2017), pp.119-157

GRAPHICAL ABSTRACT

We have been studying the soil-plant-microorganism interactions to develop different strategies for improving crop and pasture yields and quality.





Aluminum-tolerant bacteria improve the plant growth and phosphorus content in ryegrass grown in a volcanic soil amended with cattle dung manure



Maria de la Luz Mora^{a,*}, Rolando Demanet^a, Jacqueline J. Acuña^a, Sharon Viscardi^a,
Milko Jorquera^a, Zed Rengel^b, Paola Durán^a

^a Scientific and Technological Bioresource Nucleus, Universidad de La Frontera, Temuco, Chile

^b School of Agriculture and Environment, University of Western Australia, Perth, Australia

Selected bacteria were able to tolerate elevated concentration of Al⁺³ (10 mM).

Characteristics of selected aluminum-tolerant (10 mM Al) bacteria used in the consortium formulation

Isolate	Isolation Source	IAA ($\mu\text{g L}^{-1}$)	ACCD	PM	PS	SID
Klebsiella sp	endophyte	23.6	+	++	+	+
Stenotrophomonas sp RC5	endophyte	31.4	+	+	+	+
Enterobacter sp. RJAL6	rhizosphere	39.9	+	+	+	+
Klebsiella sp. RCJ4	rhizosphere	32.5	+	+	+	+
Serratia sp. RCJ6	rhizosphere	21.5	+	+	+	+

IAA: Indol acetic acid production; ACCD: 1-aminocyclopropane-1-arboxylate deaminase activity; PM: Phytate mineralizing activity; PS: P solubilization; SID: Siderophore production.



Greenhouse experiments I

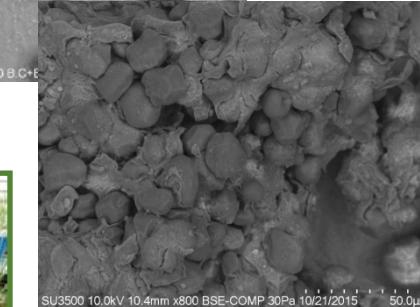
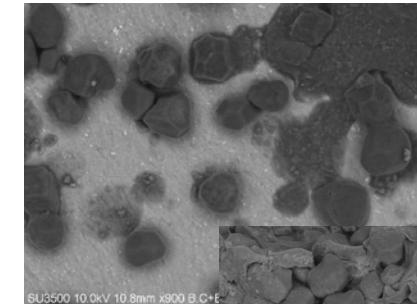
Uninoculated Control

TSP (100 kg of $P_2O_5\text{ ha}^{-1}$)

Bacterial consortium

Cattle dung

Bacterial consortium +
Cattle dung

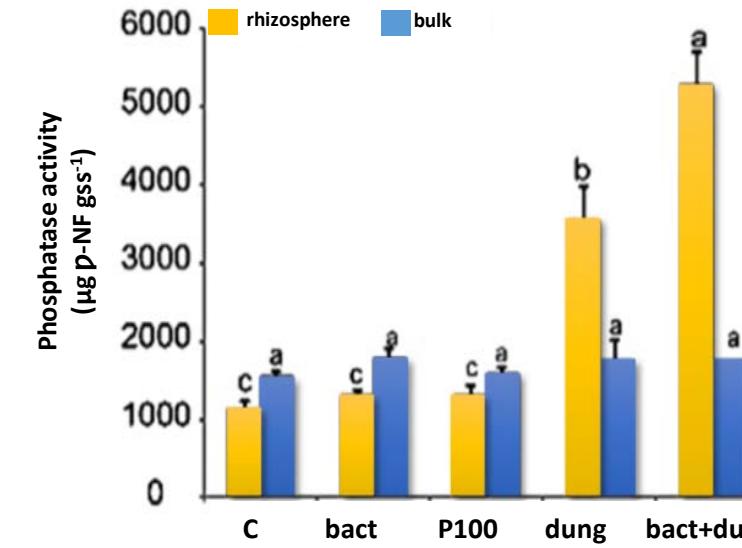
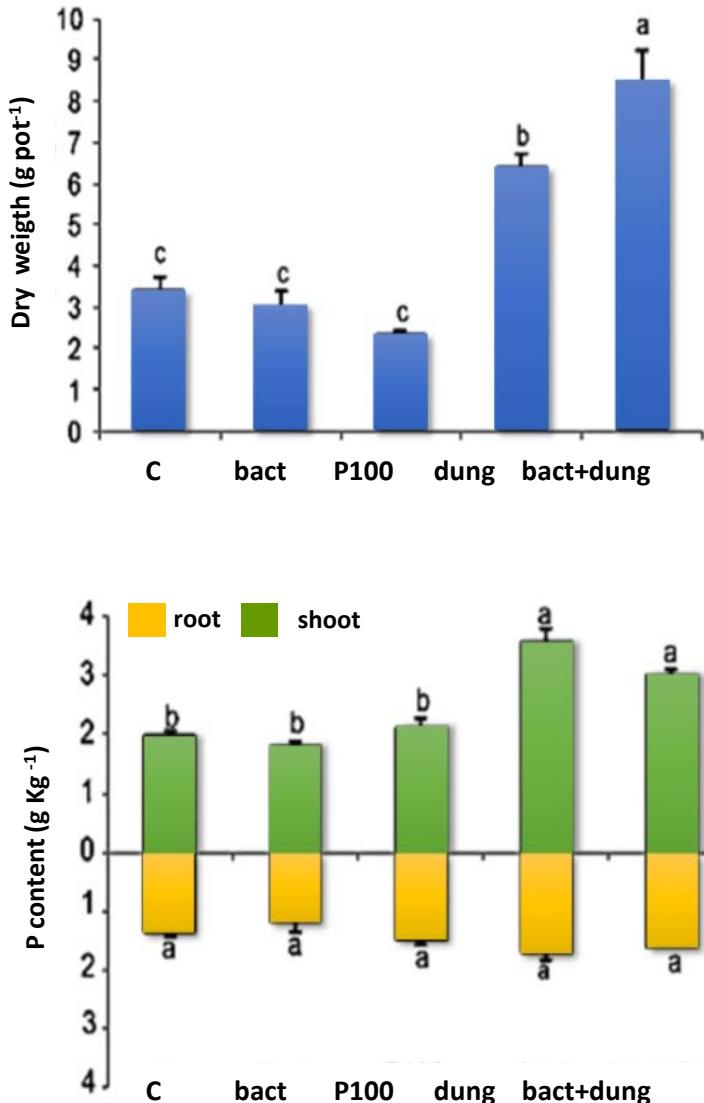


Bacteria microencapsulation

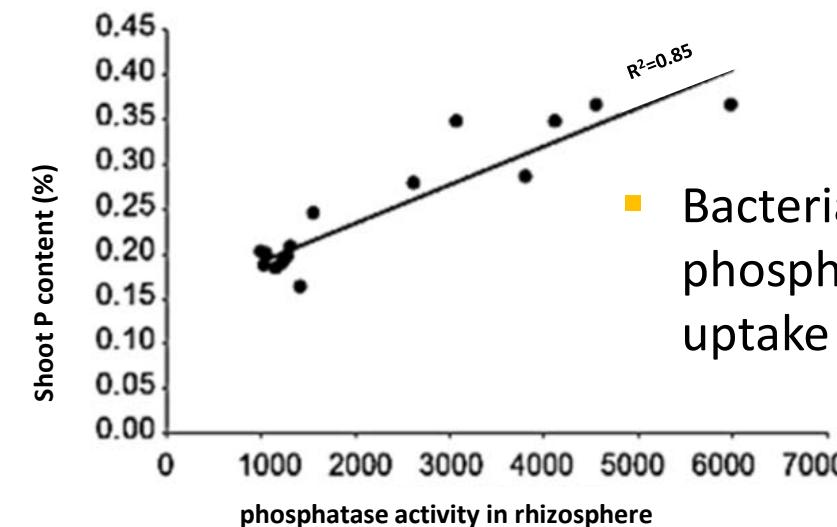
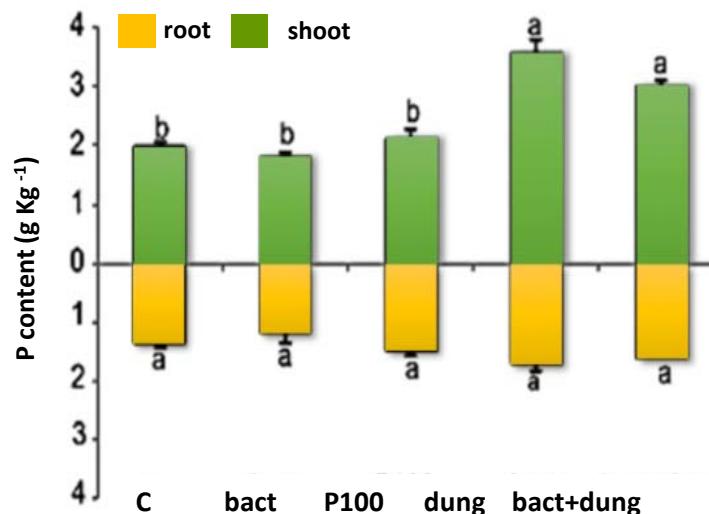
Development of Biofertilizer I



RESULTS GREENHOUSE EXPERIMENTS I



■ Bacteria consortia enhance biomass in ryegrass plant fertilized with cattle dung.



■ Bacteria consortia improve the phosphatase production and P uptake in ryegrass.

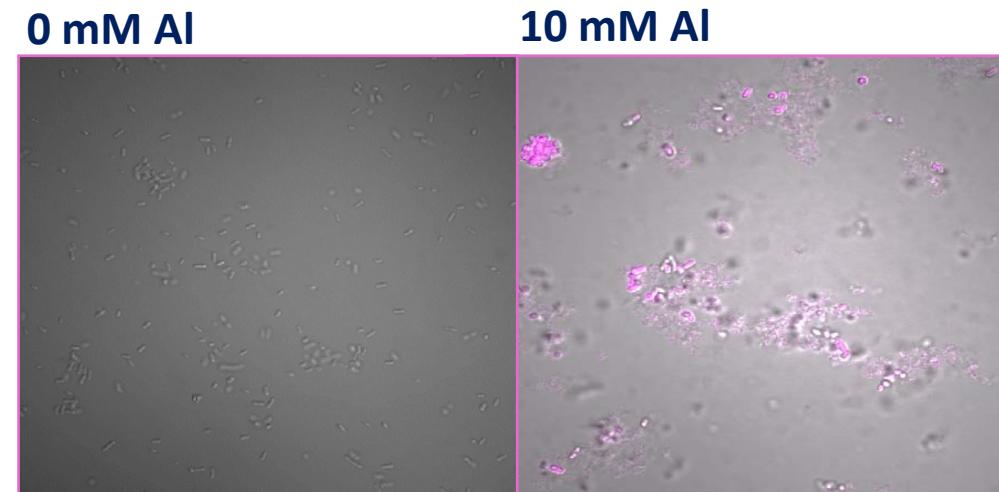


RESULTS GREENHOUSE EXPERIMENTS I

Bacterial strains used in the present study and showing the capacity to chelate Al by siderophores, have a potential to be used as biofertilizer in Andisol to ameliorate Al toxicity in crops and pastures.

