STRATEGIES TO IMPROVE PHOSPHORUS AVAILABILITY AND REDUCE ENVIRONMENTAL IMPACT IN RICE AGROSYSTEMS

ESTRATEGIAS PARA MEJORAR LA DISPONIBILIDAD DE FÓSFORO Y REDUCIR EL IMPACTO AMBIENTAL EN AGROSISTEMAS DE ARROZ

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Strategies to improve Phosphorus availability and reduce environmental impact in rice agrosystems

Estrategias para mejorar la disponibilidad de Fósforo y reducir el impacto ambiental en agroecosistemas de arroz

Memoria de tesis doctoral presentada por el Ingeniero Agrónomo Rodolfo Lizcano Toledo para obtener el grado de Doctor con mención internacional por la Universidad de Granada en Cotutela con la Universidad de Turín.

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CONTENTS

SUMMARY

Phosphorus (P) is a limiting element in the dynamics of legume-grass succession, because it fulfills different roles that imply functionality in the absorption efficiency with other elements such as Nitrogen and Iron (Fe), the latter especially in tropical soils.

Rice has shaped the diet, economy, and culture of millions of people around the world. It is considered the third most produced crop after corn and wheat on the planet. China is the largest producer and consumer of rice in the world with an area of 30 million hectares with a total amount of 145 million tons, while Italy is the main producer of rice in Europe. with an area of 234,133 ha there is a rice production of more than 1.5 million tons. Rice production faces agronomic and environmental challenges, such as the reduction of nutrient losses that can cause contamination of ecosystems, the increase of efficiency in fertilizer applications using alternative sources such as struvite, the use of friendly practices with the sustainable development of cover crops during the fallow period to improve soil fertility. Therefore, this thesis aimed to evaluate the role of P on biological N fixation (BNF) efficiency in legumes and the role of these cover crops on nutrient dynamics in temperate rice agrosystems (Northern Italy). Another objective of this thesis was developed under tropical conditions and aimed to evaluate P forms in acid soils under different water management and fertilized with conventional and alternative phosphate fertilizers.

To reach the first objective hairy vetch plants (*Vicia villosa*) were cultivated under greenhouse conditions with rhizobia in a soil with limited P and added with both inorganic and organic P at two low and high concentrations (40 and 120 mg P kg^{-1} respectively). Plant growth, nutrient uptake, and BNF (by dilution of ^{15}N isotopes) were determined at 30, 50 and 70 days after sowing. The low availability of P in the soil limited the growth of Vetch, which led to a strong response to P application, however the positive response to BNF was highly significant ($p < 0.005$) in the presence of readily available P, in addition, vetch improved N demand when P was not limiting, which indicates the strong positive relationship between BNF and P in the aerial part at maturity (70 DAS). In conclusion, the relevance of the biological and agronomic effects of P in legumes can be established, given the energy cost required by BNF, in addition to the increase in soil P availability through the addition of Pi, which improved the growth of

the plant, nodule formation, P acquisition and BNF efficiency. Something relevant is the role of Po, which can help partially increase N_2 fixation.

To reach the second objective an experiment was carried out at the Rice Research Center (Northern Italy). The experimental site was divided into 8 plots, randomly assigned and the design included a factorial arrangement of plots representing (i) with and without hairy vetch and (ii) four levels of fertilization with N. These analyzes were performed during the period between October 2018 – October 2019. Initially in the winter of 2018 the legume was sown and later in the spring it was incorporated into the soil and the rice seeded. Fertilization levels with N (Urea) were 0, 80, 120 and 160 kg N ha^{-1}. The variables determined were: yield component, N, P and K contents, SPAD, soil P forms nitrate and ammonium and potentially mineralizable N (PMN). Cover crops significantly stimulate the growth and yield of rice, with a greater benefit at lower levels of fertilization, obtaining with 80 N kg ha^{-1} a rice yield 12% higher than the control and similar to the yields obtained with 120 and 160 Nkg ha^{-1}. On the other hand, hairy vetch increased SPAD values, reflecting a higher N absorption at all fertilization levels and a reduction of grain sterility up to 27%. The net N mineralization effectively reached, in 40 days, values comparable to the contribution derived from the highest level of fertilization (160 kg N ha^{-1}) under both aerobic and anaerobic conditions. Cover crops in the cold season in succession with rice are thus highly promising, since fertilization with 80 Kg ha^{-1} could be managed, saving between 40 and 50 kg ha^{-1} of mineral N, which corresponds to an efficiency of 30% and a contribution of N of 150 kg ha^{-1}. In addition, vetch increased P availability through a high supply of Po and the increase of labile P likely due to a higher enzymatic activity.

The third experiment was carried out in mesocosms at the Nuclear Energy Center for Agriculture, located in Piracicaba, São Paulo, Brazil. The aim of this work was addressed to evaluate the effects of three P fertilizers: MAP (Monoammonium Phosphate, TSP (Triple Super Phosphate), and Struvite (all 32P labelled), using two soil types ((Typic Hapludox, and Humic Hapudult) and to water management techniques (at field capacity and continuous flooding). Isotopic quantification with ^{32}P (indirect method), maximum P adsorption capacity (MPAC), mineralogical analysis were performed. Redox processes were determined by measuring O_2, Fe^{2+}, Fe^{3+}, Fe Citrate-Ascorbate (Fe_{ca}), dissolved organic carbon (DOC). Soil C, N and P and their stoichiometric ratios, as well as SPAD measurements were also

determined. In the fertilizers, the content of total P and their chemical fractions were determined. Rhizospheric soil was collected for the determination of acid and alkaline phosphatase activity (APA), microbial C, as well as flurocein diacetate (FDA) and dehydrogenase activity (DHA). As expected, the soils were rich in kaolinite and Al and Fe hydroxides, which led to great P fixation, but in the Oxisol this was accompanied by greater Fe^{2+} activity and P desorption, under continuous flooding. In these conditions, we noticed a gradual pH increase, following E_h reduction, favouring further release of P, especially with struvite, parallel to DOC increase. It can be inferred that DOC activates microbial and enzymatic activity favouring the release of P into solution, although this occurred mostly at the end of the productive stage, leaving a remnant of labile P for the following crops. It can be concluded that the physical-chemical and biological characteristics, such as clay content, active DOC, soil P fixation capacity and mineralogical characteristics are important factors to determine the availability of P in tropical soils with high fixation of P.

RESUMEN

El fósforo (P) es un macronutriente limitante en la dinámica de la mayoría de cultivos y particularmente cumple un papel importante en la sucesión de leguminosa-gramínea, debido a que cumple diferentes roles que implican funcionalidad en la eficiencia en la absorción con otros elementos como el Nitrógeno y el Hierro (Fe), sobre todo este último en suelos tropicales.

El arroz ha dado forma a la dieta, la economía y la cultura de millones de personas en todo el mundo. Se considera el tercero cultivo más producido después del maíz y el trigo en el planeta. China es el mayor productor y consumidor de arroz del mundo con una superficie de 30 millones de hectáreas con una cantidad total de 145 millones de toneladas. Italia es el principal productor de arroz de Europa con una superficie de 234.133 hay una producción de arroz superior a 1.5 millones de toneladas. La producción de arroz enfrenta problemas a nivel agronómico y ambiental, como el de disminuir las pérdidas de nutrientes que pueden ocasionar contaminaciones en suelos y aguas, eficiencia en las aplicaciones de fertilizantes empleando fuentes alternativas como la estruvita, el uso de prácticas amigables con el desarrollo sostenible de los arrozales como la sucesión y rotación de la relación leguminosa-gramínea que altera la fertilidad de los suelos.

Esta tesis se desarrolló, tres (3) experimentos, dos en Italia y uno (1) en Brasil. Con el primer experimento hemos evaluado, el papel del P en la eficiencia de la fijación biológica del N (BNF por sus siglas en inglés) y en el segundo, la dinámica del P en arrozales en una sucesión leguminosa-gramínea en el norte de Italia. El tercer (3) experimento se desarrolló en condiciones tropicales en São Paulo (Brasil), evaluando la dinámica del P en suelos ácidos con altas concentraciones de Fe, en diferentes manejos de humedad, bajo el efecto de fertilizantes fosfatados convencionales y alternativos. Los tres experimentos se resumen a continuación:

El primer experimento se realizó en condiciones de invernadero en la facultad de Ciencias Agrarias, forestales y agroalimentarias de la Universidad de Turin en Grugliasco. Tuvo como objetivos: (a) mejorar el suministro de P a través de las entradas de P inorgánico (Pi), (b) establecer que el P orgánico (Po) puede mejorar parcialmente el efecto de limitación de P en la fijación de N_2. Para esto, se cultivaron plantas de veza peluda (*Vicia villosa*) inoculadas

con rizobios en un suelo con P limitado. Se evaluó: 1) el crecimiento de la planta, parte aérea y radicular, 2) la absorción de N y P y 3) la capacidad de BNF (por dilución de isótopos ^{15}N). Estableciendo las entradas de Pi y Po en forma de ortofosfato o ácido fítico, en bajas y altas concentraciones (40 mg P kg-1 y 120 mg P kg^{-1}) respectivamente. Las evaluaciones de la planta (veza), suelo y determinación de la capacidad de Fijación de N_2, se realizaron en tres tiempos (30, 50 y 70 días después de la siembra). Las bajas disponibilidades de P en el suelo limitaron el crecimiento de la Veza, lo que conllevó a una fuerte respuesta a la aplicación de P, no obstante, la respuesta positiva a la BNF fue altamente significativa ($p < 0.005$) en presencia de P fácilmente disponible. Además, la veza incrementó el contenido de N, cuando el P no era limitante, lo que indica la fuerte relación positiva entre la BNF y el P en la parte aérea en la madurez (70 DDS). Como conclusión se puede establecer la relevancia en los efectos biológicos y agronómicos del P en las leguminosas, dado el coste energético que requiere la BNF, además, del aumento de la disponibilidad de P del suelo mediante la adición de Pi, mejoró el crecimiento de la planta (Tres veces), la formación de nódulos (16 veces), la adquisición de P (6 veces) y la eficiencia de la BNF (7 veces). Respecto al Po se observó que puede ayudar a aliviar parcialmente el efecto de limitación de P en la fijación de N_2, de acuerdo a los resultados obtenidos tanto en bajas como en altas concentraciones.

El segundo experimento se llevó a cabo en un centro experimental cerca del Centro de Investigación del arroz del Ente Nazionale sul Riso, ubicado en la región de Lombardía en el Noreste de Italia. La investigación tuvo como objetivos: (a) establecer el rendimiento de los cultivos de arroz y sus componentes de producción y calidad, (b) determinar la recuperación aparente de N del fertilizante y el nivel óptimo de fertilización nitrogenada con veza, y (c) definir la variación de las formas de N y P durante la temporada de cultivo de la leguminosa y el arroz. El sitio experimental se dividió en 8 parcelas de 20 x 80 m, asignadas al azar y el diseño consistió en una distribución factorial de parcelas divididas 2 x 4 la cual correspondía a: (i) con y sin veza vellosa y (ii) cuatro niveles de fertilización con N. Estos análisis se realizaron durante el periodo comprendido entre Octubre de 2018 – Octubre de 2019. Inicialmente en el invierno del 2018 se sembró la leguminosa y posteriormente en la primavera (Mayo) del 2019 se incorporó al suelo y se sembró la semilla de arroz. Los niveles de fertilización con N (Urea) fueron: 0, 80, 120 y 160 kg N ha^{-1}. Las variables a determinar fueron: componente de rendimiento y contenidos de N, P y K en el arroz y en el suelo, también se realizaron fraccionamientos de P (Olsen, Citrato y NaOH) y determinaciones de nitratos y amonios. Además, se estableció el N potencialmente mineralizable (PMN por sus siglas en inglés). Por último se midió con un clorofilómetro, los valores SPAD (Análisis del desarrollo de la planta en el suelo, por sus siglas del inglés *Soil Plant Analysis Development*) correspondiente a valores del índice de clorofila en la planta, a los 31, 42, 55, 67, 81, 94, y 109 días. La veza determinó el rendimiento de arroz en función de los niveles de fertilización con N. Se puede establecer que los cultivos de cobertura estimulan significativamente el crecimiento y rendimiento del arroz. El beneficio del cultivo de cobertura fue mayor a menores niveles de fertilización, obteniendo el cultivo del arroz un gran rendimiento con 80 kg ha^{-1} siendo superior un 12 % al tratamiento control y similar a los rendimientos obtenidos con la fertilización 120 y 160 kg ha^{-1}. Por otro lado, los valores de índice SPAD reflejaron una mayor absorción de N con la veza en todos los niveles de fertilización. Por otra parte, el uso de la cobertura como la veza, puede influir positivamente en reducir disturbios fisiológicos como la esterilidad del grano de arroz hasta un 27%, reduciendo además el disturbio de grano yesoso. La mineralización neta de N alcanzó en 40 días, valores comparables al aporte derivado del nivel más alto de fertilización (160 kg N ha^{-1}), tanto en condiciones aeróbicas como anaeróbicas. Se concluyó que es recomendable usar cultivo de cobertura (Leguminosa) en la estación fría en sucesión con arroz, ya que se podría manejar una fertilización con 80 kg ha^{-1}, ahorrando entre 40 y 50 kg ha^{-1} de nitrógeno mineral, lo que corresponde a una eficiencia del 30 % y un aporte de N en biomasa de 150 kg ha^{-1}. En el caso del P la veza disminuye las relaciones C:N y C:P, aumentando el P disponible a través de un alto aporte de Po y la transformación de P lábil a partir de una actividad enzimática activa.

El tercer experimento se realizó entre los meses de Octubre de 2019 hasta Enero del 2020 en condiciones de invernadero en el Centro de energía nuclear para la agricultura, ubicado en Piracicaba, São Paulo, Brasil. El diseño experimental fue en bloques completamente al azar, en arreglo factorial 4x2x2 con cuatro (4) fertilizantes fosfatados: MAP (Fosfato monoamónico, TSP (Super fosfato triple), Estruvita y el control (Sin fertilizante fosfatado) x dos (2) tipos de riego, un riego a capacidad de campo y otro por inundación en dos (2) suelos ácidos tropicales que se clasifican (USDA, 1998) como Oxisol (Typic Hapludox) en la región de São Paulo y Ultisol (Humic Hapudult) de la región de Mato Grosso. El experimento see desarrolló en macetas de 5 kg forradas con bolsas negras de polietileno. A los 75 días de cultivo se cortaron las plantas a 1 cm a ras del suelo, para tomar el material aéreo y realizar los respectivos análisis. Las variables determinadas fueron: cuantificación isotópica con ^{32}P (método indirecto), capacidad máxima de adsorción de P (MPAC), y análisis mineralógico en la fracción de arcilla. Los procesos Redox se determinaron tomando muestras a los 0, 0.5, 1, 2, 5, 10, 20, 35, 50, 75. Se determinaron las concentraciones de O_2, Fe^{2+}, Fe^{3+}, Fe Citrato-Ascorbato (Fe_{ca}), carbono orgánico disuelto (DOC), además de las concentraciones de C, N y P y sus relaciones estequiométricas, como también las mediciones de SPAD. En los fertilizantes se determinó el contenido de P total y las fracciones químicas de P, de acuerdo a las directrices brasileñas (Embrapa, 1999). Por otro lado, se recolectó suelo rizosférico, para la determinación la actividad fosfatasa ácida y alcalina (APA), carbono microbiano, además de diacetato de fluroceína (FDA) y actividad deshidrogenasa (DHA).