(Mora et al., 2017)

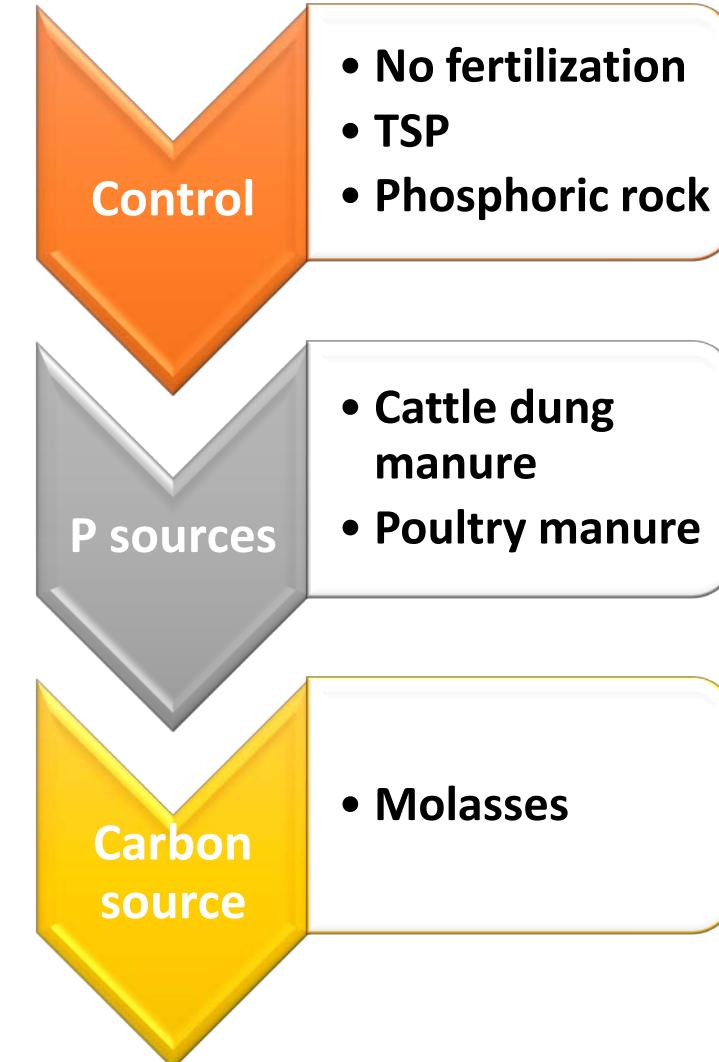
Selected bacteria were able to form Al^{+3} siderophore complexes in vitro conditions

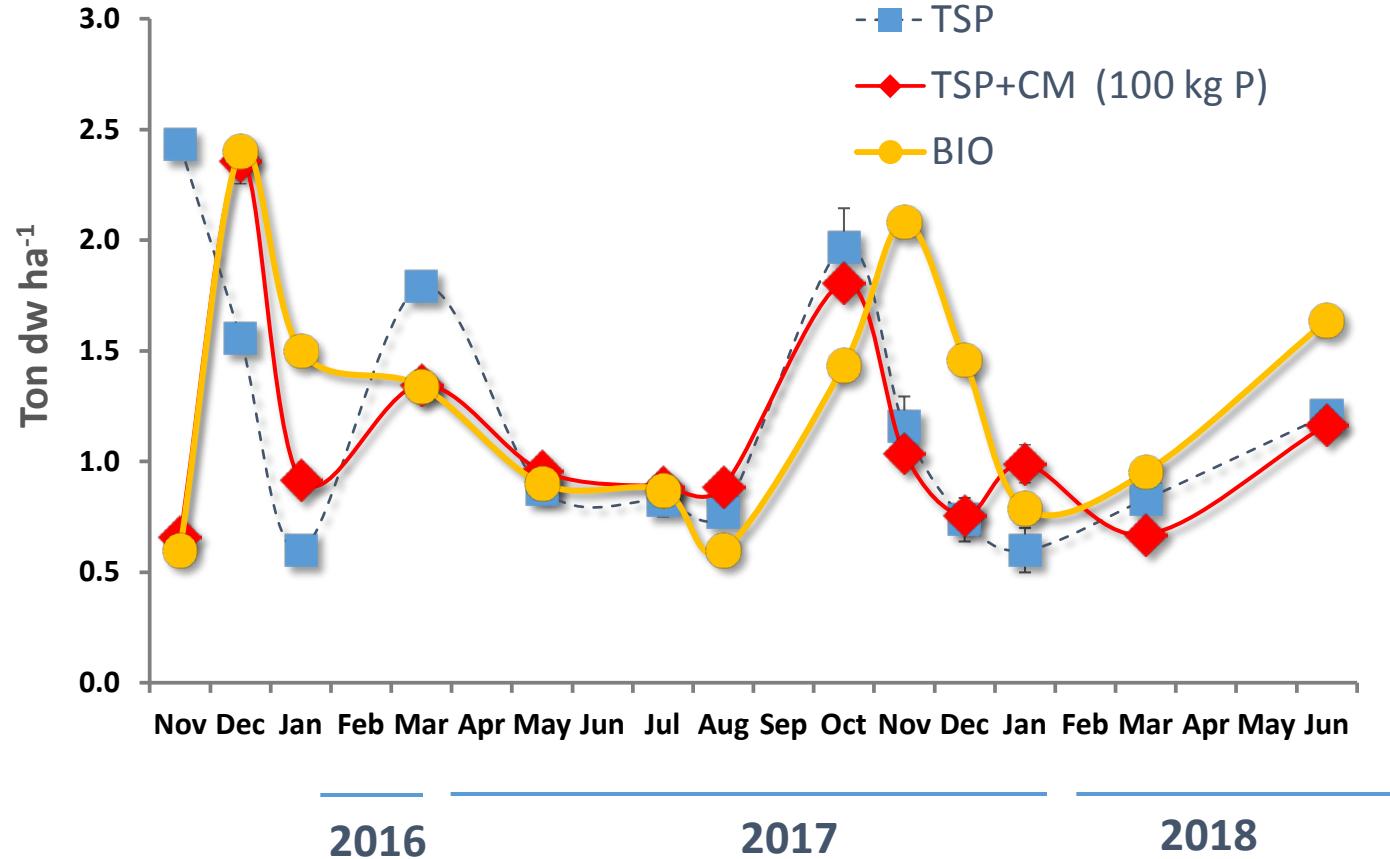


Confocal laser scanning micrographs of the siderophore complex forming in bacteria (*Klebsiella* sp RCJ4) in the presence of 0 mM (control) and 10 mM aluminum



FIELD EXPERIMENT





Lolium perenne field production (ton dw ha⁻¹) under Triple superphosphate (TSP), TSP and cattle manure (100 kg ha⁻¹) and BIOact (BIO) P fertilization.

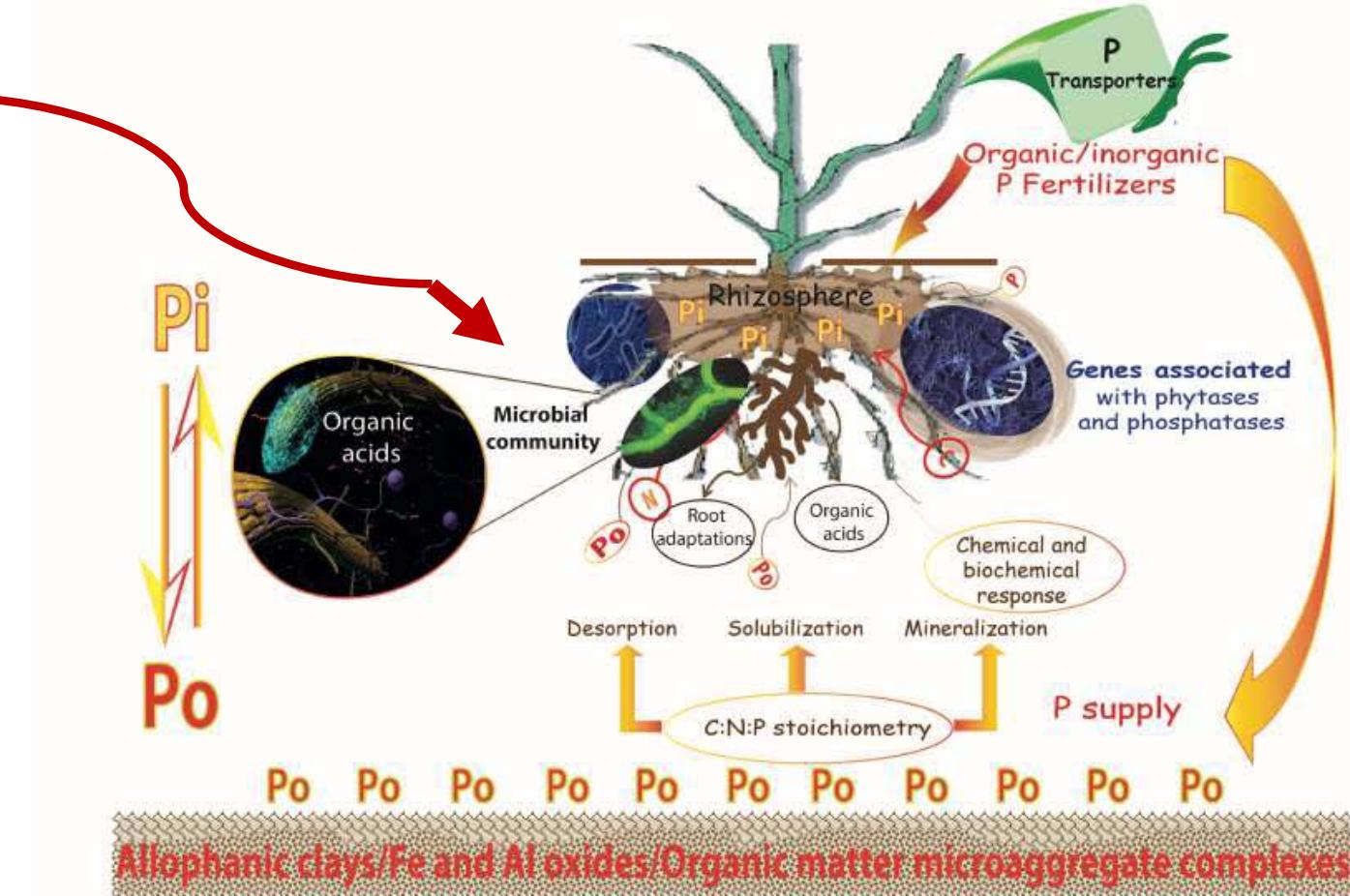
The result showed that during summer, BioAct increased the production of *Lolium perenne*.

Soil-plant-microbiome interactions

What is the role of microbes into plant nutrition?

Applying genomics:

- **Microbial diversity**
- metagenomics
- Metatranscriptomics
- Microbial genomics

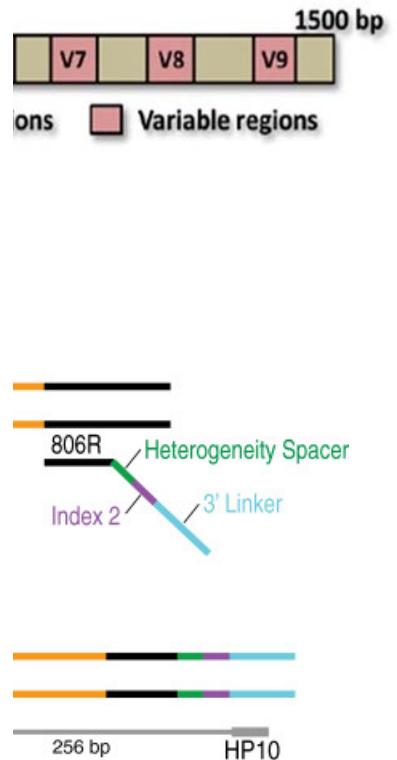
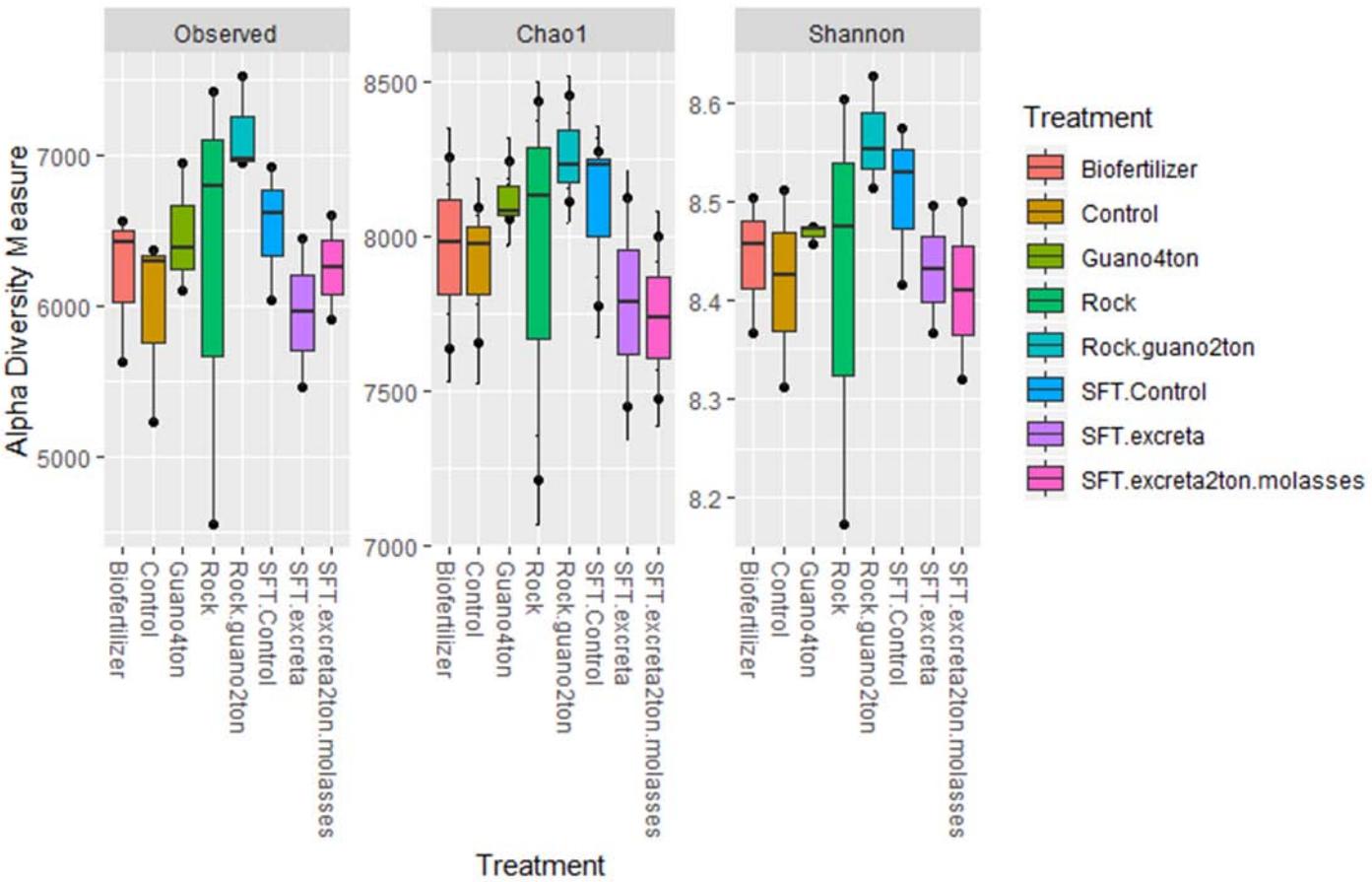


Key microbial actions

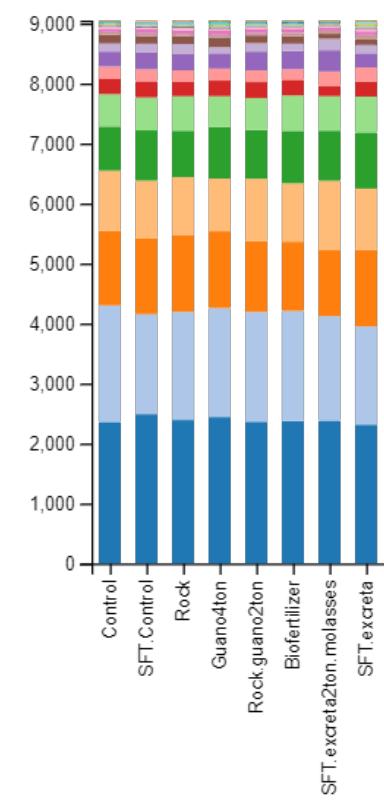
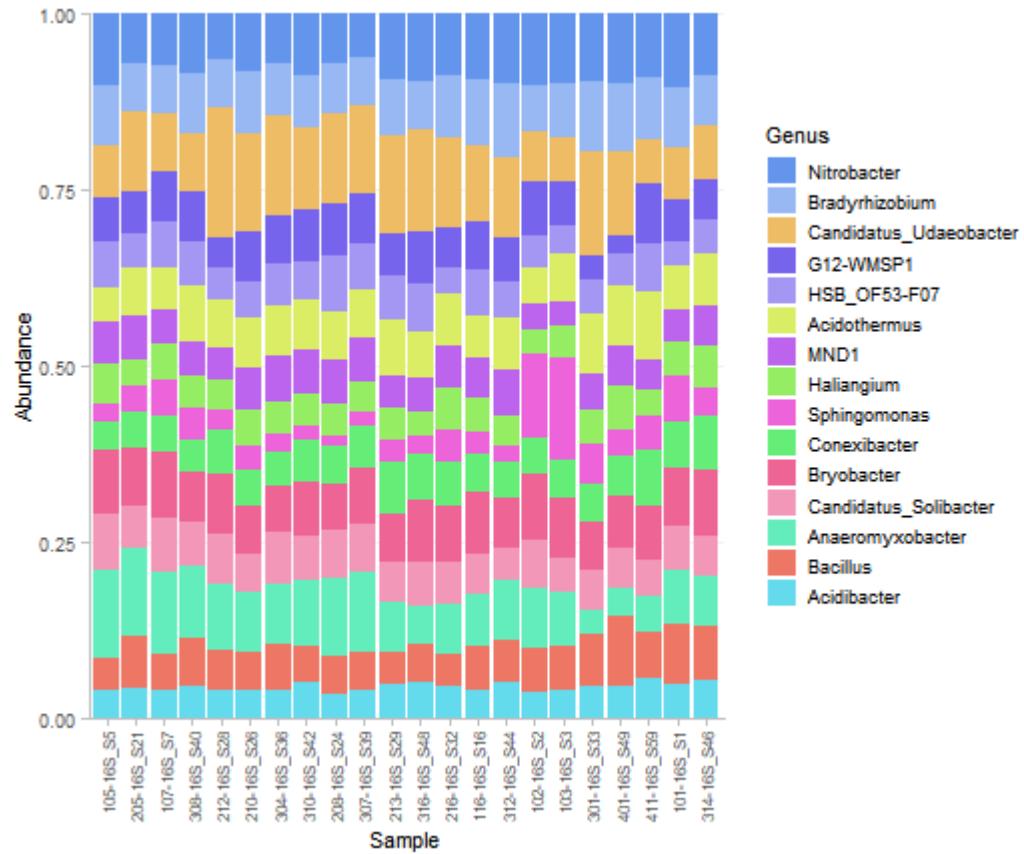
- Microbes perform key functions such as **Nitrogen fixation** and **Phosphate solubilization**
 - Nitrogen in the air is not bio-available to plants
 - Plants can't use organic phosphates in the soil
 - Nitrogen and organic phosphates in the soil are converted by microbes into a form that plants can use
- Microbes act as **Nutrient Cyclers** making soil nutrients more bio-available to the plants
 - Microbes break down organic matter in the soil, freeing carbon and other key nutrients, returning them to biological circulation

Assessing microbial composition by NGS

- The experimental treatment consists of 8 treatments totaling 64 samples.
- The amplification of the rRNA 16S gene was performed using Illumina Miseq.
- Bioinformatics analysis includes quality control, merging of paired-end reads, identification of operational taxonomic units (OTUs), and a pipeline for taxonomic classification.
- Taxonomy classification was based on 16S rRNA genes.