En el análisis mineralógico se observaron mayores niveles de caolinita e hidróxidos de Fe y Al en el Oxisol que en el Ultisol, lo que condujo a una mayor fijación de P en el Oxisol, en el que también se observó mayor actividad de Fe^{2+} y mayor desorción de P. Hubo mayor desorción de P en suelos manejados con riego por inundación y DOC. Cabe destacar, el aumento gradual de pH en los dos suelos que recibieron riego por inundación. También se observó una reducción del Eh, que condicionó la reducción de Fe^{3+} a Fe^{2+}, causando liberación de P. La reducción se hizo más fuerte cuando existió una alta fertilidad activa y en el caso de altas concentraciones de DOC activo. La estruvita presentó mayores incrementos de pH y un mayor contenido de P lábil al final del experimento, lo que permite establecer, que a pesar de que el P de la estruvita es de lenta liberación, el contenido de carbono orgánico, mejoró el procesos al incrementar la actividad microbiana y enzimática. Esto repercute en que haya mayor P asimilable al final de la etapa productiva, dejando un remanente de P lábil para los siguientes cultivos. Se puede concluir que las características físico-químicas y biológicas de los suelos ácidos tropicales como el contenido de arcilla, DOC activo, capacidad de fijación de P de los suelos y características mineralógicas, son factores importantes para determinar la disponibilidad de P en suelos tropicales con alta fijación de P.

CHAPTER 1

INTRODUCTION

Phosphorus (P) is a key macronutrient for the growth and development of crops. It participates in the synthesis of nucleotides, which are structural components of cells and their membranes. It is also involved in membrane permeability, photosynthesis, respiration, glycolysis, redox reactions and signal transductions (mechanisms that enable plants to obtain information from the environment and adapt themselves to its constant changes). Additionally, P participates in lipid metabolism, carbohydrate transportation and the maintenance of osmotic potential (Abel *et al*., 2002; Wang *et al*., 2018; Paz-Ares., 2021). On the other hand, a reduction of bioavailable P causes changes in its relationships with other elements such as carbon (C) and, especially, nitrogen (N) (Fernandes *et al*., 2014; Chen *et al.,* 2015; Zhang *et al*., 2019).

As a dynamic element in nature, P is vital in agriculture, where it is a basic fertilizer (Adimassu *et al*., 2012). In most soils, the P constrains are related to its limitations and losses that depend on soil factors such as pH, salinity (Bai *et al*. 2017), high concentrations of toxic elements, interaction with micronutrients (Dimkpa *et al*., 2019), runoff, leaching (Qin *et al*., 2010), total P concentration, remaining P in the solution and as such assimilable for the plants (Fink *et al*., 2016), organic matter in soil, redox potential (li *et al*., 2016), soil structure and texture, especially the clay content (Barbieri *et al*., 2013; Oliveira *et al*., 2014), mineralogical composition (Fink *et al*., 2016), P-solubilizing microorganisms (Kumar *et al*., 2019; Rezakhani *et al*., 2019), enzymes and mycorrhizas (Jansa, *et al*., 2011).

Low P concentrations in the soil require high applications of P fertilizers, although this can turn in, especially in case of excessive fertilization, dramatic environmental problems, due to the so-called agricultural diffuse pollution of P (Everaert *et al*., 2018) – that is, water pollution arising from a broad array of human activities for which the pollutants have no obvious point of entry into receiving watercourses (Moor & Miller, 1994). This pollution comes mainly from crop fertilizers applied to the soil (Bergström *et al*., 2015; Schindler *et al*., 2016).

Crops absorb P from the soil through the diffusion process, but the diffusion coefficients of this element are very low, around 0.3-3.3 x 10^{-13} m^2 s^{-1}, and its concentration in the soil solution is limited, 0.02 ppm according to several authors (Roy *et al*., 2017; Cavalcante *et al*., 2018; Cui *et al*., 2019). Hence P fertilizers must be applied as close as possible to plant roots, either applied in bands (especially six-month crops) and/or half or full circle (Huygens *et al.*, 2018).

Conversely to its activity in soil, P is highly mobile within the plant (Manghabati, *et al.* 2019). Phosphorus deficiency causes a characteristic color ranging from orange to reddish tones in the old leaves, due to a reduction of the chlorophyll biosynthesis and to a higher production of pigments such as anthocyanins (Wang *et al.*, 2018; Mo *et al.*, 2019). Soluble P is transported through the xylem to all growth and drainage points, depending on the P concentrations, which are located within the adequate range of 0.1-0.3 g Kg^{-1} for most crops (grasses and agro-industrial crops, including fruit, legumes and others) (Cao *et al.*, 2018).

The most used P fertilizers are water soluble and neutral ammonium citrate soluble or citric acid soluble phosphates (Menon *et al.*, 1989; Prochnow *et al.*, 2003; Barreto *et al.*, 2017). Simple superphosphates (SSP), triple superphosphates (TSP), monoammonium phosphate (MAP) and diammonium phosphate (DAP), apart from being used in traditional ways, are the most soluble P sources and the ones that provide the highest efficiency for crops, although they are also the ones that contribute most to environmental pollution (Khan *et al.*, 2018).

These fertilizers are costly as their production demands a high amount of energy apart from requiring other elements to be soluble, such as sulphuric and nitric acid (imported raw material, which increases the selling price of the product even more) (Huang *et al.*, 2017). Hence research is needed on P controlled-release fertilizers which can minimize the losses outside the crop and improve the agronomic effectiveness of the fertilizer use (Shaviv & Mikkelsen, 1993; Shaviv, 2001). In this context, materials such as biochar are being tested (Eduah *et al.*, 2020), or others with water-soluble P coatings with an insoluble layer, which creates a physical barrier to slow down the release rate of P. The polymers applied include graphene oxide, a nanomaterial that is being studied as a middle-term P carrier for the crops (Zhang *et al.*, 2014; Andelkovic *et al.*, 2018).

1.1 PHOSPHORUS CYCLING IN THE SOIL-PLANT AGROSYSTEM

1.1.1 Soil P processes

Phosphorus can be found in two different forms in soil, either organic, as a component of organic matter, or inorganic (Fardeau, 1996). Losses and limitations of P are quite important in most soils, as both inorganic and organic P forms have a low solubility rate and can be retained by solid phase through physical-chemical and microbiological processes such as adsorption, precipitation and microbial immobilization (Roberts & Johnston, 2015).

The processes affecting the adsorption and desorption are numerous. Phosphate sorption is governed by iron (Fe) and aluminium (Al) (hydr)oxides below $pH < 5.0$, mainly due to low crystallinity forms and with unbalanced charges. This adsorption occurs at Lewis acid sites, where the OH and OH_2^+ groups, mono- or tricoordinatedly bound to the metal (Fe and Al) are exchanged by the phosphate (Barrow, 1983). The sorption is related to soil pH (Gustafsson *et al*., 2012). Depending on the pH, P exists in the soil solution as orthophosphate ($H_2PO_4^-$ and HPO_4^{2-}). According to Bai *et al*. (2017), the soils of the Yellow River delta in China showed a maximum P-sorption rate (43.9 mg kg^{-1}, 45.9 mg kg^{-1} and 40.5 mg kg^{-1} in different types of soils) with optimal pH levels of 5.5, 6.2 and 5.2, respectively. On the other hand, at high pH values, P can precipitate with Ca. The mineral forms of Ca-P can be various: amorphous calcium phosphates, octocalcium phosphates and apatites (hydroxyapatites or fluorapatites) (Penn & Camberato, 2019). The precipitation or dissolution of these minerals can cause a negative pH dependency, with an increased solubility of P at a lower pH (Hesterberg, 2010).

Crop productivity has been estimated to decrease by as much as 40% with marked deficiencies of P in soil (Balemi & Negisho, 2012), especially in some environments such as in Asia (Han *et al*., 2005; Vengavasi & Pandey, 2018), America (Chi *et al*., 2017; Bezerra *et al*., 2018), Europe (Noë *et al*., 2018), Africa (Adam *et al*., 2018) and Oceania (Vandamme *et al*., 2016).

The P release in the agricultural soils represents an important threat for water quality (Liu *et al*., 2019). P losses can be due to runoff, leaching and erosion in soluble forms (<0.45 mm) and particles (>0.45 mm). Normally, P particles are the main forms transported from cropped soils to waterbodies, representing 80% of all transported materials (Edwards & Withers, 1998; Jahanzad *et al*., 2019). This is because the selective erosion of the P-rich particles caused by rainfall, splash and cutting forces of water flow over the land surface according to the topography (Withers *et al*., 2015; Uusitalo *et al*., 2018). On the other hand, losses resulting from surface runoff (in dispersed particles), subsurface flow (leaching and flow through the soil matrix and macropores), drainage flow, or even from groundwater, can boost the eutrophication of waters that is sensitive to P concentrations (Bieroza & Heathwaite, 2015; Huang *et al*., 2017).

Various studies have confirmed that the high risk of surface-water pollution arising from processes such as runoff during periods of high precipitations (rainfall regime) is linked also to the high solubility of P fertilizers (Hart *et al*., 2004; Tian *et al*., 2020; Kumaragamage *et al*., 2011). Liu *et al*. (2013) found out that the P loss due to runoff was similar for different soluble mineral fertilizers such as MAP, DAP and KH_2PO_4.

The high fixation capacity of P in most soils worldwide and the low efficiency of use of P-based fertilizers (around 10 – 15%) in most crops mean that the most part of P fertilizers tends to accumulate in soil (Valdas *et al.,* 2009). This accumulation can become a problem due to the environmental impact and to the high cost of the phosphatized fertilizers.

In Europe and Oceania, for instance, there is an accumulation of P fertilizers input in agricultural soils (ranging between 560 and 1.115 kg P ha^{-1}, respectively for the period 1965-2007) and these were much higher than the uptake by crops (100 and 350 kg P ha^{-1} respectively) (Sattari *et al*., 2014). At the same time, this leads to changes in the P dynamics in the soil solution, in the microbial and enzymatic activity of the crop rhizosphere and in the absorption, extraction and transportation of P into the plant.

1.1.2. Plant P processes

As already mentioned, P is an irreplaceable and essential element for life. In plants, it is one of the 16 essential elements for their vital functions, and thus without this element, no high-quality or abundant harvests could be achieved. Nevertheless, the potential content in soil is limited, due to the low P reserves worldwide (Filippelli, 2008).

As a macronutrient for plants, P is fundamental to the development of many cellular components, such as the nucleic acids, proteins, phospholipids and ATP (adenosine triphosphate) (Hammond & White, 2008). It is essential for the enzymatic regulation and for the interpretation of metabolic signals, and its important mobility within the plant causes the signs of P nutritional deficiency to be reflected first in the old leaves (Cakmak & Marschner, 1987; Netzer *et al*., 2019), although recent works (Carstensen *et al*., 2018) point out that plants can be heavily affected by P deficiency for weeks without reflecting any visual symptoms on the leaves. On the other hand, all photosynthetic processes that are influenced by P deficiency seem to be fully reversible and can be restored in less than 60 min by providing P to the leaf tissue.

To continue growth and the development under P deficiency, plants have developed several adaptation strategies (Laliberté *et al*., 2014). These can be classified into two groups: (i) those improving P absorption in monocotyledons and dicotyledons, and (ii) those limiting P use in vegetal organs, exclusively in monocots, (Ramaekers *et al*., 2010). Plants increase their P absorption from the soil by modulating their root system architecture by inducing and secreting acids such as phosphatases, exuding organic acids and increasing soil P availability through activities of carriers in the rhizosphere (Hinsinger *et al*., 2011, Fernandes *et al*., 2014). By contrast, plants limit the use of P as their growth rates decrease, by accumulating sugars and anthocyanins, by modifying their glycolytic and respiratory pathways, and by alternating vegetal hormones (Vance *et al*., 2003; Zhang *et al*., 2014).

Other special strategies that plants have developed to take up P include the capacity to produce different types of special roots called proteoid roots or radicles and basal roots (Ramaekers *et al*., 2010; Haling *et al.,* 2013; Neumann, 2016). The second one is the development of associations with mycorrhizas, especially arbuscular mycorrhiza, forming a symbiosis with the plant roots, so the hyphas of these fungi increase the spatial availability of P (Shen *et al*., 2011; Brown *et al*., 2013). The third strategy consists of modifying the environment of the rhizosphere in order to boost P mobilization. This includes the release of exudates by the plant roots, acidifying the rhizospheric area so there is more $H_2PO_4^-$, which is the easier form absorbed by plant and formed only in acid soils. The exudation into the rhizosphere of phosphatases can also increase P availability, as they hydrolyze the different forms of organic P (Li *et al*., 2011; Lynch, 2011; Bayuelo-Jimenez, 2014).

Plants produce many secondary metabolites that are fundamental for survival under environmental stress. These include flavonoids and especially anthocyanins. The biosynthesis of anthocyanins is modulated not only by a number of intracellular signals but also by environmental stress (Yuan & Liu, 2008; Khan *et al*., 2016), such as P deficiency. These adaptive responses are due to protein production, arising from the differential expression of many stress-sensitive genes (Li, *et al*., 2019). Therefore, identifying the genes and/or proteins involved in the adaptive responses to P deficiency is essential in order to understand the molecular mechanisms of the adaption. Wang *et al*. (2018), on the basis of the iTRAQ-based proteomic analysis with *Arabidopsis* plants, executed tests to determine the molecular mechanisms for the anthocyanin accumulation in induced plants under P suppression. The authors concluded that, under P deficiency, anthocyanins increased its metabolite concentration, with high levels of messenger adenosine triphosphate (mRNA) of seven proteins mapped in the experiment. This suggests that P deficiency promotes the accumulation of anthocyanins by prompting their biosynthesis. Hence, when plants undergo early deficiencies of this element, the mature leaves (due to the high mobility of P within the plant) present violet, orange or red tones due to the increase of anthocyanin concentration around the leaf area, with a remarkable decrease of photosynthesis.

1.1.3 Microbial P processes

Microorganisms have an important role in controlling P bioavailability in soil. Some microorganisms, especially associated to the roots (**Figure 1.1. 1**), have the ability to promote the plant growth and productivity. These are recognized as plant-growth-promoting microorganisms (PGPM) (Rosas *et al*., 2006). Phosphate-solubilizing microorganisms (PSB) constitute an important PGPM group, as they are involved in a wide range of processes affecting P transformation, being integral components of the edaphic cycle of this nutrient (Fankem *et al*., 2006).