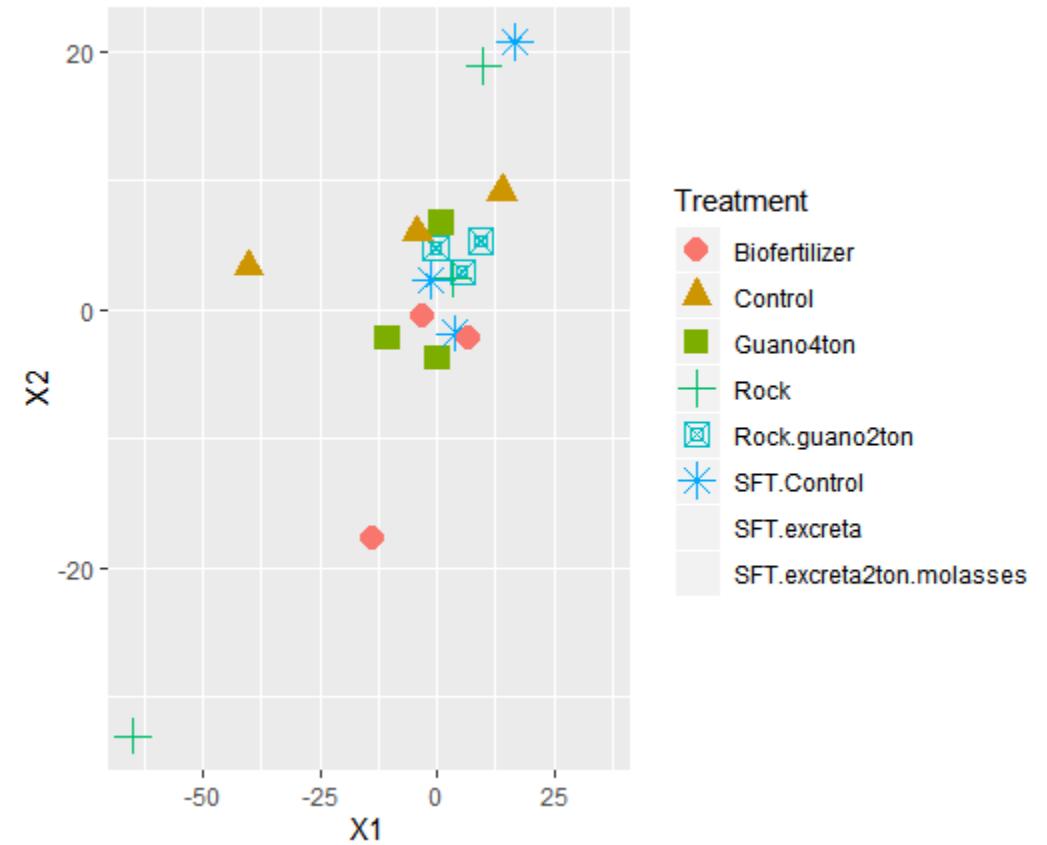
Top 15 most abundant bacteria taxa



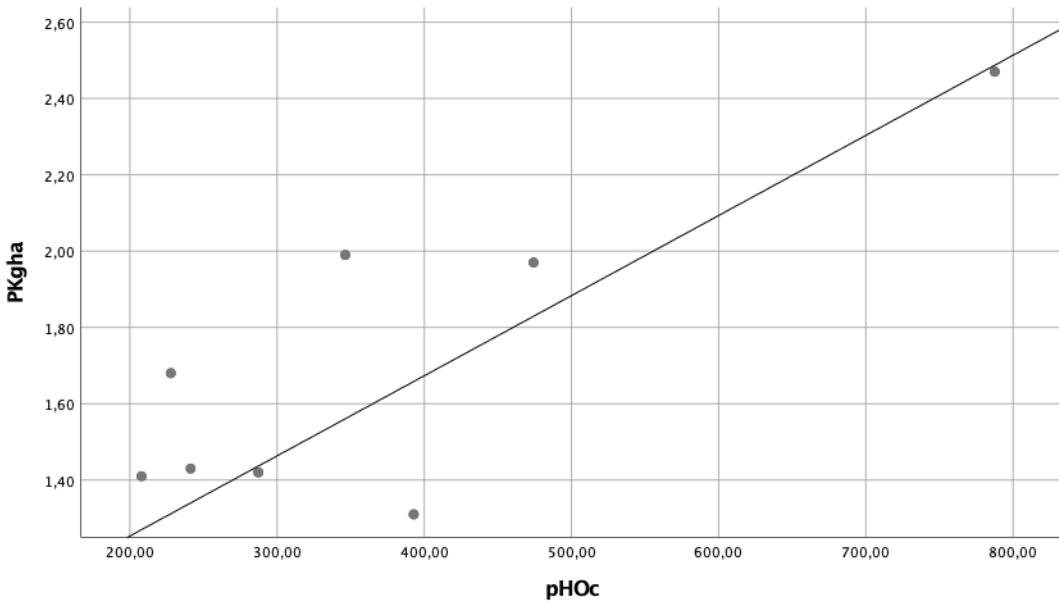
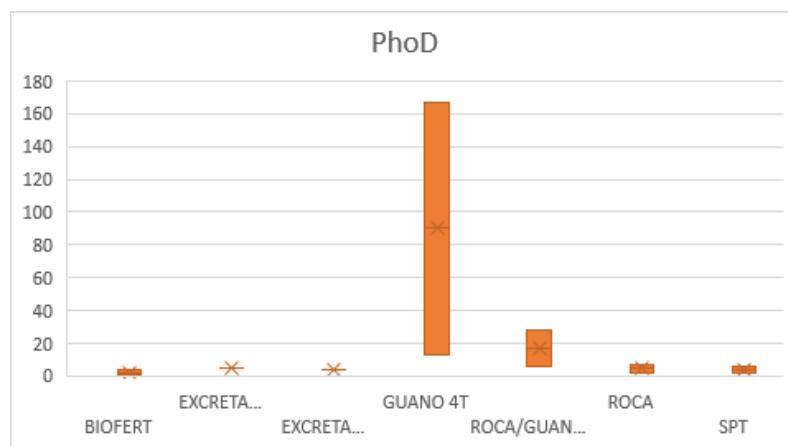
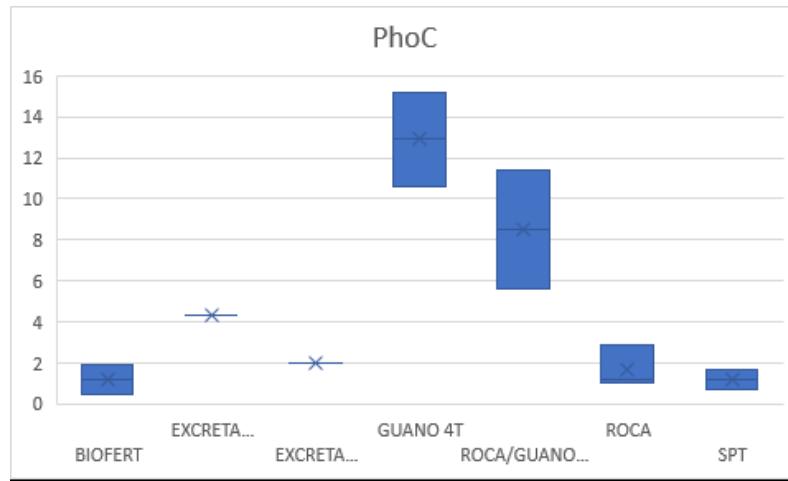
Beta diversity

tSNE plot using Bray-Curtis distance between samples

t-Distributed Stochastic Neighbor Embedding - tSNE. This method emphasizes local distances instead of global distances, thus generating greater resolution or separation between points or samples



Relative expression of *phoC* and *phoD* genes



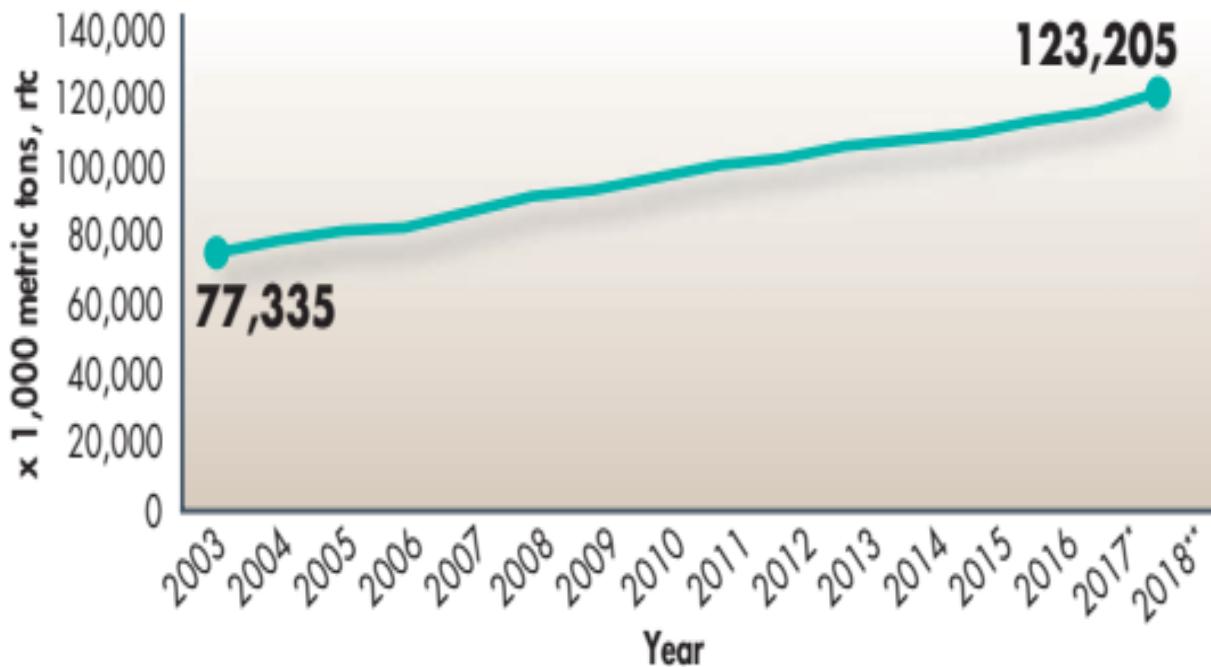
- Cow dung manure increase alkaline phosphatase (*phoD*) more than acid phosphatase (*phoC*)
- Due to manures are generally slightly alkaline, and therefore, bacterial alkaline P-propeller phytases (BPP) would be the most suitable in this environment (Menezes-blackburn, 2016).
- *phoC* is more efficient in support available P to plant uptake.

Introducción



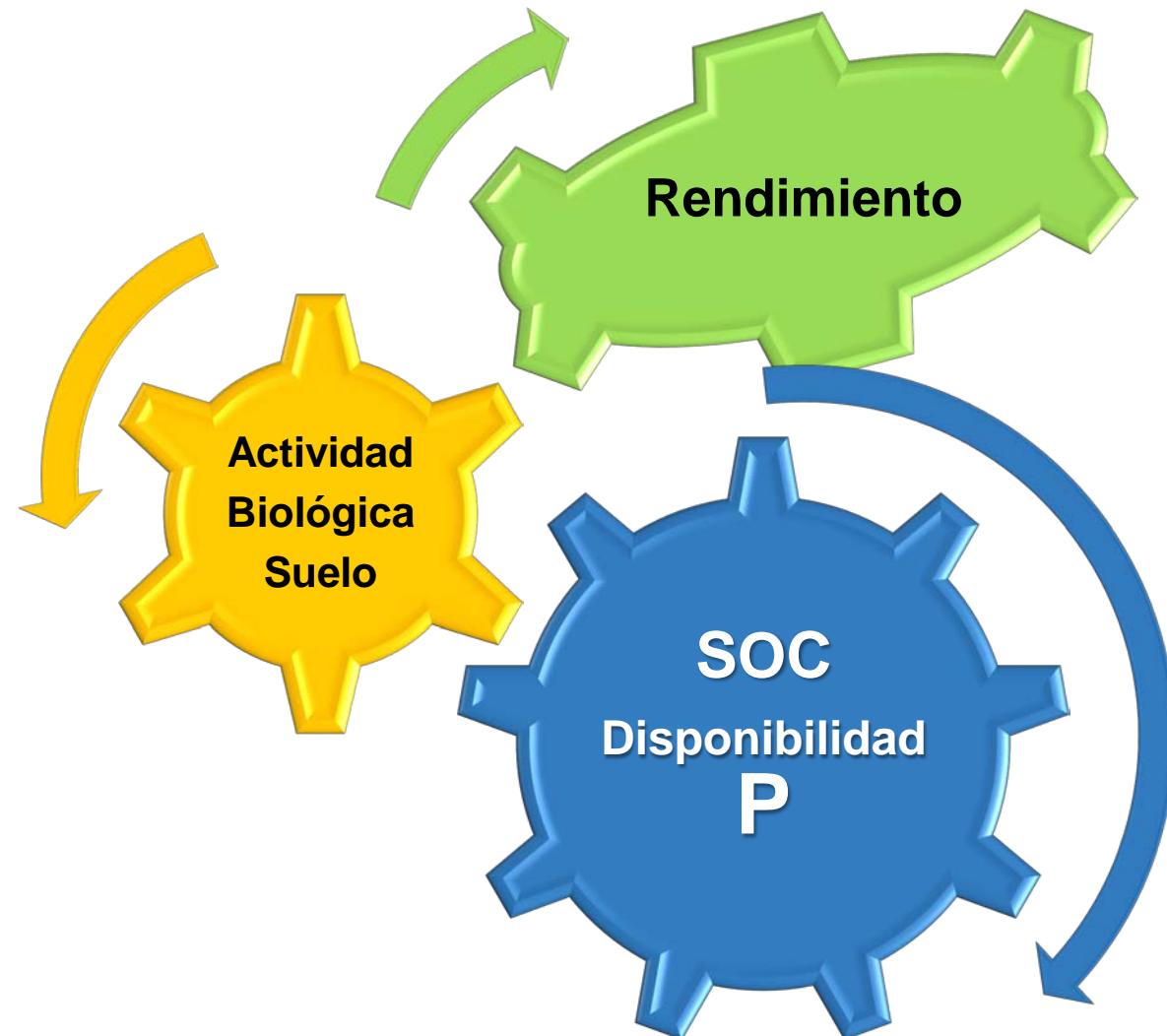
World poultry meat production trend 2003-18

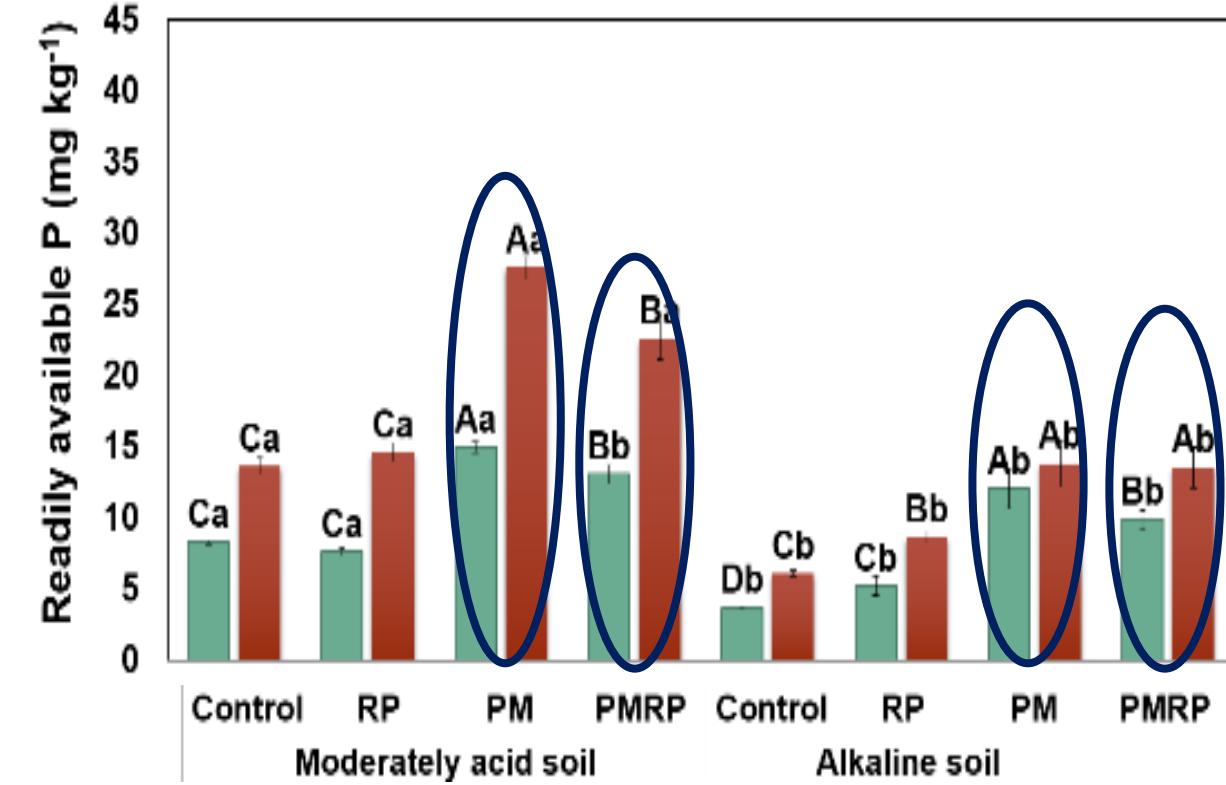
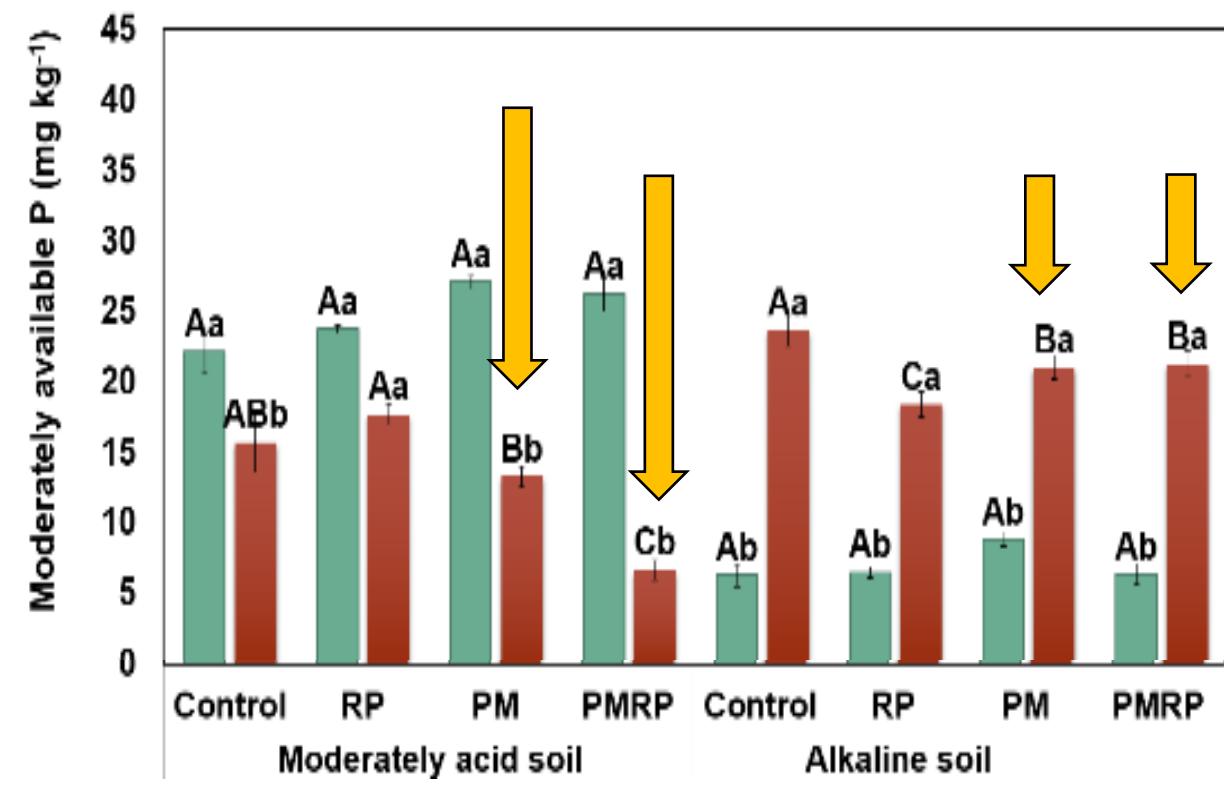
Copyright WATT Global Media 2018