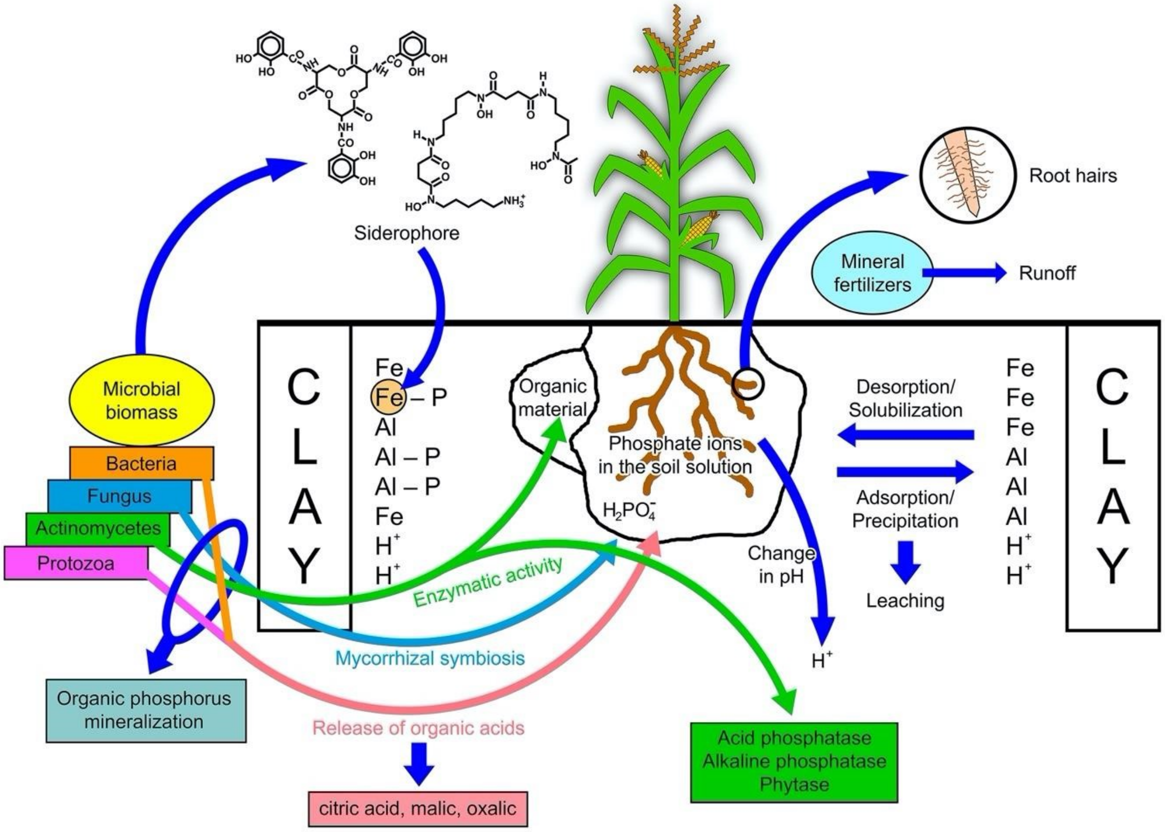

Figure 1.1. 1 Different sources of phosphate fertilizers on the market, their agronomic efficiency and environmental impact on the soil.

Bacteria are the predominant microorganisms that solubilize mineral phosphate in soil when compared to fungi (Kucey & Janzen, 1983; Rawat *et al.,* 2021), constituting between 1 and 50% and 0.1 - 0.5% of their respective total populations, respectively. In general, solubilizer bacteria outnumber fungi from 2- to 150-fold (Banik & Datta, 1988; Richardson & Simpson, 2011). On the other hand, most solubilizing microorganisms can solubilize calcium phosphate complexes and only some of them can solubilize Al or Fe phosphates (Long *et al*., 2018).

As plants, microorganisms have developed several mechanisms to solubilize and mobilize P. These mechanisms include the release of: (i) protons; (ii) organic acid anions such as oxalate or citrate that solubilize inorganic P by chelating Fe^{2+}, Al^{3+} and basic cations (Ca^{2+}, Mg^{2+}, K^+, Na^+) bound to P; and (iii) extracellular phosphatases that hydrolyze organic P (Illmer *et al*., 1995; Richardson *et al*., 2009; Hinsinger *et al*., 2011). While plants can produce only acid phosphatases, microorganisms are able to produce both acid and alkaline phosphatases (Dick & Tabatabai, 1983; Juma & Tabatabai, 1988; Nannipieri *et al*., 2011), including bacteria and also fungi and archaea (Ragot *et al*., 2015).

1.1.4 Phosphorus dynamics and effects of climate change

Different environmental conditions can modify the P dynamics in the soil-plant system, such as temperature, water stress, wet/dry cycles, drought, and O_2/CO_2 concentration (Field *et al.*, 1995). These conditions can affect the dynamics in the absorption, transportation and distribution of P from the soil into the plant with contrasting effects. Global warming involves important changes of these factors with important impacts on P cycling (Zhu *et al.*, 2018). The high CO_2 concentration reached in the atmosphere (Hou *et al.*, 2016), anticipates an improvement in the photosynthetic rate of the crops, and some physiological variables, such as the Net Assimilation Rate, which reflects CO_2 concentration, can act as a fertilizer for the crops, contributing to increase the net primary productivity (Tian *et al.*, 2013; Hidayati *et al.*, 2019). Nevertheless, an increase in the plant growth causes a higher nutrient demand, leading P to become the most limiting nutrient (Zhao & Wu, 2014).

The worldwide demand for fertilizers in 2017–2018 was 187 million tons of P, and this number is expected to increase up to 199 million tons for 2022–2023 due to demographic growth, which requires a higher productivity (Jia *et al.*, 2020). In general, the response of fertilizers in the irrigated areas, especially the areas used to grow rice throughout the world, decreased around two-thirds, from 13.4 in 1970 to 3.7 kg grain/kg of nutrient applied (NPK) in 2005 (Khan *et al.*, 2020). These fertilizers, due to their alkaline nature, also affect the dynamics of enzymes and bacteria that solubilize P. This is due to the alkaline-residual effect that those fertilizers present when entering in contact with water and dissolving in the soil, which leads also to changes in the microbial respiration (Zhu *et al.*, 2018). In addition, an indiscriminate use of P fertilizers such as MAP (12-52-0) and DAP (18-46-0), when applied in productive agricultural systems, generates a high amount of nitrous oxide (N_2O), which is one of the most dangerous greenhouse gases, with a warming potential 298 times higher than that of CO_2 (Garzón & Cárdenas, 2013). The Colombian Institute for Hydrology, Meteorology, and Environmental Studies reported that in 2004, the agricultural industry in Colombia produced 94.91 gigagrams (Gg) of N_2O.

There is an uneven distribution of high precipitations in different parts of the world, and the fact that 70% of soils worldwide are acid (Jiang *et al.*, 2018) should be also taken into account when applying P fertilizers acidulated with H_2SO_4, HNO_3, or H_3PO_4, which can further intensify soil acidity and hence P precipitation due to increasing amount of soluble Al and Fe (Jia *et al.*, 2020;

Nash *et al.*, 2019). **Table 1.1. 1** shows some worldwide research on the main processes in which P intervenes in agricultural systems.

Table 1.1. 1 Main processes in which P intervenes in agricultural systems.

P Forms	**Area**	**Process**	**P Activation response on Soil or Plant**	**Crops**	**Reference**
C:P:N	Stoichiometric relationships	Physiological, cellular, morphologycal and molecular factors		*Oryza sativa* *Leymus chinensis* *Panicum maximum* *Bouteloua gracilis* *Populus deltoides*	Dias Filho *et al*., 1992; Elser *et al*., 2010; Bell *et al*., 2014; Li *et al*., 2016; Shang *et al*., 2018; Zhu *et al*., 2018.
P deficiency $H_2PO_4^-$	Radicle and proteoid root emission	Physiological, cellular, morphologycal and molecular factors	mRNA and ethylene synthesis	*Arabidiopsis* sp.	Ramaekers *et al*., 2010; Haling *et al.,* 2013; Neumann, 2016.
	Alteration of biochemical processes	Biosynthesis of secondary metabolites. Radicle emission (Proteomic hairs)	Anthocyanines	*Arabidiopsis* sp.	Wang *et al*., 2018
Effect of global change on the P dynamics	P dynamics with temperature, water stress, greenhouse gases.	CO_2 N_2O O_3	Photosynthesis Metabolic expenditure Transpiration Microbial activity	*Zea maiz* *Triticum vulgare* *Orysa sativa*	Goufo *et al.,* 2014 Wang and Wesche, 2016 Hidayati *et al.,* 2019
Environmental impact of phosphatized fertilizers	Coating of the P molecule with different materials from different sources	Loss of soil Runoff Leaching Eutrophication		*Zea maiz* *Triticum vulgare*	Shaviv & Mikkelsen, 1993; Novoselov *et al*., 2012; Andelkovic *et al*., 2018
Agronomical efficiency in the use of phosphatized fertilizers	Dynamics of different sources of phosphatizd fertilizers	Acidic phosphatic fertilizers Struvite Graphene oxide Thermophosphates	Acidification of the rhizosphere	*Zea maiz* *Triticum vulgare* *Glycine max* *Saccharum officinarum*	Soteras *et al.,* 2013; Degryse, *et al*., 2016; Xu *et al*., 2017; Everaert *et al*., 2018.
Relationship Pmicrobiology of the soil	Microbial activators biological nitrogen fixation	Enzyme dynamics (phosphatases and phytases) and effect on the rhizobium dynamics	Release of organic acid exudates	*Zea maiz* *Triticum vulgare* *Oryza sativa* *Vicia villosa*	Teng *et al*., 2018

1.1.5. Efficiency of P fertilizers in agrosystems and their environmental impact

The agronomical efficiency of fertilizers is not yet adequate. This is partly due to the low nutrient-absorption rate by plants and partly to losses (Reetz, 2016). Over 70% of the arable surface on Earth needs a great supply of P to produce crops. On the other hand, its absorption by plant is slow over short distances of approx. 0.1 to 0.15 mm (Eghball *et al.,* 1990).

The amount of fertilizer applied to a crop but not used by the plant implies certain high environmental and economic costs. The environmental costs include pollution due to eutrophication and groundwater or runoff water hypoxia (Li, *et al*., 2018). The P enrichment due to human activities is one of the main reasons and causes eutrophication of surface waters, which leads to an explosion in autotrophic organisms that can remarkably affect the quality of the aquatic ecosystems (Yan *et al*., 2016).

This problem has led to the development of different types of technologies to obtain P fertilizers with a great agronomic efficiency, high yields and low environmental impact. The choice of P fertilizer depends on its capacity to meet the crop needs during their phenological phases and on its solubility rate. Other aspects (quality traits of the fertilizers) can be considered when choosing P fertilizers, such as supply of other nutrients (synergy), pH, granulometry, mechanical resistance, density, hygroscopicity, saline index and physical and chemical compatibility in the mixes. **Figure 1.1. 2** shows the agronomic efficiency of different sources of P fertilizers, the potential pollution, as well as new alternative sources of fertilizers.

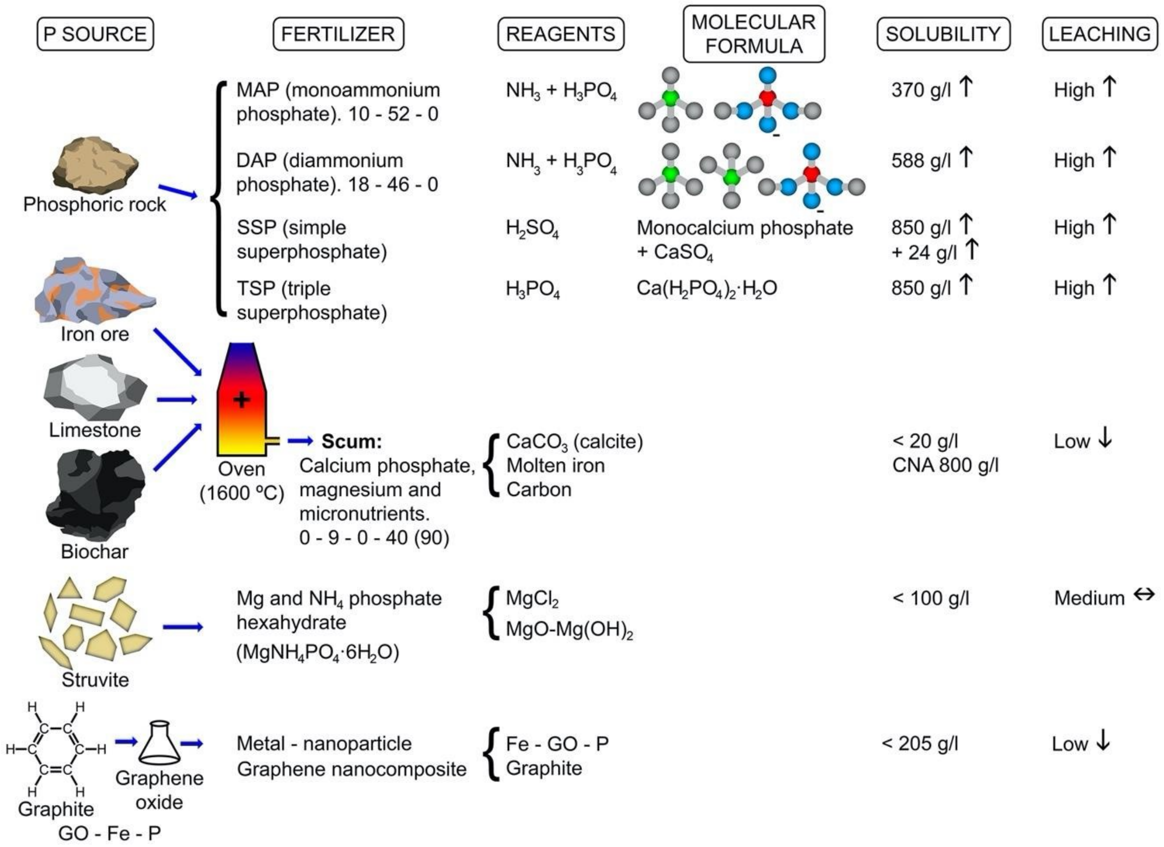

Figure 1.1. 2 Effect of Phosphorus (P) in the biological fixation of the Nitrogen (N) by leguminose.

1.1.6. Alternative sources of P fertilizers of potential use in agriculture

Of the total amount of P applied into the soils, only a small part can be recovered in the first harvest, leaving 70 - 95% of residual P, which is not bioavailable and can be used by the plants during the following seasons (Barrow & Shaw, 1976; Bolland & Gilkes, 1998). This is an important reason for the attention dedicated to developing phosphatized controlled-release fertilizers, as these can minimize the loss outside the application area and improve the fertilizer use efficiency (Shaviv, 2001; Shaviv & Mikkelsen, 1993).

A new approach to face this challenge involves coating soluble P in water with an insoluble layer of different materials. This creates a physical barrier that can slow down the release rate of P, reduce its leaching and diminish other critical processes that degrade soils such as the surface runoff. A number of natural and synthetic polymers have been studied, ranging from starch (Zhong *et al*., 2013; Jin *et al*., 2016), cellulose (Tomaszewski*, et al*., 2002; Wu & Liu, 2008), chitosan (Wu & Liu, 2008) and P acrylic acid acrylamide / kaolin (Ma *et al.,* 2007).

Other types of materials have been tested as well, such as struvite ($MgNH_4PO_4 \cdot 6H_2O$), which is a mineral phosphate that often precipitates spontaneously in wastewaters of treatment plants. In the last decade, struvite has gained interest as a method to recover P from wastewaters so it can be used as a source of phosphatized fertilizer (Le Corre *et al.,* 2009). During the manipulation and recovery of this material, different processes may be applied. One option is to use a magnesium (Mg) salt, most frequently $MgCl_2$ and the pH is adjusted by using NaOH. This Mg water-soluble salt reacts quickly with P in the wastewaters during the crystallization process, thus enabling the formation of a highly pure product called struvite. Britton *et al.* (2005) used alternatively MgO and $Mg(OH)_2$ as Mg source, which is more economic than other Mg-based salts. They also removed or reduced the use of NaOH to increase the pH of the solution. These reactions are slower than those of other Mg salts, as they have to dissolve first, so this is normally added excessively in order to boost the struvite, precipitation resulting into a product with an excess of MgO or Mg $(OH)_2$ (Chimenos *et al*., 2003; Capdevielle *et al.,* 2013).

Experimental tests have been also made in different crops with products derived from metallurgy in order to decrease the use of phosphoric rocks. Metallurgic industries of Ta and Nb produce a waste of acids with an acidity of 5.8 mol L^{-1}, a high content of S (Mattiello *et al*., 2016) and a pH of around 0 (Santos *et al*., 2016). This type of acid source has been used to solubilize low-reactivity apatites, involving a lower price and lower-solubility P.

The potential to improve the formulations of fertilizers by using nanomaterials has been explored as well (Andelkovic *et al*., 2018). Carbon-based materials are often applied in agriculture (Mauter & Elimelec, 2008). Their lower solubility makes them more profitable, environmentally friendly and chemically and physically advantageous. One of these materials applied at industrial scale is biochar. This material has been recently investigated to capture P and to recover wastewasters (Chen *et al.,* 2017; Marshall *et al.,* 2017).

An emerging carbon-based material recently discovered is graphene (Novoselov *et al*., 2004). It has attracted widespread attention for a broad range of applications including the use of energy-related materials (Kuhn & Gorji, 2016), medication-administration systems (Kiew *et al*., 2016), sensors (Liu *et al*., 2012; Xu *et al*., 2017) and membranes (Jiang *et al.,* 2016). Graphene and its oxidized form, graphene oxide have a range of reactive oxygen, a functional group and a high specific surface. Furthermore, it has been confirmed by many studies as a non-toxic and biocompatible material (Novoselov *et al*., 2012; Liu *et al*., 2013). On the basis of their high specific surface (2600 m^2 g^{-1}) and a unique 2D structure, graphene-based products present an ideal form to retain nutrients, due to their surface negative charge. Other formulas using graphene oxide together with Fe are currently being tested, which would work as phosphate ion carrier. In this way, the supply of nutrients to plants can be improved and at the same time the losses by leaching minimized, due to the slow P release (Andelkovic *et al*., 2018).

1.2. PHOSPHORUS DYNAMICS IN RICE SYSTEMS

Cereals largely represent the most diffused staple food and, among them, rice feeds over 50% of the global population with a consumption of about 500 Mt (Garcia *et al.,* 2020). With around 159 Mha under cultivation globally, rice (*Oryza sativa* L.) is typically grown in flooded paddies. The traditional way of growing rice involves rice transplantation and flooding from crop establishment to one month before harvest (Huang *et al*., 2015; Huang *et al*., 2018). Thus, rice soils are largely anaerobic for the most part of the cropping season (Khan *et al*., 2019). Nevertheless, rice roots are well recognized for their ability for radial O_2 loss (ROL) and formation of a narrow aerobic or microaerobic zone in the rhizosphere (Wu *et al*., 2012). ROL leads to a gradual decrease of oxidation processes from the root surface to the bulk soil, which substantially affects the biogeochemistry of the rice rhizosphere (Khan *et al*., 2016). As suggested by Faulwetter et al. (2009), the redox potential (E_h) near the root surface of flooded plants is approximately 500 mV and can decrease to approximately -250 mV at 1– 20 mm from the root surface, depending on factors such as ambient O_2 concentration, root permeability and soil and root respiration (Larsen *et al*., 2015; Armstrong & Armstrong, 2001). This leads to significant E_h and pH changes (Kögel-Knabner *et al*., 2010) with important consequences for rice cultivation, since they interfere with fertility, mainly altering the biogeochemistry of C, N, P and Fe (Long *et al*., 2018).

Thus, P dynamics in flooded soils involves complex processes, that make difficult to evaluate P availability to biota and potential losses into waters. A first increase followed by a decrease in solution P is often observed after flooding (Ponnamperuma, 1972; Ishida *et al*., 2020). This behavior has been ascribed to the reductive dissolution of Fe^{3+} to $Fe^{2+,}$ which increases the solubility of Fe-P minerals (Mitra *et al*., 2016; Xu *et al*., 2019). According to Long *et al*. (2021), redox potential controls Fe reduction, whereas pH governs dissolution-precipitation of Fe compounds and, consequently, P sorption-desorption in waterlogged soils (**Figure 1.2. 1**). However, later the phosphate released by reduction reactions can be readsorbed, mainly by clay minerals, gibbsite, and Fe (hydr)oxides, including the freshly precipitated poorly crytalline Fe (hydr)oxides. Therefore, P mobilization in solution depends on the stability of the soil adsorbents. Silicate clays, gibbsite, and some crystaline Fe (hydr)oxides constitute stable adsorption matrices under waterlogging, whereas the poorly crystalline Fe (hydr)oxides are unstable. Thus, P dynamics in rice paddies is strongly influenced by poorly crystalline Fe (hydr)oxide content (Jan *et al.*, 2015; Claudio *et al.*, 2017).

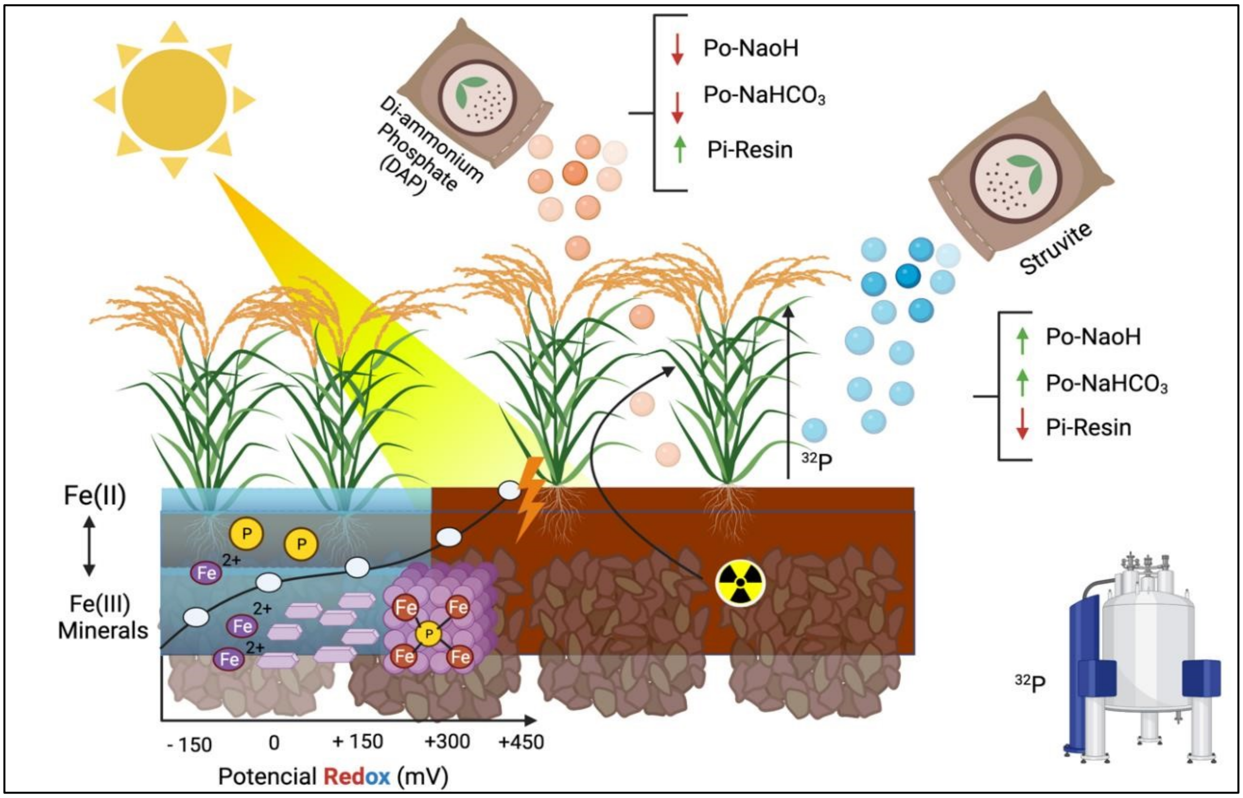

Figure 1.2. 1 Effects of redox conditions on P processes, as related to the application of different P fertilizers.

The various soil P pools are affected also by vegetation cover and agronomic management techniques. When phosphate fertilization is carried out, a sequence of physical-chemical events occurs, transforming the applied P into complex substances, which govern its availability in soil.

Another important factor is the degree of soil evolution. Tropical soils are characterized to be P sinks due to their advanced degree of weathering, and thus to the high content of crystalline Fe and Al oxides. Under these conditions, P is found in very low concentrations in the soil solution, limiting the development of crops. On the other hand, in highly weathered acid soils, the Fe^{3+} reduction mediated microbial action and associated Fe^{2+} mobilization result in substantial P release (Amarawansha *et al.*, 2015). Therefore, the concentration of P in the soil solution increases together with the soluble Fe^{2+} (Wu *et al.*, 2020). However, the intensity of Fe^{3+} reduction depends on organic C as an energy source for microorganisms and as an electron donor (Li *et al.*, 2017), and varies with the type of soil and environmental conditions, such as the E_h state of flooded soils (Bai *et al.*, 2020; Haque *et al.*, 2018). The availability of organic C and Fe dynamics may also affect hydrolysis of organic P by phosphatases. We have to notice also that in the rice rhizosphere there is a high input of C from the roots (Kuzyakov & Blagodatskaya, 2015). The portion of C derived from photosynthesis that is transported to the subsoil and becomes soil organic C through rhizodeposition varies between 7.6% and 23.5% of the rice biomass, according to the fertilization, water management and the growth stage of rice (Atere *et al*., 2017; Ge *et al*., 2015).

1.2.1 Effect of cover crops on P dynamics in rice cropping systems

Rice is mostly cultivated under flooding conditions involving the use of large amounts of fertilizers with a low use efficiency (Cassman *et al*., 1998; Eagle *et al*., 2000). The pressing challenge of providing sufficient food for a rapidly growing world population while enhancing environmental sustainability is leading to an increase in the adoption of alternative agronomic techniques (Montgomery, 2007; Weerasekara *et al*., 2017) that can restore the balance in soil carbon (C) input/output and nutrition status (Amundson *et al*., 2015).

In this regard, cover crops have been shown to have enormous potential to improve yields and quality of the crop that follows, such as corn (*Zea mays* L.) and soybean (*Glycine max* L. Merr.) (Delgado & Gantzer 2015). Cover crops can be used as green manure to add nutrients to a cropping system. Leguminous cover crops can additionally improve soil fertility through biological nitrogen fixation, i.e., the conversion of atmospheric dinitrogen gas (N_2) into plant available ammonium (NH_4^+) (**Figure 1.2. 2**) inside legume root nodules occupied by symbiotic rhizobia N-fixing bacteria (Prell & Poole, 2006).

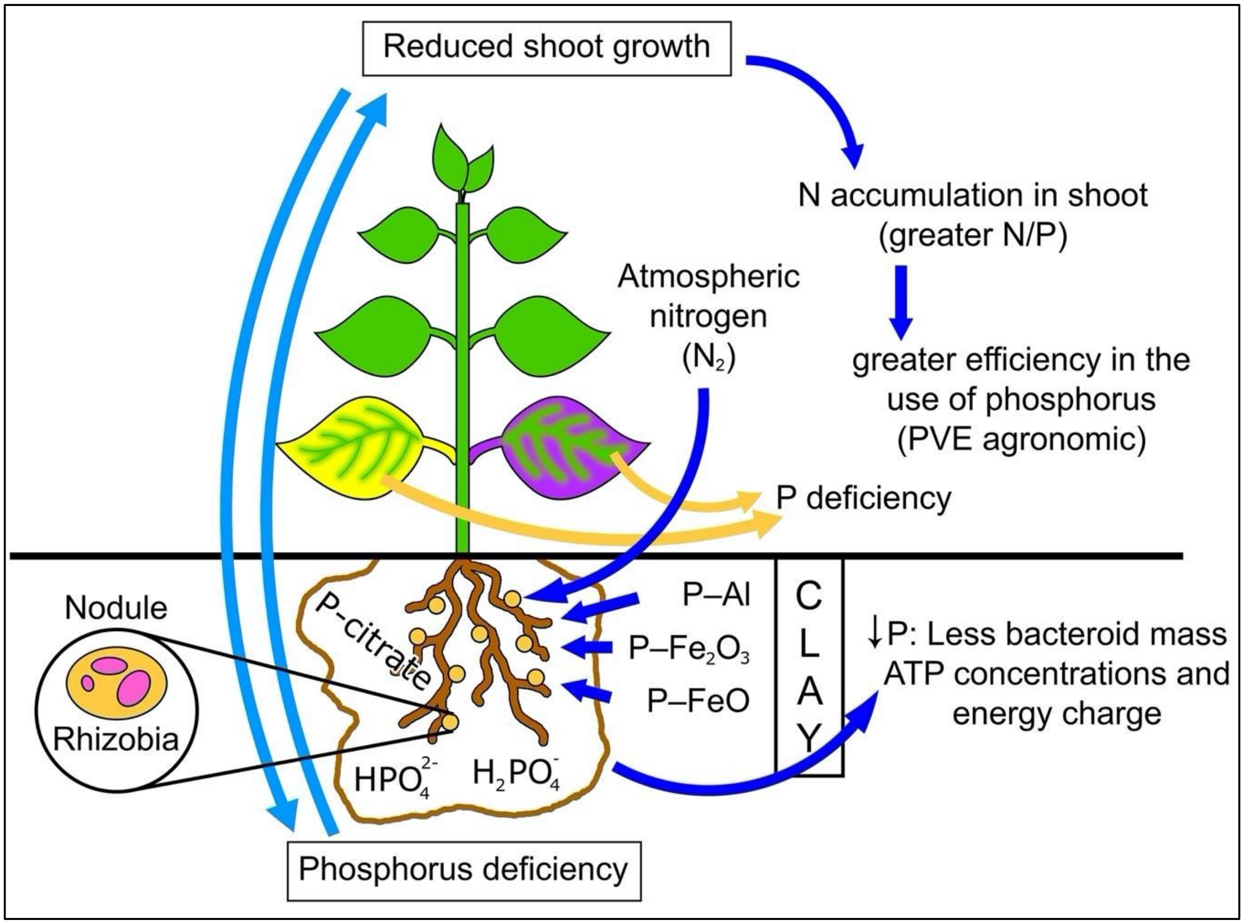

Figure 1.2. 2 Effect of Phosphorus (P) in the biological fixation of Nitrogen (N) by leguminose

Vicia villosa Roth (hairy vetch) is a broadly winter adapted annual legume, that can provide significant quantities of N to farming systems (100 to 130 kg N ha^{-1} yr^{-1}) (Parr *et al*., 2001; Butler & Muir, 2012). This is especially important in rice paddies where N is one of the most yield-limiting nutrients, due to its low recovery efficiency, being globally less than 40% (Wei *et al.*, 2021). Aside from limiting nitrate losses during winter time compared to fallow soils (Hanrahan *et al*., 2018), the incorporation of vetch residues may further influence the processes that control N availability in soil. The incorporation of vetch residues with a relatively low C:N ratio represents a direct source of N due to a fast N mineralization rate (Bonanomi *et al*., 2019). In addition, vetch is expected to act as primer in accelerating the decomposition of the more recalcitrant straw residues (Li *et al*., 2020) with strong short-term changes in their turnover. This may further favor the release of the N pool immobilized by the soil microbial biomass under anoxic conditions (Devêvre & Horwáth 2001; Cucu *et al*., 2014; Said-Pullicino *et al*., 2014), although it is poorly known how these processes affect temporal variations in N forms during the cropping season and provide a prompt source of N for rice plants.