*Estimate **Projection

Source: Adapted from OECD-FAO Agricultural Outlook publications

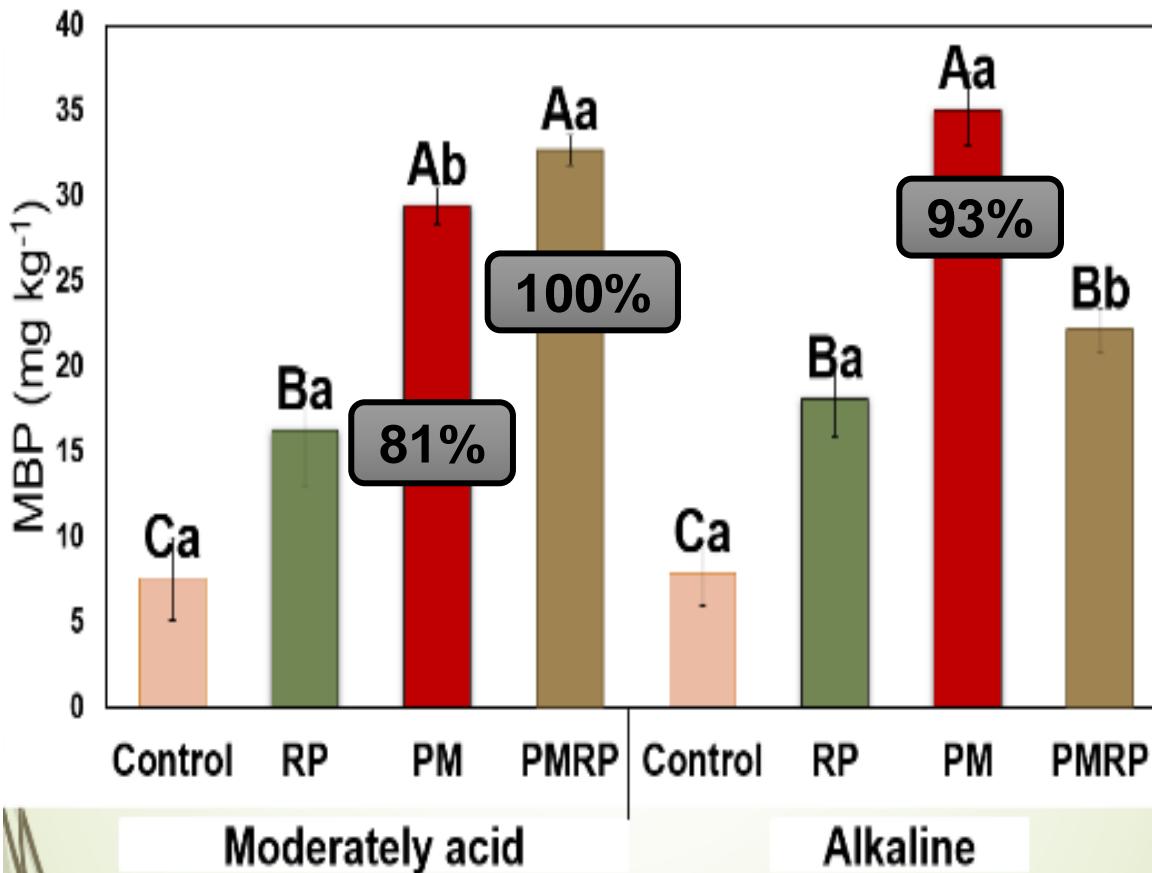


**A)****B)**

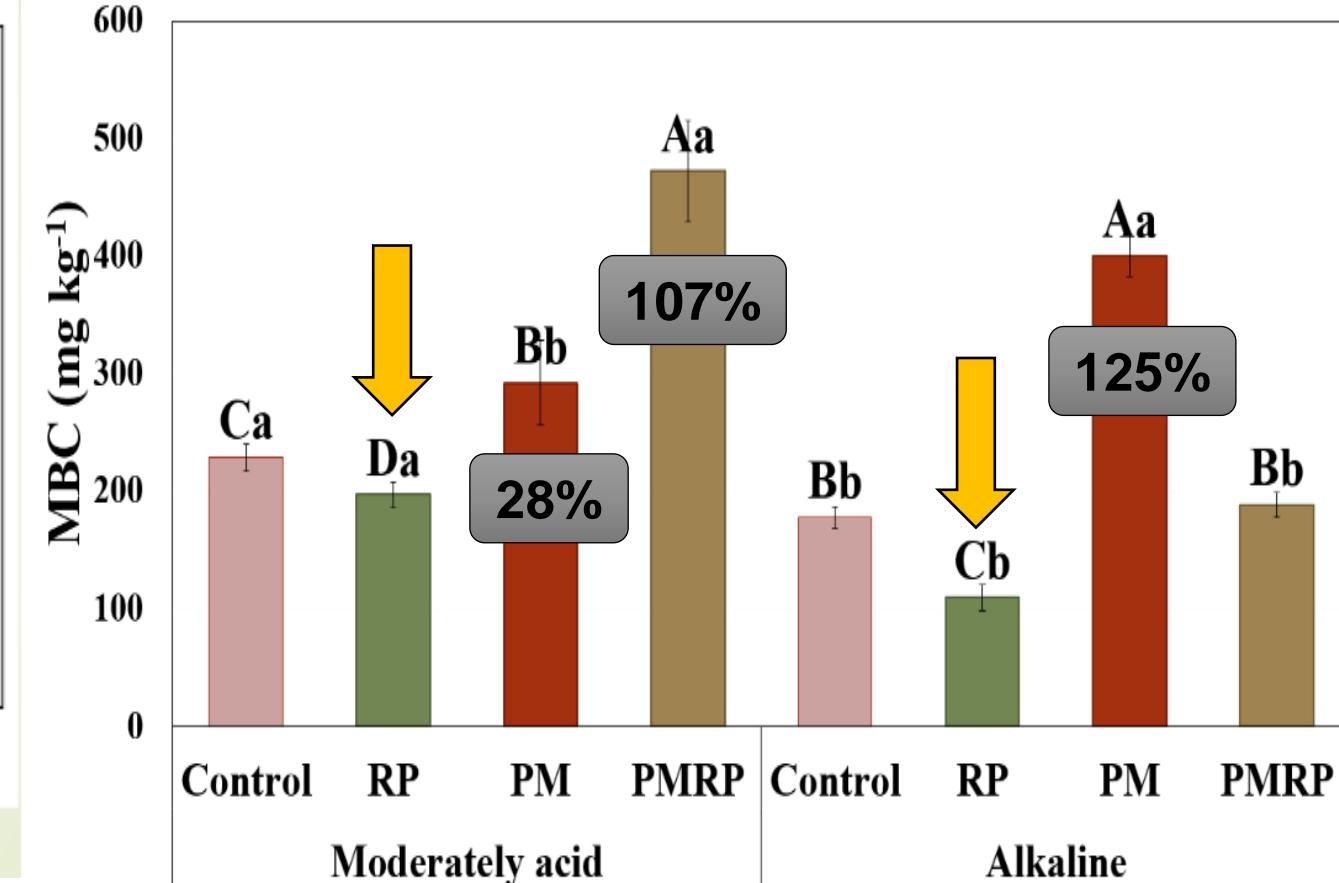
Inorganic and organic P concentration of fractions (A,B). Upper case letters mean differences between treatment in each soil. Lower case letters mean differences between soil in each treatment. $p \leq 0.05$



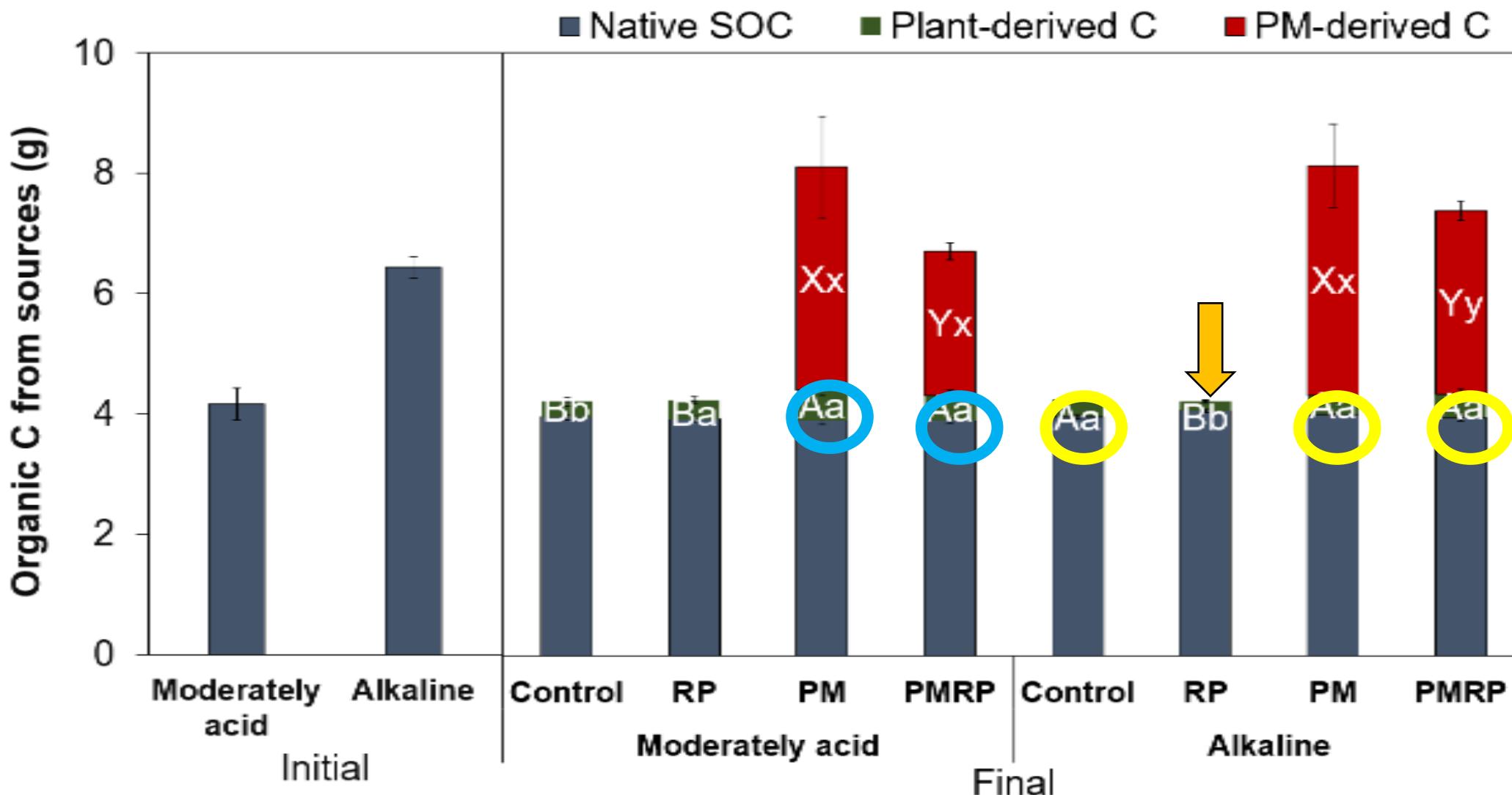
A)