Cover crops can also act as scavengers to recover sparingly available nutrient forms in the soil, even in deeper horizons, increasing the fertility in the rhizosphere surrounding. In particular, P can be taken up by cover crops and released into more surface soil horizons following plant residues decomposition and mineralization of the microbially immobilized pool (Alamgir *et al*., 2016; Damon *et al*., 2014; Oehl *et al*., 2001). Cover crops with high P uptake and a substantial amount of P released in plant available forms normally have a positive effect on P cycling (Damon *et al*., 2014).

In rice paddies, although P availability is rarely limiting under continuous flooding, the balance between P mobilization during the reductive dissolution of Fe (hydr)oxides and retention by formation of P-Fe coprecipitates on the rice roots due to radial O_2 can actually limit P availability more than supposed until now (Li *et al*., 2021). In this context, cover crops can further increase P availability: the enhanced microbial activity following the increase of plant residues and N may indeed favor the release of P from minerals due to the production of protons and organic acids that can dissolve or compete with phosphate for the same sorption sites of Fe and Al-hydroxides (Oberson *et al*., 2005; Celi *et al*., 2020). However, these effects have been poorly investigated.

The data presented in Chapter from 1 to 4 have been published in:

Lizcano-Toledo, R., Reyes-Martín, M. P., Celi, L., & Fernández-Ondoño, E. (2021). Phosphorus Dynamics in the Soil–Plant–Environment Relationship in Cropping Systems: A Review. *Applied Sciences*, *11*(23), 11133.

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1.3. AIM OF THE WORK

Rice is one of the most important and representative cereals in the world in Asian, European and American subtropical regions. Appropriate management practices can overcome P limitation and create a more equilibrate distribution between P pools, especially in tropical soils. In this regard, the association of rice with legumes generate a series of benefits and reactions that can improve the equilibrium between P availability and losses within an agricultural system. The general objective of the thesis is to determine the dynamics of P in rice systems under American and European conditions, proposing alternative management techniques that can increase the availability of P and its relationship with N, also evaluating the effect of different P fertilizers. on agronomic and biological efficiency and its environmental impact.

The CHAPTER 2 deals with cover crops which offer various ecosystem services that contribute to the sustainability of intensive cropping systems. In particular, leguminous cover crops may represent an important source of N for the following income crop. Through the symbiotic association between plant roots and N_2 fixing bacteria, biological N fixation (BNF) may contribute as much as 65-95% of the total plant N. However, BNF requires considerable amounts of energy in the form of ATP, and its efficiency could therefore depend on soil P availability. Apart from the uptake of readily available P, leguminous plants can favour the release of P from poorly available forms, through the exudation of organic acid anions, and/or the hydrolysis of organic P sources, or the production of phosphatases. However, there is scant information on the possible limiting effect of low P availability on BNF, and it is still unclear how, and to what extent, the different forms of P in soil (Pi vs Po) can affect BNF. The aim of this work was to understand how the availability and forms of soil P can affect the production of biomass and BNF efficiency of a typical cover crop (*Vicia villosa*). We hypothesized that increasing soil Pi contents would promote plant growth with a higher shoot/root ratio, and enhance BNF efficiency, with similar but less expressed trends than with increasing Po contents.

In the CHAPTER 3 cover crops were evaluated at field scale. Indeed, although the results showed positive benefits on nutrient dynamics, the effects of cover crops on rice grain yields and nutrient use efficiency may render the proper evaluation of these management practices rather complex. This study therefore aims to investigate the effects of cover crops grown before rice in a temperate agro-ecosystem (NW Italy) on: (i) crop yields and yield components, (ii) apparent fertilizer N recovery and optimal level of N fertilization with hairy vetch; and (ii) temporal variation of N and P forms during the vetch and rice cropping season.

To complete this research, we moved from temperate to tropical systems, to understand i) the different Fe-P relationship in such a weathered soils subjected to different water management, and ii) P dynamics and use efficiency of low and high solubility fertilizers through P fractionation and ^{32}P isotope labeling (CHAPTER 4). All the goals of the three investigations belonging to the thesis are summarized in **Figure 1.3. 1**.

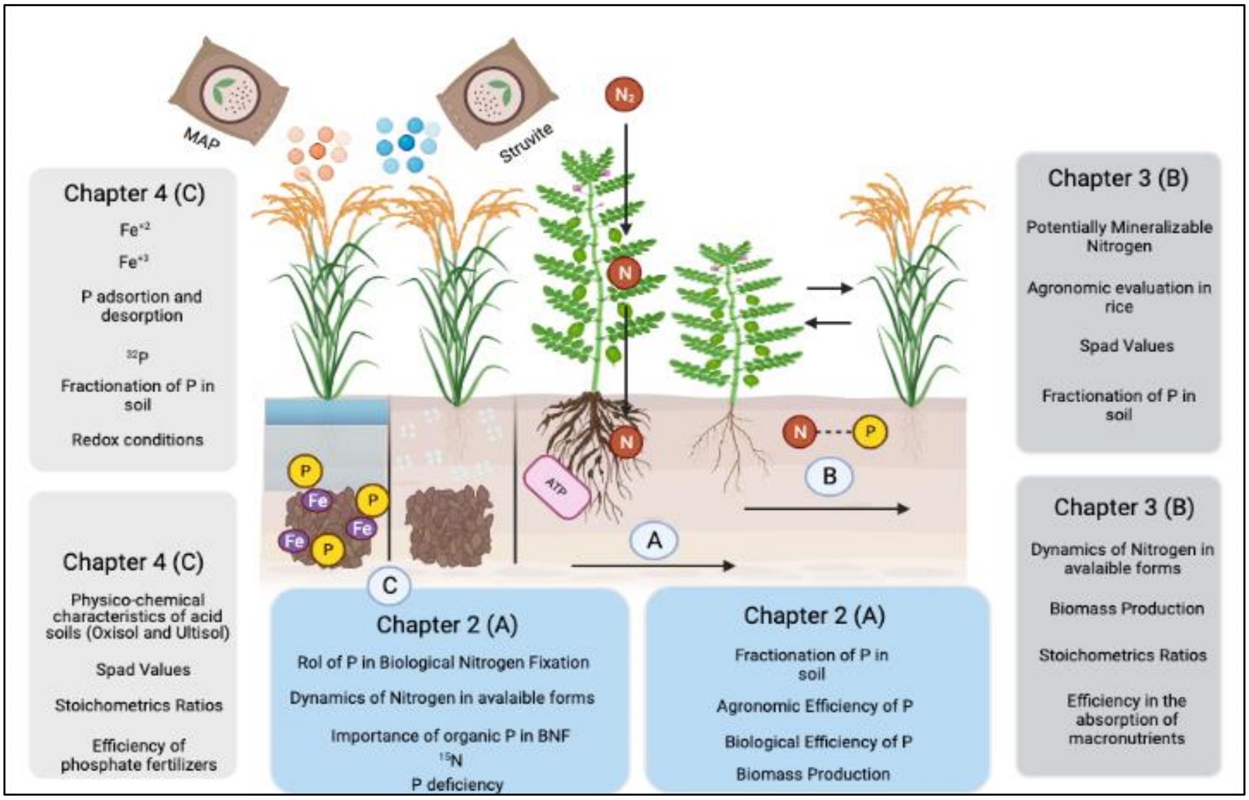


Figure 1.3. 1 The general objectives of this doctoral thesis were a) to determine the effect of organic and inorganic P on the biological fixation of N in the legume *Vicia villosa*; the importance of the legume-rice succession on the N and P dynamics in relation to agronomic rice productivity in northern Italy (B); and the P dynamics in tropical soils subjected to different water management and high and low release fertilizers (C).

1.4 AIM (Objetivos)

Objetivo General

Determinar la dinámica de P en sistemas de arroz bajo condiciones de América y Europa, proponiendo técnicas alternativas de manejo que puedan incrementar la disponibilidad de P y su relación con el N, evaluando además el efecto de diferentes fertilizantes P sobre la Eficiencia agronómica, biológica y su impacto ambiental.

Objetivo específicos

1. Comprender cómo la disponibilidad y las formas de P del suelo pueden afectar la producción de biomasa y la eficiencia de BNF de un cultivo de cobertura típico (*Vicia villosa*).
2. Determinar los efectos de los cultivos de cobertura sembrados antes que el arroz en un agroecosistema templado (noroeste de Italia) sobre el rendimiento de los cultivos y los componentes del rendimiento.
3. Establecer la recuperación aparente de N y el nivel óptimo de fertilización nitrogenada con la Veza.
4. Estudiar la variación temporal de las formas de N y P durante la temporada de cultivo de la veza y el arroz.
5. Analizar la dinámica del P bajo condiciones de suelos tropicales manejando fertilizantes fosfatados convencionales y alternativo.
6. Identificar los efectos Redox en la disponibilidad de P, bajo la dinámica del Fe en suelos ácidos del Brasil.

CHAPTER 2

Effects of inorganic and organic P inputs on nutrient uptake and biological N_2 fixing capacity of hairy vetch (*Vicia villosa)*

2.1. Introduction

Legume cover crops are often included in agricultural cropping systems for enhancing soil N availability and can be higly promising in rice systems where N use efficiency is very low. Apart from increasing inputs of organic matter to the soil, growing legumes as winter cover crops may increase the net N inputs through biological N_2 fixation (BNF), a process that involves the establishment of a symbiotic relationship between plants and rhizobia N-fixers hosted in their roots (Taylor *et al*., 2020).

Hairy vetch (*Vicia villosa* Roth) is widely used in agroecosystems as a winter legume cover crop and green manure contributing between 100 and 230 kg N ha^{-1} with biomass incorporation and having a BNF capacity, expressed as the proportion of N derived from the atmosphere by symbiotic association over the plant growth period (%Ndfa), ranging between 60 and 100% (Wagger, 1989; Parr *et al*., 2011). It has been shown to perform better than other vetch cultivars in terms of aboveground biomass production, root morphological characteristics, P and K uptake and N_2-fixing activity (Solangi *et al*., 2019), with an appropriate adaptability to both P-limiting and non-limiting conditions (Anugroho *et al*., 2010).

The BNF capacity of a leguminous crop is known to greatly depend on soil fertility (Romanyà & Casals, 2020). Adequate phosphorus (P) nutrition is an important component of legume production systems due to their greater P requirements with respect to cereals (Pang *et al*., 2018). The dependence of symbiotic performance on P availability was reported to depend on the overall nutritional status of the soil, with a stronger effect of P addition on BNF in soils with a low P status where rhizobia compete with plants for available P, but not in P rich soils (Raji *et al*., 2019). Soil P availability can affect BNF capacity directly by modulating nodule growth, formation and functioning as a result of the high P costs of symbiotic N fixation (Raven, 2012; Divito & Sadras, 2014), or indirectly by affecting plant growth and allocation of photosynthetically assimilated C to the symbionts (Walley *et al*., 2011; Püschel *et al*., 2017).

Nonetheless, legumes are also known to hold an advantage in P acquisition due to their capability to mobilize sparingly soluble P in the soil through a variety of root mechanisms in order to provide for the great P demand necessary to maintain the rhizobial symbiosis (Jakobsen, 1985). Under P-limited conditions, leguminous plants have been reported to respond by acidification of the rhizosphere, increasing the exudation of organic acids, up-regulating the production of extracellular phosphatase enzymes, as well as favouring symbiotic mycorrhizal associations (Hinsinger, 2001; Houlton *et al*., 2008; Nasto *et al*., 2014). In particular, considering that organic P (Po) may account for 30–65% of the total P in arable soils (Tiessen, 2008) primarily in the form of inositol phosphates and other phosphate monoesters (Turner *et al*., 2002), the greater investment in phosphatase enzymes with respect to non-N_2-fixing species could facilitate P acquisition by catalyzing the hydrolysis of organic P esters releasing Pi for uptake by plant roots (Tarafdar & Claassen, 1988; Olde Venterink, 2011). However, Pang *et al*. (2018) have recently evidenced that the greater root phosphatase activity of legumes, particularly at low soil P availability, was likely a phylogenetic trait of rhizobial legumes rather than being directly related to their N_2-fixing capacity.

Various studies have evidenced a positive effect of P supply on BNF by N_2-fixing plant species (e.g. Isaac *et al*., 2011), with increasing P supply improving nodule number, nodule biomass, and BNF rates (Chekanai *et al*., 2018; Olivera *et al*., 2004). In particular, Bukovsky-Reyes *et al*. (2019) reported a positive relationship between soil available P and vetch root N content when vetch was P-limited at soil available P contents below 70 mg kg^{-1}. However, little is known on the responses of *Vicia villosa* to P limitations, the consequences for N_2 fixation rates and adaptive plant strategies for coping with nutrient deficiencies in low fertility soils. Moreover, whereas most studies focused on understanding the effects of inorganic P availability on the mechanisms controlling P acquisition and implications on BNF rates under low P supply, there still remains a lack of evidence on role of organic P sources in controlling BNF in leguminous plants. These Po sources may contribute to partially offset the dependence on labile inorganic P to satisfy plant P requirements by leguminous plants (Turner, 2008).

Based on these considerations we hypothesized that under severe P limitation, (a) improving P supply through Pi inputs can favor plant growth and C allocation to the root nodules, and consequently enhance the BNF capacity of hairy vetch plants, while (b) increasing Po inputs can partially alleviate the P limitation effect on N_2 fixation, thanks to the ability of vetch plants to access organic P sources. We tested these hypotheses by growing rhizobia-inoculated hairy vetch plants in a P-limited soil and evaluating plant growth, N and P uptake, and BNF capacity (by isotope dilution), as a function of Pi and Po inputs in the form of orthophosphate or phytic acid, respectively.