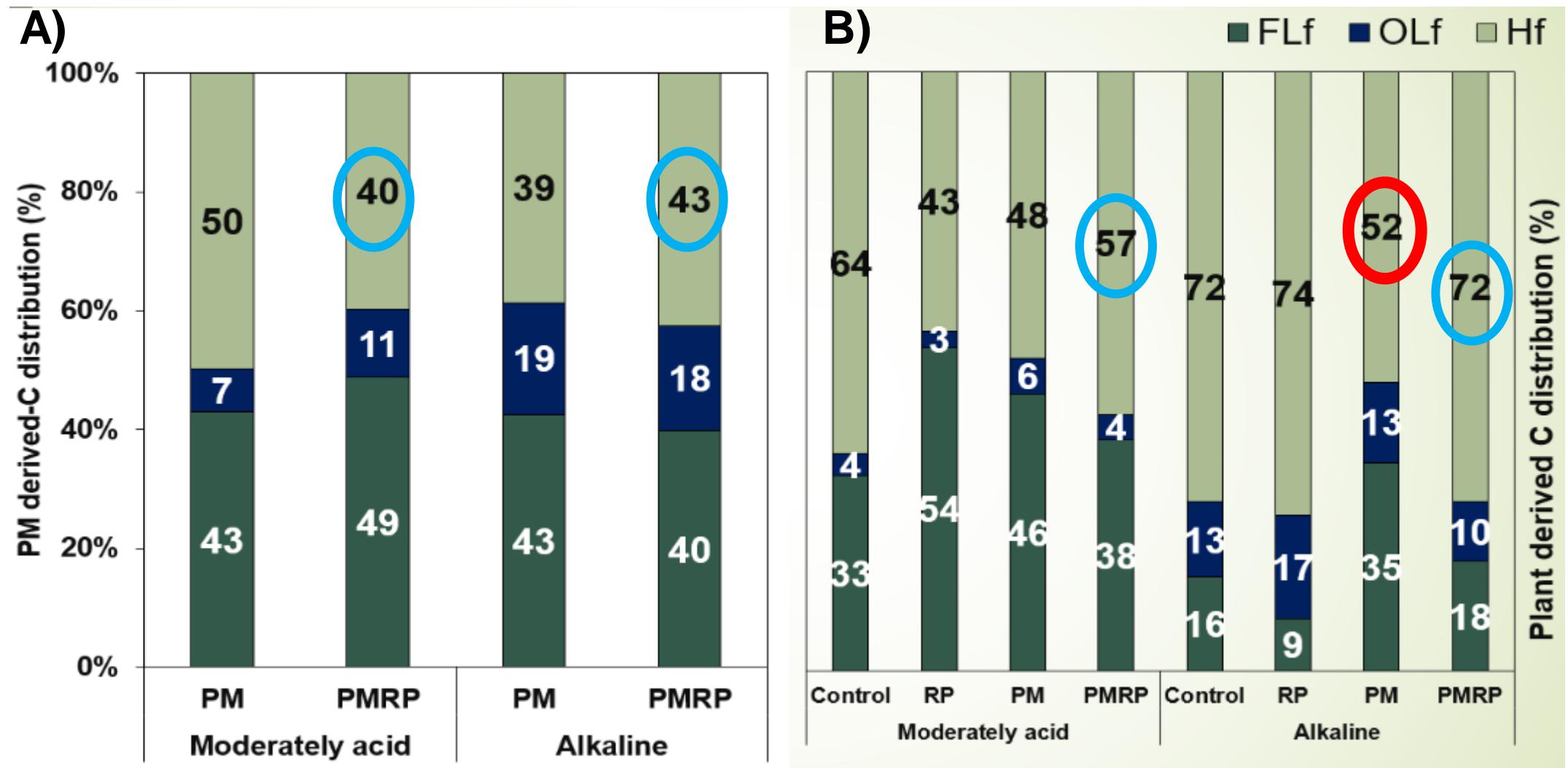
B)



Soil microbial biomass phosphorus (A) and carbon (B). Upper case letters means differences between treatment in each soil. Lower case letters means differences between soil in each treatment. $p \leq 0.05$



Organic carbon from sources (A) in the rhizosphere soil. Upper case letters means differences between treatment in each soil. Lower case letters means differences between soil in each treatment. $p \leq 0.05$



Poultry manure (A) and plant (B) derived-C distribution from free light (FLf), occluded light (OLf) and heavy fraction (Hf) of SOM.

SOM stability increases from FLf (active pool) to Hf (mineral-associated SOM) (Von Lützow et al., 2007)

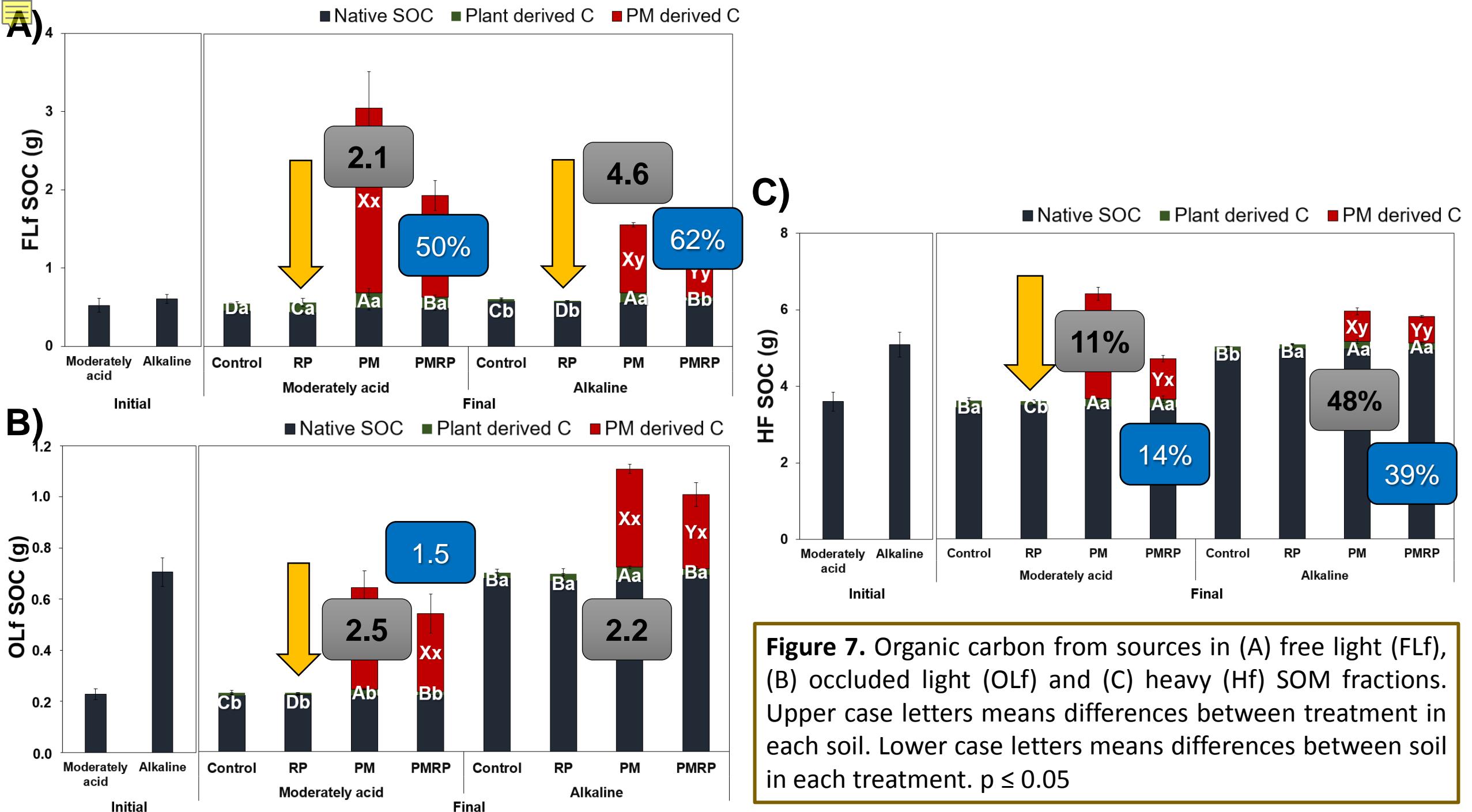
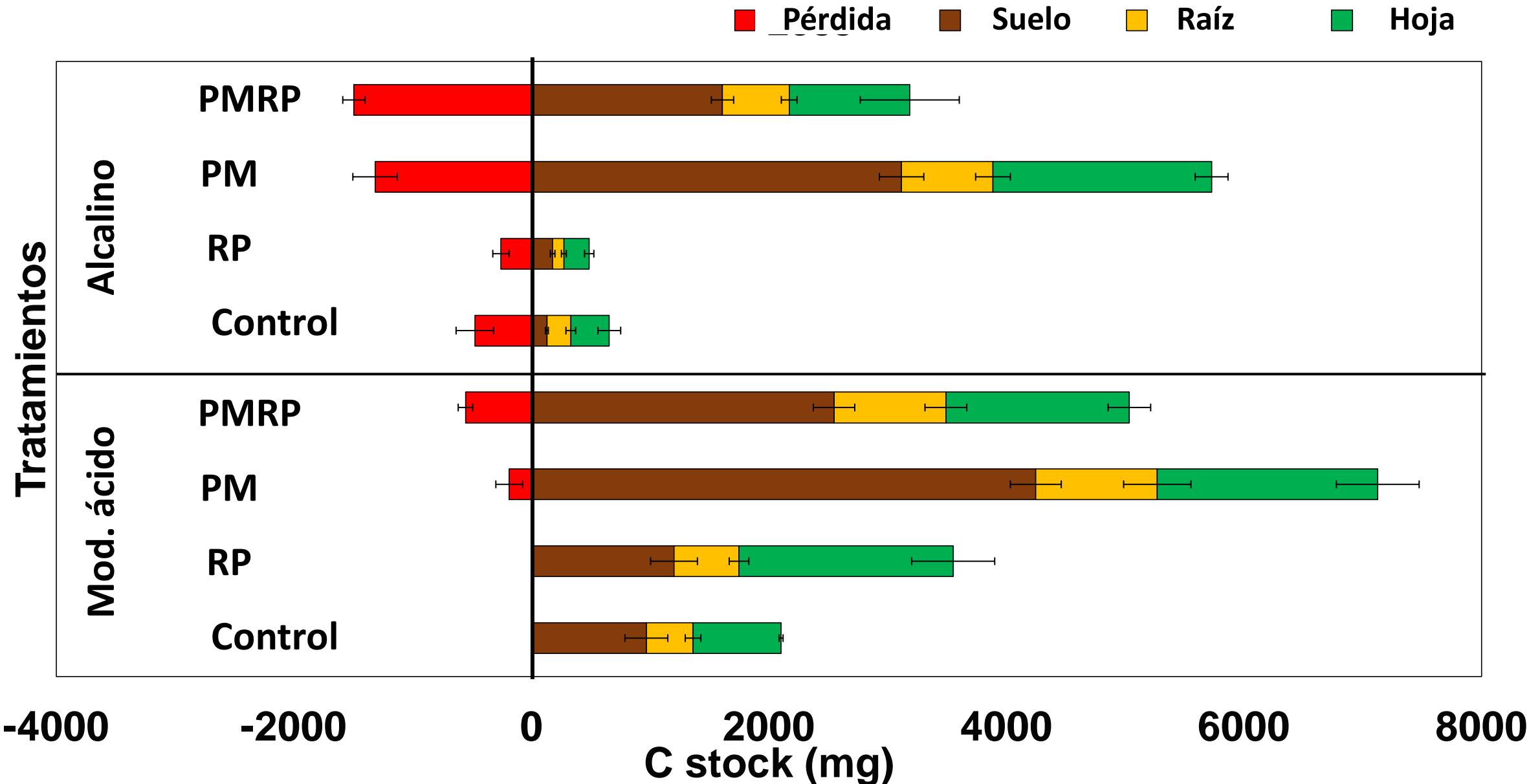