2.2. Materials and methods

2.2.1. Experimental setup

The effects of P availability on nutrient uptake and BNF capacity of *Vicia villosa* Roth were evaluated by means of a greenhouse pot experiment in which hairy vetch was planted in a P poor agricultural soil amended with two levels of added P in the form of inorganic (Pi) and organic P (Po), as well as an unfertilized control (CNT). The experiment was conducted in a fully factorial experimental design with three sampling times over the plant growth period and five biological replicates per treatment, and thus comprised a total of 75 pots. The plants were seeded in mid-April and were grown for 10 weeks in 1.2-litre pots (containing 800 g of soil) positioned randomly in a greenhouse under natural light conditions. The soil used was collected from the topsoil (0-30 cm) of an acidic, sandy loam agricultural soil having a pH of 5.0, a clay and sand content of 8.1 and 56.4 %, respectively, an available P content (Olsen P) of 2.94 mg kg^{-1}, an organic C content of 2.62 g kg^{-1} a C:N ratio of 11.8 and a cation exchange capacity of 4.5 $cmol_{(+)}$ kg^{-1}. Prior to use, the soil was air-dried and passed through a 2 mm sieve.

A gradient of P supply was obtained by applying 40 mg P kg^{-1} (PiL and PoL) or 120 mg P kg^{-1} (PiH and PoH) in the form of inorganic (KH_2PO_4) or organic P (potassium *myo*-inositol hexaphosphate, <2% hydrolysed P) prior to planting. Ten seeds of hairy vetch were planted in each pot, and after 10 d these were thinned to 5 plants per pot. All plants were inoculated with rhizobia compatible with the host plant species. During the growing period a nutrient solution (50 ml) containing 235 ppm K, 200 ppm Ca, 64 ppm S, 50 ppm Mg, 0.5 ppm B and Mn, and 0.05 ppm of Zn and Mo was applied weekly, while the soil moisture was regularly adjusted and maintained around 60% of the water-holding capacity.

In order to distinguish N uptake by plant via root and BNF pathways, and to calculate the BNF capacity of hairy vetch as a function of P availability by isotope dilution, a relatively small amount of isotopically labelled N fertilizer was applied to each pot as $K^{15}NO_3$ (10 at% ^{15}N; 10 mg N kg^{-1}) 20 days after seeding (DAS) to prevent potential suppression of nodulation at the early stages of plant development.

2.2.2 Plant and soil sampling and analyses

Five pots per treatment were destructively sampled 30, 50 and 70 DAS. During harvesting, hairy vetch plants were removed without damaging roots and biomass. The shoots were cut at the soil surface, while the roots were carefully removed from pots, gently shaken to remove most of the soil and subsequently carefully washed in water to remove adhered soil particles. Visible nodules growing on the roots were collected aseptically and pooled. Fresh samples were dried at 65°C until constant mass, weighed, and then milled for subsequent analysis. Soil samples were collected after plant harvesting, air-dried and ground (2-mm sieve) prior to analyses.

Total N contents and the N isotopic ratio in plant tissues were measured by high temperature combustion using an elemental analyzer (Vario Isotope Select, Elementar Analysensysteme GmbH, Hanau, Germany) coupled to an isotope ratio mass spectrometer (IsoPrime 100, Elementar Analysensysteme GmbH). Total P concentrations in the plant shoots, roots and nodules were determined by sulphuric-perchloric sample digestion followed by spectrophotometric analysis using the malachite green method (Ohno & Zibilske, 1991). Phosphorus-acquisition efficiency (PAE) was calculated as the ratio of total plant P uptake to soil available P (P Olsen), while P-utilization efficiency (PUE) was calculated as the ratio of dry biomass to P content in the plant tissues (Neto *et al.*, 2016).

Soil P fractions were determined by extraction with 0.5 M $NaHCO_3$ (Olsen *et al.*, 1954), 10 mM citric acid and 0.1 M NaOH/1 M NaCl. The extraction with 0.5 M $NaHCO_3$ simulates available P pool while diluted citric acid mimics the effect of organic acids within rhizospheric exudates in P mobilization. The fraction extracted with 0.1M NaOH is typically defined as (quite strongly) associated with the surfaces of Fe and Al minerals.

Nitrate and ammonium concentrations in the soil were determined by extraction in 1 M KCl followed by spectrophotometric quantification using modified Greiss and Berthelot methods, respectively as described by Cucu *et al.* (2014). Isotopic enrichment of the extracted mineral N pool was determined by a combination of micro-diffusion and ^{15}N stable isotope analysis (Vario Isotope Select and IsoPrime 100) as described by Schleppi *et al.* (2006), and subsequently used to calculate biological N fixation by the plants.

2.2.3 Calculation of biological N fixation

Biological nitrogen fixation (BNF) was calculated as the product of plant N biomass and the proportion of N derived from the atmosphere (%Ndfa). The %Ndfa was calculated by isotope dilution (Unkovich *et al.*, 2008; Chalk & Craswell, 2018), which compares the isotopic signature of the leguminous plant biomass with that of the isotopically labelled plant-available soil mineral N pool, according to the equation:

$$\%\text{Ndfa} = \left(1 - \frac{\text{atom \% }^{15}\text{N excess}_{\text{legume}}}{E^*}\right) \times 100$$

where $E*$ is the time-integrated pool enrichment of the soil mineral N available for plant uptake and that takes into account the exponential decline in the ^{15}N enrichment (atom % excess) of the mineral N pool over the plant growth period due to the supply of unlabelled N through the mineralization of soil organic N. The initial soil mineral N isotopic enrichment (E_0) and the first-order rate constant (k in d^{-1}) for the decline in the ^{15}N enrichment of the soil mineral N pool over the growth period, were estimated by fitting the change in ^{15}N enrichment (atom% excess) over time (t) into the exponential equation:

$$E_t = E_0 e^{-kt}$$

$E*$ over a specific time interval (i.e. over 30, 50 and 70 DAS) was obtained by mathematical integration of the exponential equation using the formula:

$$E^* = \frac{E_0(e^{-kt_1} - e^{-kt_2})}{k(t_1 - t_2)}$$

2.2.4. Enumeration of culturable soil microorganisms

Different groups of soil microorganisms were enumerated by plating serial dilutions of fresh soil on different agar media. A total heterotrophic count was performed on Plate Count Agar (PCA, Oxoid, Hants, UK); total fungi were enumerated on Malt Extract Agar (MEA, Merck

KGaA, Darmstadt, Germany) supplemented with Tetracycline (50 mg L^{-1}, Sigma-Aldrich, Milan, Italy). For soil diazotrophs enumeration, a nitrogen-free solid medium (NF, Döbereiner *et al.*, 1995) was used, coupled with an incubation under hypoxic conditions in anaerobic jars (Mirza & Rodrigues, 2012).

2.2.5 Statistical analysis

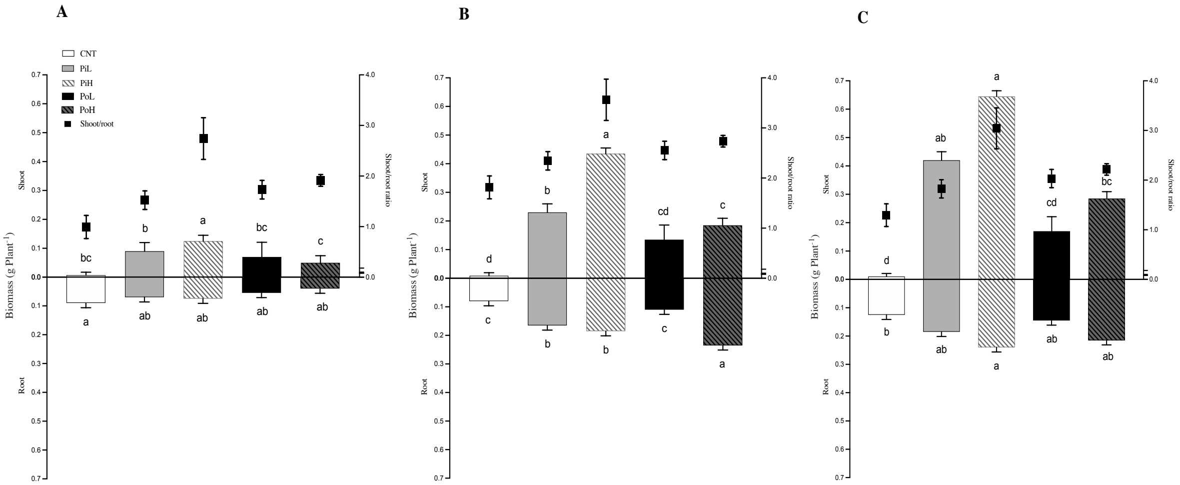
Prior to analysis of variance (ANOVA) the data sets were tested for normality and homogeneity of variance by Shapiro-Wilk ($p > 0.05$) and Levene-test ($p > 0.05$), respectively. Any data that were not fit for normal distribution were log transformed. One-way ANOVA was used to assess the effects of P forms and doses on all measured parameters separately for each sampling time. Significant ($p < 0.05$) differences between means were identified using the post hoc Tukey HSD test. All ANOVA analyses were performed using SPSS version 19.0 (SPSS Inc., USA).

2.3. Results

2.3.1 Plant growth and nodulation

Marked changes and visual differences were observed in the root and shoot biomass of hairy vetch subjected to different levels and forms of P (**Figure 2.3. 1**), with Pi treatment showing the best performance and the control the lowest. However, if the control treatment had the greatest root length after 30 DAS, the PoL showed the greatest length after 70 DAS. The plants treated with PiL and PiH reached the flowering stage 30 DAS and the fruiting stage 60 DAS. P deficiency could be evidenced 20 DAS, with mature purple leaves as shown in **Figure 2.3. 1.D.**

Figure 2.3. 1 Phenological development of hairy vetch under different treatments. Root and shoot biomass after 30 (a), 50 (b) and 70 DAS. Signs of P deficiency in hairy vetch leaves.



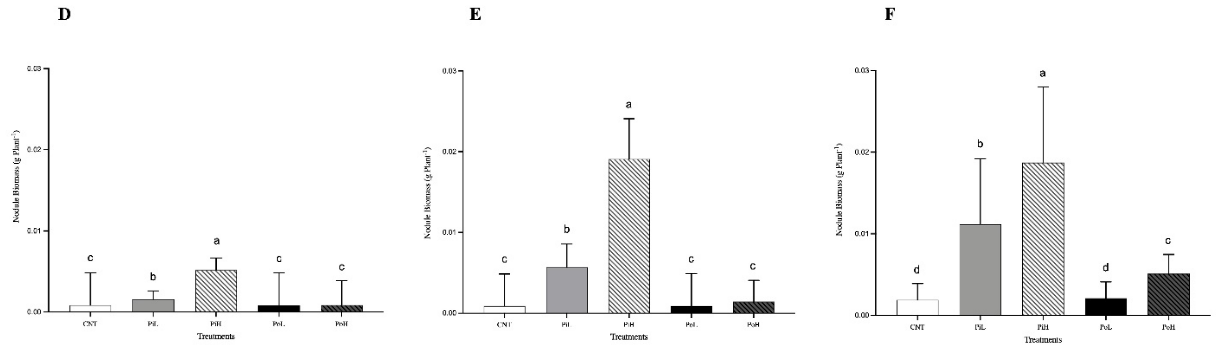


Figure 2.3. 2 Biomass distribution. Root and shoot biomass in the different treatments after 30 (a), 50 (b) and 70 (c) DAS. Nodule biomass after 30 (d), 50 (e) and 70 (f) DAS.

Aboveground biomass was significantly influenced by P application over the whole growth period (*p* <0.001). Application of Pi strongly enhanced shoot growth with respect to the control even when applied at low doses, and at 70 DAS greatest shoot biomass was observed for PiH followed by PiL (**Figure 2.3. 2**). On the other hand, application of Po did not result in significant differences in shoot growth with respect to the control, even though shoot biomass for PoH at 70 DAS was slightly greater than CNT (**Figure 2.3. 2**). Root growth showed a different response to P addition when compared to shoot growth. After 50 DAS, only PoH showed a significantly greater root biomass with respect to all the other treatments including the control, resulting in the highest root:shoot (R:S) ratio of 1.5. However, these differences were no longer observed at 70 DAS when both root biomass and R:S ratios of Po treated soils were not different from those of the control. Application of Pi did not influence root growth but resulted in significantly lower R:S ratios with respect to the control (0.3 – 0.6). Nodulation was significantly affected by the application of Pi that always led to greater nodule biomass with respect to the control, proportional to the dose of applied P. By day 50, nodule biomass was already 6 and 18 times greater than that in the control for PiL and PiH, respectively (**Figure 2.3. 2** D, E, F). In contrast, application of Po did not influence nodulation except for a slightly greater nodule biomass observed for PoH after 70 DAS with respect to the control, that however was not statistically significant.

2.3.2 Soil P fractions and available N

Plant available P generally reflected the application of P with the different treatments. Lowest Olsen and citrate-extractable P were obtained for the untreated control, while highest contents were observed for the Pi treated soils, with PiH showing significantly higher values with respect to PiL (**Figure 2.3. 3** and **Figure 2.3. 4**). Irrespective of the dose, application of Po did not result in significantly different Olsen P contents with respect to CNT, while citrate-extractable P was slightly higher than CNT but not different between PoL and PoH treatments. These differences were consistent over the entire growth period. Most of the Pi applied to the soils was recovered in the NaOH-extractable fraction resulting in proportionally higher P contents in the PiL and PiH treatments with respect to CNT (**Figure 2.3. 5**). On the other hand, NaOH-extractable P in soils treated with PoL and PoH was not significantly different from CNT.

Soil mineral N contents (sum of ammonium and nitrate N) were generally relatively low (<5 ppm) with little or no differences between treatments throughout the growth period expect for the earliest sampling time (30 DAS) (**Figure 2.3. 6** and **Figure 2.3. 7**). At this time mineral N concentrations were generally >5 ppm with highest concentrations of 18.2 mg N kg^{-1} measured for PoH, that were slightly but significantly higher than that obtained for CNT (13.2 mg N kg^{-1}).

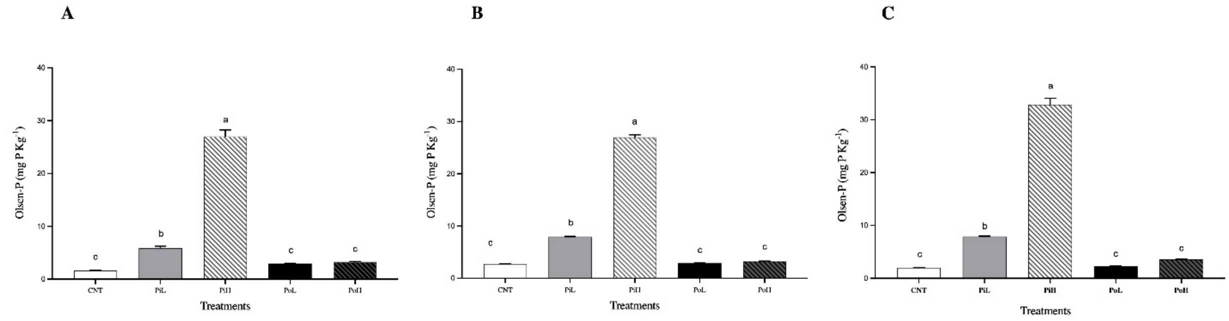


Figure 2.3. 3 Olsen P concentration in the different treatments after 30 (a), 50 (b) and 70 (c) DAS.

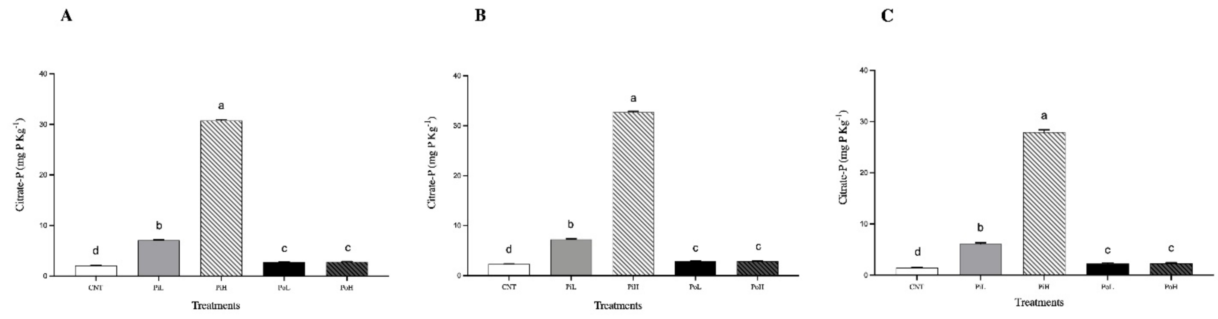


Figure 2.3. 4 Phosphorus-Citrate concentration in the different treatments after 30 (a), 50 (b) and 70 (c) DAS.

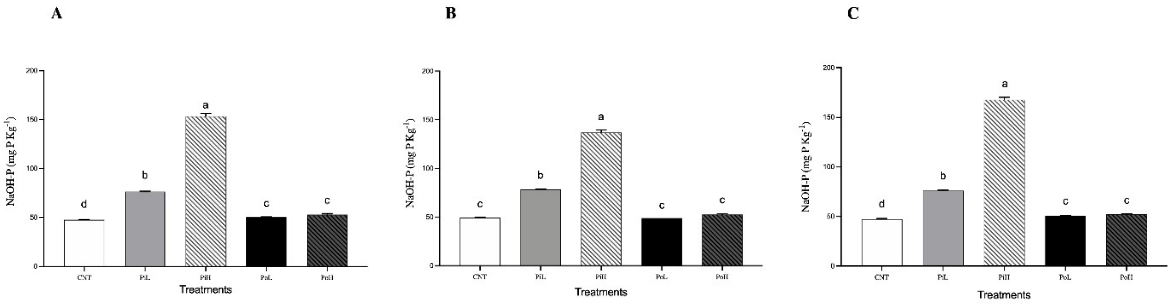


Figure 2.3. 5 Extracted NaOH-P fraction in the different treatments after 30 (a), 50 (b) and 70 (c) DAS.

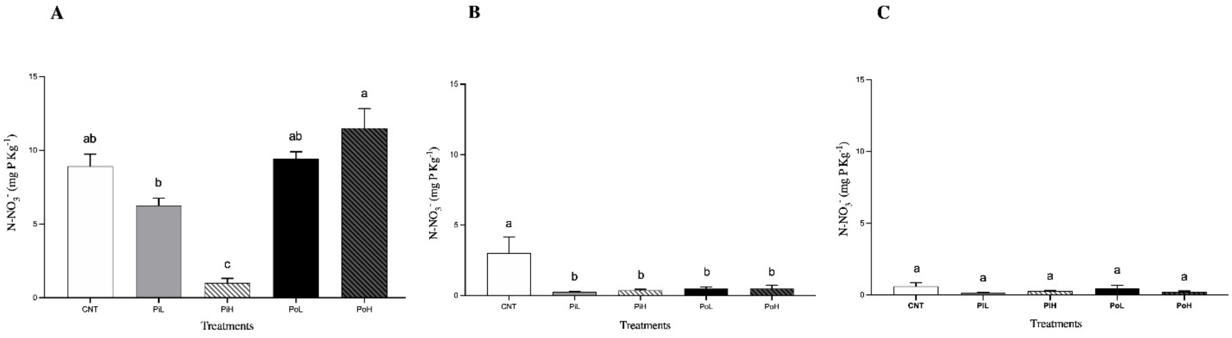


Figure 2.3. 6 Extracted N-NO$_3^-$ in the different treatments after 30 (a), 50 (b) and 70 (c) DAS.

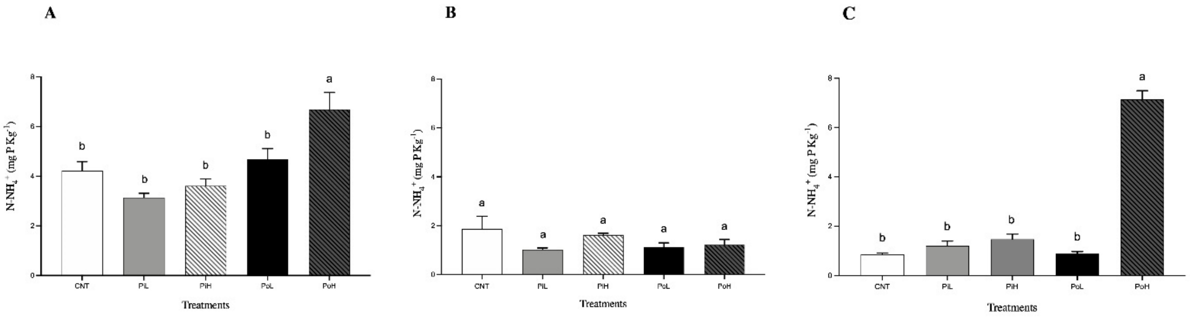


Figure 2.3. 7 Extracte N-NH4$^+$ in the different treatments after 30 (a), 50 (b) and 70 (c) DAS.

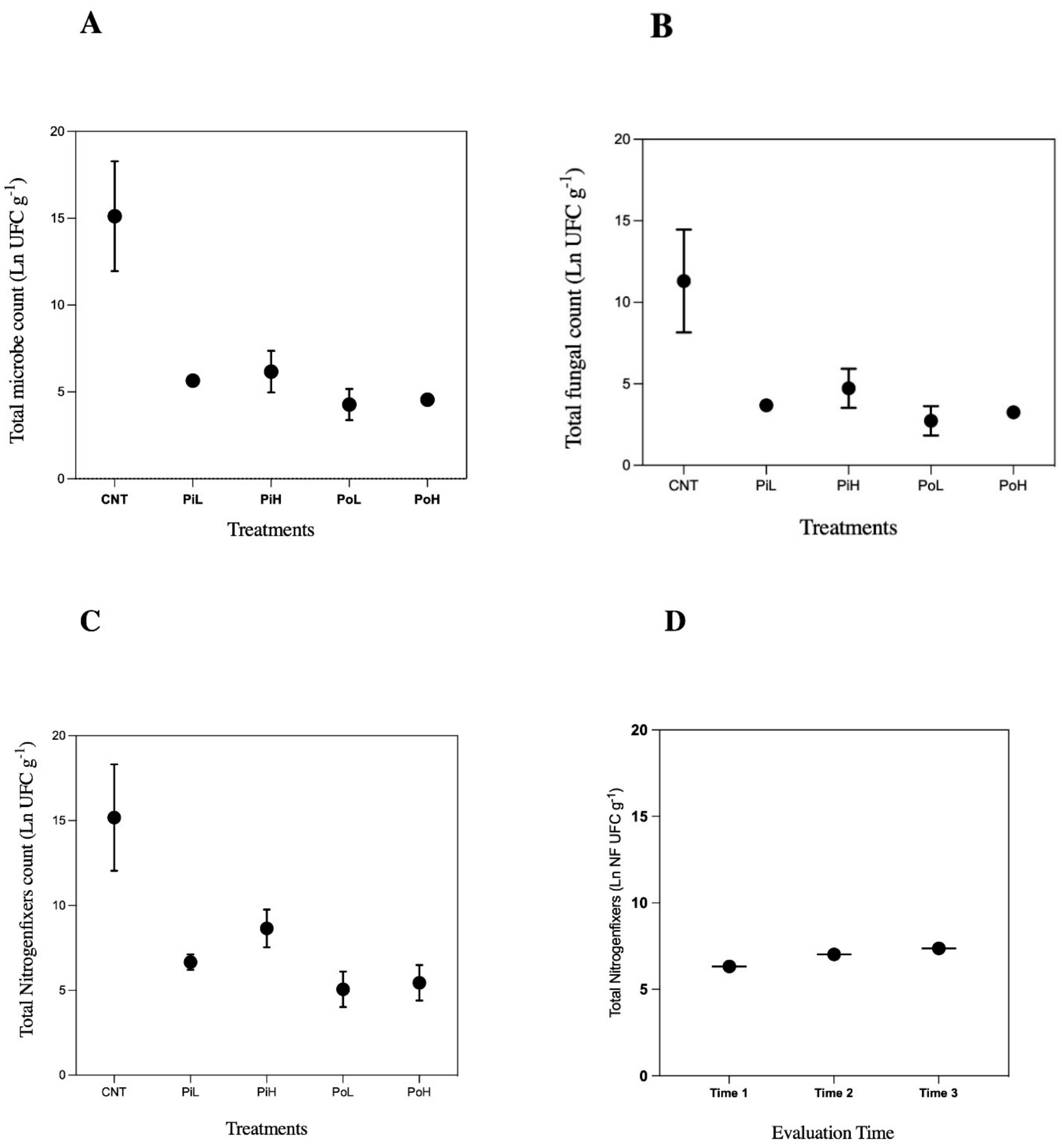
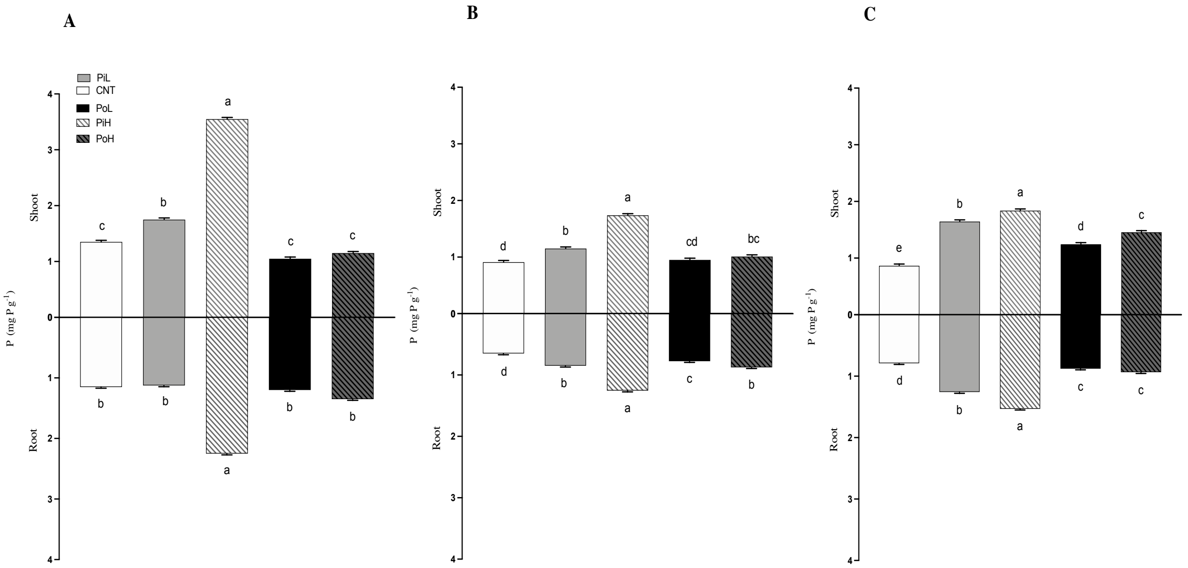


Figure 2.3. 8 Total microbial determination (a) and distribution between fungi content (b), total nitrofixer bacteria at 70 DAS (c) and at different times.

The microbiological analysis showed significant differences in the total microbial counts, fungi and Nitrofixer bacteria (p <0.001). The highest values were observed in CNT, followed by PiH **(Figure 2.3. 8).**

2.3.3 Plant P uptake

With the addition of Pi, vetch plants always showed higher total P uptake with respect to the untreated control (**Figure 2.3. 9**; p <0.001) due to the combined effects of a higher biomass as well as a higher P content. This higher P uptake was observed for both above and belowground biomass as was proportional to the amount of applied Pi. The increase in plant P acquisition with increasing P availability however resulted in a PAE that was always lower or equal to the control (**Figure 2.3. 10a**), and a PUE that was always lower with respect to the control (**Figure 2.3. 10b**) throughout the growth period, particularly for PiH. In the case of treatment with Po, only application of high doses resulted in a plant P uptake after 50 DAS that was slightly but significantly higher than that observed for the untreated control (p <0.001; **Figure 2.3. 10**). This led to a higher PAE with respect to both the control and the Pi treated plants, that was however only significant at 50 DAS. When compared to the addition of Pi, plants grown in the presence of Po also showed a generally higher PUE that was most evident at 70 DAS, particularly for PoL.



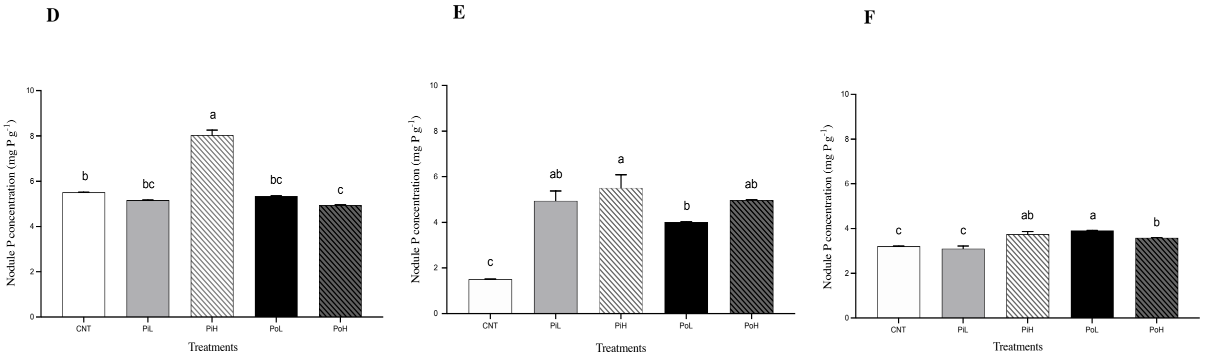


Figure 2.3. 9 Phosphorus content in shoot and root in the different treatments after 30 (a), 50 (b) and 70 (c) DAS. Phosphorus content in nodule biomass after 30 (d), 50 (e) and 70 (f) DAS.

Table 2.3. 1 Proportion of N derived from the atmosphere (Ndfa) in the shoots, roots and nodules of hairy vetch as a function of different forms (Pi, inorganic P; Po, organic P) and amounts (CNT, no P; L, Low P; H, High P) of applied P after 30, 50 and 70 days after seeding (DAS). Values represent the mean ± standard error (n = 5) while different letters indicate significant differences between treatments within each sampling date ($p < 0.05$).