Figure 7. Organic carbon from sources in (A) free light (FLf), (B) occluded light (OLF) and (C) heavy (Hf) SOM fractions. Upper case letters means differences between treatment in each soil. Lower case letters means differences between soil in each treatment. $p \leq 0.05$

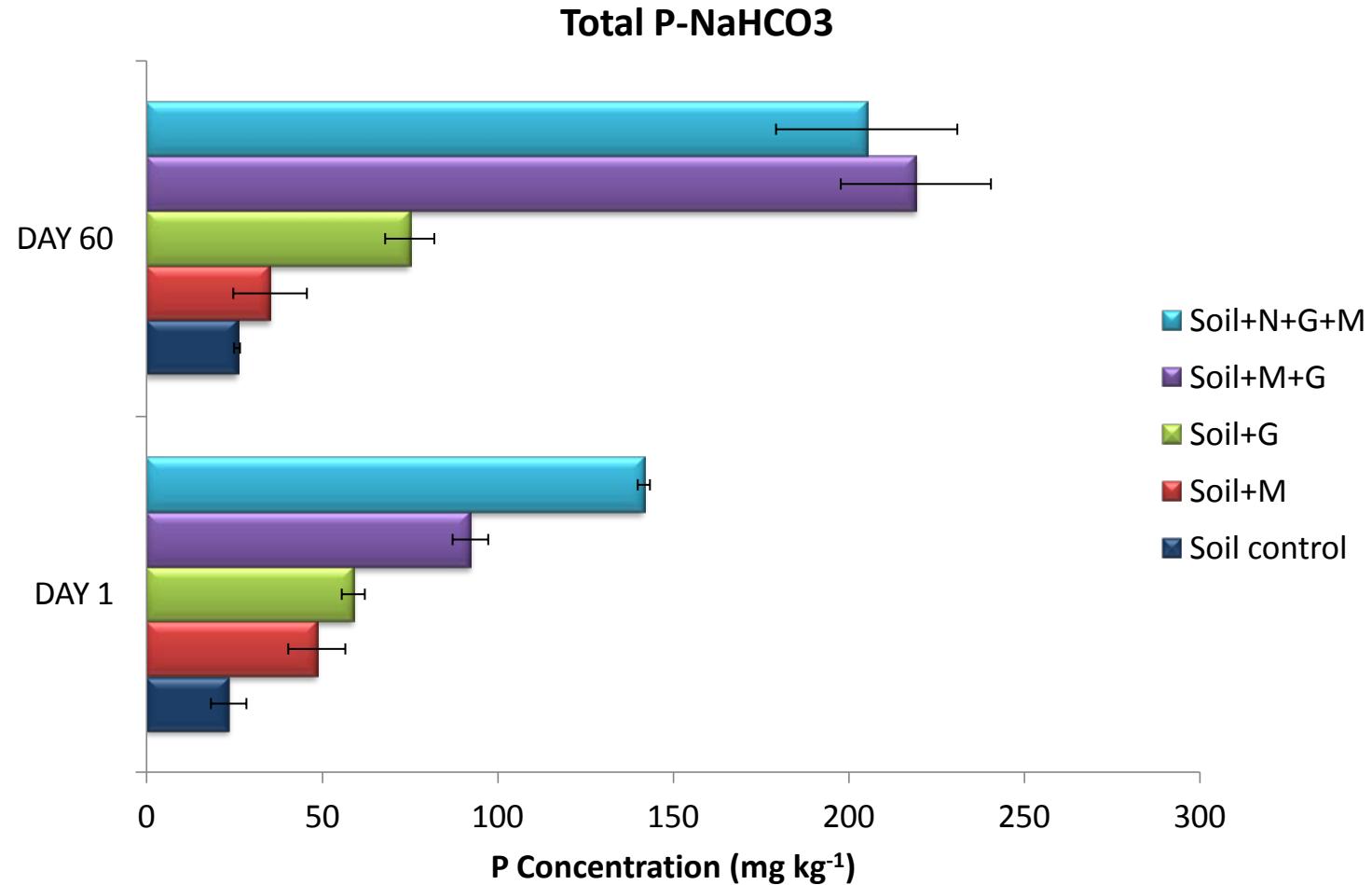
Figura 9. Stock de C (suelo-planta) y pérdida de C





Relationships between Al / Fe forms in the soil with different P fractions

		$\text{NaHCO}_3\text{-P}_i$	$\text{NaHCO}_3\text{-P}_o$	NaOH-P_i	NaOH-P_o	HCl-P_i	HCl-P_o	$\bullet \text{P}_i$	$\bullet \text{P}_o$
Al	Pyrophosphate	-0.385**	-0.255	-0.217	-0.051	-0.334*	-0.295*	-0.354*	-0.259
	Oxalate	-0.003	0.388**	-0.054	0.253*	-0.066	-0.096	-0.184	0.095
Fe	Pyrophosphate	-0.064	-0.188	-0.238	0.392**	-0.367**	0.224*	-0.322*	0.301*
	Oxalate	0.513**	0.343*	0.480**	0.228	0.420**	0.040	0.577**	0.276





Phosphorus residual porcentaje

TREATMENTS	TIME INCUBATION					
	Day 1	Day 3	Day 7	Day 15	Day 30	Day 60
Soil	56.77±0.65a	59.27±1.08a	50.76±1.40a	63.68±0.70a	56.25±0.14a	60.78±0.96a
Soil+G	48.07±0.73cd	50.60±0.50bc	48.18±2.02a	57.59±1.58b	52.09±0.34ab	53.80±0.58b
Soil +N	45.21±1.25d	50.38±0.45bc	46.84±1.06a	57.26±0.69b	49.29±0.32bc	50.04±0.17bcd
Soil+N+G	50.69±1.14bcd	41.12±1.23d	46.69±0.32a	51.79±1.03cd	42.17±0.36d	44.73±1.92d
Soil+300M	55.61±1.08ab	56.12±1.95ab	48.80±0.92a	56.41±0.67bc	50.40±2.34bc	60.09±0.90a
Soil+ 300M+G	48.98±1.38cd	50.97±1.97bc	50.68±0.39a	55.70±1.33bcd	45.18±1.90cd	50.67±0.58bc
Soil +300M+N	50.83±0.52bc	47.69±2.22cd	47.99±1.73a	51.91±0.88cd	46.73±0.93bcd	45.53±1.38cd
Soil+300M+N+G	48.58±1.68cd	41.49±1.45d	47.56±0.51a	51.16±1.22d	42.38±0.92d	46.85±1.33cd

Development of Biofertilizer I



Development of Biofertilizer II





Summary

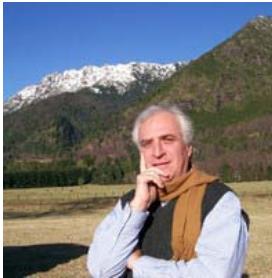
- ¤ Positive P priming effect is clearly in bicarbonate P soil fraction
- ¤ Negative priming effect in NaOH organic fraction is regulated by Al oxalate (Allophane) and is the main factor controlling P organic storage in soil
- ¤ Phosphorus in residual fraction decreased about 15% in soil amended with manure, glucose and nitrogen treatment. This is equivalent to apply approx. 384 kg P ha⁻¹ year⁻¹ and with 100 kg N ha⁻¹ year⁻¹ in intensive pasture management.

Acknowledgments

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Thanks for your attention