Treatment	Shoot Ndfa (%)	Root Ndfa (%)	Nodule Ndfa (%)
30 DAS			
CNT	76.0 ± 1.7^{a}	56.8 ± 3.1^{ab}	86.8[†]
PiL	72.5 ± 2.1^{ab}	59.5 ± 2.5^{ab}	85.7[†]
PiH	64.7 ± 3.4^{b}	50.6 ± 3.7^{b}	83.2 ± 1.6
PoL	78.1 ± 1.0^{a}	67.4 ± 1.6^{a}	100.0[†]
PoH	77.1 ± 2.7^{a}	67.9 ± 3.5^{a}	91.8[†]
50 DAS			
CNT	45.1 ± 3.4^{c}	43.2 ± 2.7^{b}	73.1[†]
PiL	58.7 ± 3.2^{b}	55.1 ± 2.4^{ab}	77.7 ± 1.9
PiH	82.9 ± 2.5^{a}	67.4 ± 7.0^{a}	90.8 ± 1.0
PoL	38.7 ± 2.2^{c}	41.1 ± 1.9^{b}	75.4[†]
PoH	41.5 ± 2.3^{c}	51.8 ± 5.5^{ab}	67.8[†]
70 DAS			
CNT	40.3 ± 2.9^{c}	45.2 ± 1.6^{bc}	72.4[†]
PiL	72.1 ± 7.0^{ab}	59.0 ± 5.1^{ab}	80.1 ± 3.5
PiH	82.4 ± 4.6^{a}	69.4 ± 5.9^{a}	86.6 ± 2.4
PoL	34.7 ± 2.4^{c}	42.4 ± 1.7^{c}	56.4[†]
PoH	56.8 ± 7.4^{bc}	52.7 ± 2.6^{bc}	73.9[†]

[†] Insufficient sample mass for replicated analysis

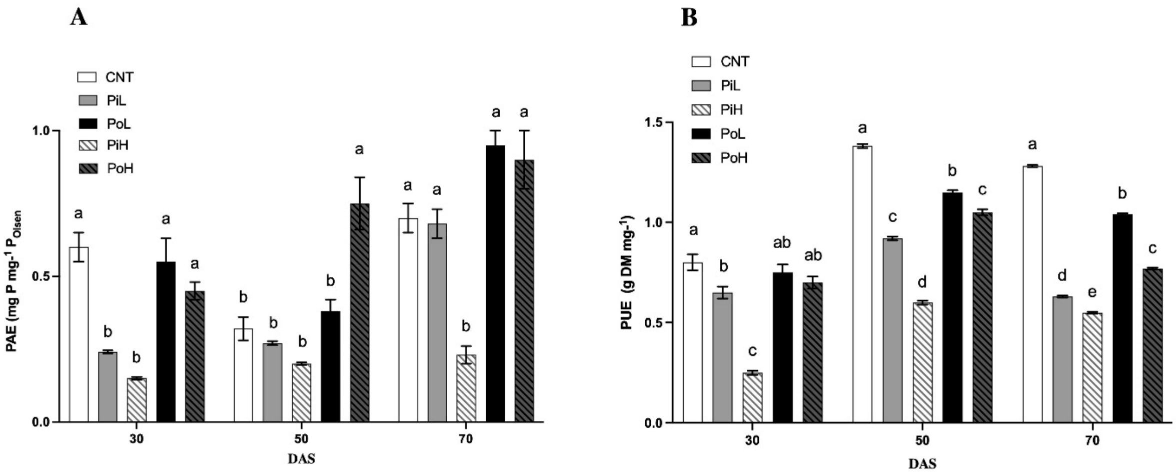


Figure 2.3. 10 P-acquisition efficiency (a) and P-utilization efficiency (b) as a function of different forms (Pi, inorganic P; Po, organic P) and amounts (CNT, no P; L, low P; H, high P) of applied P after 30, 50 and 70 days after seeding (DAS). Values represent the mean (n = 5) while error bars represent the standard error. Different letters indicate significant differences between treatments within each sampling date (p <0.05).

2.3.4 Plant N uptake and biological N fixation efficiency

Plant N uptake was influenced by the addition of Pi as the supply of plant available P led to an increase in N uptake that was proportional to the amount of Pi added. This was more appreciable in the shoot N content that was significantly higher with respect to the control already by day 30, while differences in the root N content were only noted after 70 DAS (**Figure 2.3. 11**). In contrast, application of Po did not affect plant N uptake even when applied at high doses, and shoot and root N contents were generally similar to those observed in the control, except for a higher root N content in plants treated with PoH after 50 DAS (**Figure 2.3. 11**). Similar results were also obtained for the amount of plant N derived from the atmosphere through BNF. Application of Pi had a positive effect on BNF with shoot Ndfa reaching values of around 72-82% by day 70, compared to only 40% in the untreated control and 35-55% in the Po treated soils (**Table 2.3. 1**). Similar trends were observed for root Ndfa although the differences were less marked as fixed N represented a smaller proportion of total plant N in the roots. Here, a maximum of 67-69% Ndfa was observed in PiH treated soils, while the proportions of fixed N in the roots of the other treatments were not significantly different from the control (**Table 2.3. 1**). Root nodules always showed relatively high contents of fixed N (on average 82%) though the difference between treatments was not easy to decipher due to a lack of sufficient sample for analysis leading to non-replicated results. Nonetheless, nodule Ndfa values with the addition of Pi were always somewhat larger than the other treatments.

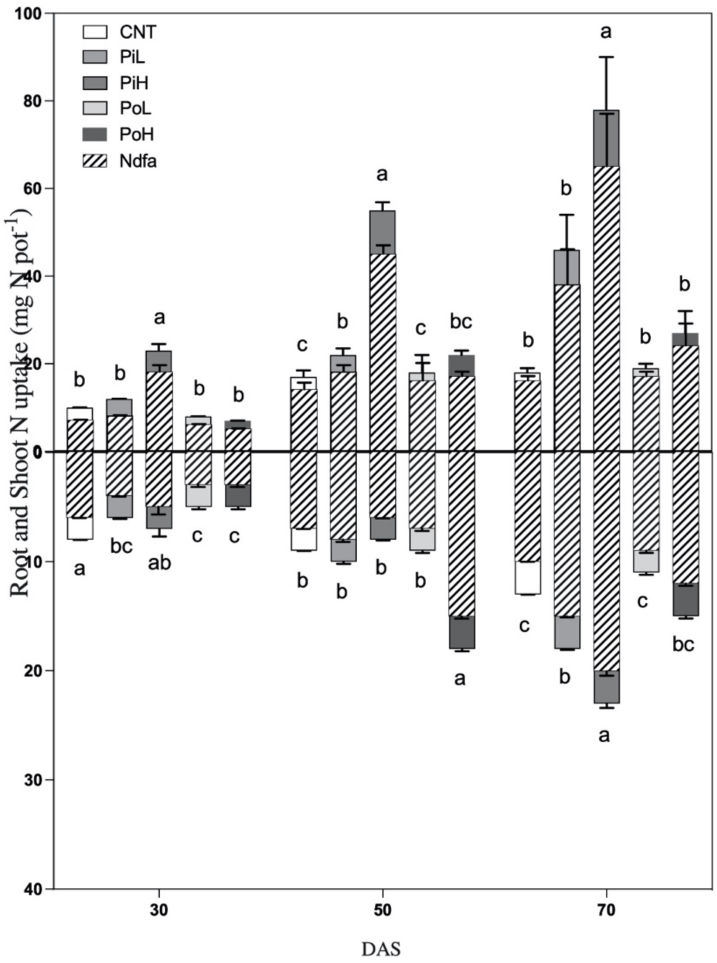


Figure 2.3. 11. Total root and shoot N acquisition and proportion of N derived from atmosphere (Ndfa) as a function of different forms (Pi, inorganic P; Po, organic P) and amounts (CNT, no P; L, low P; H, high P) of applied P after 30, 50 and 70 days after seeding (DAS). Values represent the mean (n = 5) while error bars represent the standard error. Shaded areas represent the proportion of plant N that derives from the atmosphere (Ndfa). Different letters indicate significant differences in both total N and Ndfa between treatments within each sampling date (p <0.05), except for differences in root Ndfa at 30 DAS that were not significant.

2.4. Discussion

In general, plant growth is greatly influenced by soil mineral P availability, and in leguminous plants, an increase in P availability can positively affect BNF rates. This can be due to the high P demand of N_2-fixing bacteria as well as to the effect of P availability on the plant photosynthetic capacity and belowground C allocation to roots and nodules (Divito & Sadras 2014). In this work, low soil P availabilities strongly limited hairy vetch growth, such that increasing P supply through the addition of Pi resulted in a strong positive effect on biomass production, primarily through enhanced shoot growth. This inevitably led to hairy vetch plants investing less resources towards root development (lower R:S ratios) and showing lower P acquisition and utilization efficiencies with respect to plants grown in P-deficient conditions.

In the presence of readily available P, hairy vetch enhanced N_2-fixation suggesting an increase in the plant's N demand when P was not limiting, as indicated by the strong positive relationship between Ndfa and shoot P at maturity (i.e. 70 DAS). Increasing N uptake was previously linked with a growth response to P supply in various other leguminous crop species (e.g., *Trifolium repens* L. by Almeida *et al*. 2000 and Høgh-Jensen *et al*. 2002, *Medicago* spp. by Püschel *et al*. 2017). Moreover, the increase in nodule biomass with increasing P availability suggests that whereas nodulation was strongly P limited under deficient conditions, plants allocated more resources to the symbiosis with the N_2-fixing bacteria when P was readily available. This was reflected in a higher BNF efficiency and a greater allocation of fixed N into the aerial parts of the plant, confirming our first hypothesis. In contrast, fixed N in the roots was rather conservative, in line with the lower shoot-to-root C allocation. Nonetheless, even though nodule growth was strongly limited under sever P deficiency (≤2 ppm Olsen P), approximately 40% of the total N assimilated by these plants was due to symbiotic N_2 fixation, in line with the findings of Almeida *et al*. (2000) for white clover. BNF showed a stronger relationship with biomass production rather than with Ndfa across all P treatments, confirming that in P deficient conditions, BNF was primarily regulated by plant growth rather than a direct effect of P availability on N_2 fixation. However, the effects of P deprivation are also known to depend on the duration of the stress and plant age (Høgh-Jensen *et al*., 2002). Similar effects of P availability on BNF were reported for other vetches like bitter vetch (*Vicia ervilia*) grown in low-fertility soils (Romanyà & Casals 2020).

An increase in BNF with increasing P uptake was also observed as a result of the addition Po (at higher application doses), even though only a minimal increase in citrate-extractable P was recorded. Although this effect was less marked with respect to the addition of Pi, it suggests that hairy vetch can to some extent access organic P sources for its P requirements, and this can partially alleviate the P limitation effect on N_2 fixation. However, addition of Po did not significantly affect plant growth and nodulation, although slightly higher P uptake and shoot, root and nodule biomass were observed for the higher Po application dose after 70 DAS with respect to the control. After the addition of Po, hairy vetch plants generally showed a slightly higher P acquisition efficiency though this did not correspond to a higher P utilization efficiency that was often lower with respect to the control, although always higher than that observed for plants receiving Pi. These findings suggest that although the plants allocated resources to improve P acquisition from organic forms, this did not lead to a commensurate increase in plant growth over the duration of the experiment. Consequently, neither plant N uptake nor BNF benefitted from the slightly higher P uptake following Po addition, even though the proportion of shoot Ndfa was nonetheless higher in mature plants receiving the highest dose of Po with respect to the control. Our second hypothesis was therefore only partially confirmed, as evidence for the acquisition of P from organic resources by hairy vetch over the growth period from seeding to flowering under severely P limited conditions was weak, and BNF was probably limited by the reduced photosynthetic capacity. These findings are in line with those of Adams & Pate (1992) who showed that although *myo*-inositol phosphate is a potential source of P for the growth of *Lupinus* spp. that are capable of up-regulating root phosphohydrolase enzymes, it represents a much poorer P source in soil where availability depends on the susceptibility to soil phosphatases and the interaction with soil minerals (Celi, 2020; Giaveno *et al*., 2010). This could have well been the case in our experiment where even the highest dose of added *myo*-inositol was well below the maximum sorption capacity of the soil used and did not result in any substantial increase in plant available P pools (**Figure 2.3. 3**, **Figure 2.3. 4**, **Figure 2.3. 5**). Garcia-López *et al*. (2021) further showed that the adsorption of inorganic P released during the hydrolysis of Po on soil minerals can also negatively affect plant P uptake even in the presence of elevated hydrolytic activity.

CHAPTER 3

Cover crops increase N and P availability in temperate rice agrosystems

3.1. Introduction

In the previous chapter we observed a great potential of cover crops to improve soil fertility and N and P cycling in soil. This can have important consequences on crop yields and quality of the crop that follows (Delgado & Gantzer 2015). Hairy vetch provided also significant quantities of N to soil, and this is especially important in rice paddies where N is one of the most yield-limiting nutrients, due to its low recovery efficiency, being globally less than 40% (Cassman & Doberman, 2022). Aside from limiting nitrate losses during winter time compared to fallow soils (Girondé *et al*., 2015), the incorporation of vetch residues may further influence the processes that control N availability in soil. These residues with a relatively low C:N ratio represent indeed a direct source of N due to a fast N mineralization rate (Ameloot *et al*., 2015). In addition, vetch is expected to act as primer in accelerating the decomposition of the more recalcitrant straw residues with strong short-term changes in their turnover. This may further favour the release of the N pool immobilized by the soil microbial biomass under anoxic conditions (Ren *et al*., 2019; Cucu *et al*., 2014; Said-Pullicino *et al*., 2014), although it is poorly known how these processes affect temporal variations in N forms during the cropping season and provide a prompt source of N for rice plants.

In the previous chapter we observed also a different dynamics of P forms and the influence determined by both inorganic and organic P forms. In this context, cover crops can further increase P availability: the enhanced microbial activity following the increase of plant residues and N may indeed favour the release of P from minerals due to the production of protons and organic acids that can dissolve or compete with phosphate for the same sorption sites of Fe and Al (hydr)oxides (Oberson *et al*., 2015; Celi *et al*., 2020).

The larger P availability may overcome many serious problems faced with P deficiency in rice crops. A reduction in the number of fertile grains has been observed; with fewer seeds formed, the plant increases the supply of nutrients per seed, thus improving its viability. When P is not added during the initial stage or the vegetative stage of the crop, there is less tiller production than that obtained in plants without P restriction. The development of secondary roots follows the same behavior, demonstrating the need for the plant to have P available at the beginning of growth to allow maximum root development (Miguel *et al*., 2015; Vejchasarn *et al*., 2016). However, these effects have been poorly investigated, combining cover crops with rice cropping.

Based on these considerations, although cover crops generally show positive benefits, their effects on grain yields and nutrient use efficiency may render the proper evaluation of these management practices rather complex. This study therefore aims to investigate the effects of cover crops grown before rice in a temperate agro-ecosystem (NW Italy) on: (i) crop yields and yield components, (ii) apparent fertilizer N recovery and optimal level of N fertilization with hairy vetch; and (ii) temporal variation of N and P forms during the vetch and rice cropping season.

3.2. Materials and methods

3.2.1. Study site

The research was carried out in an experimental platform located close to the Rice Research Centre of Ente Nazionale Risi at Nicorvo (45°14'49"N, 8°42'05"E), NW Italy) **(Figure 3.2. 1).**

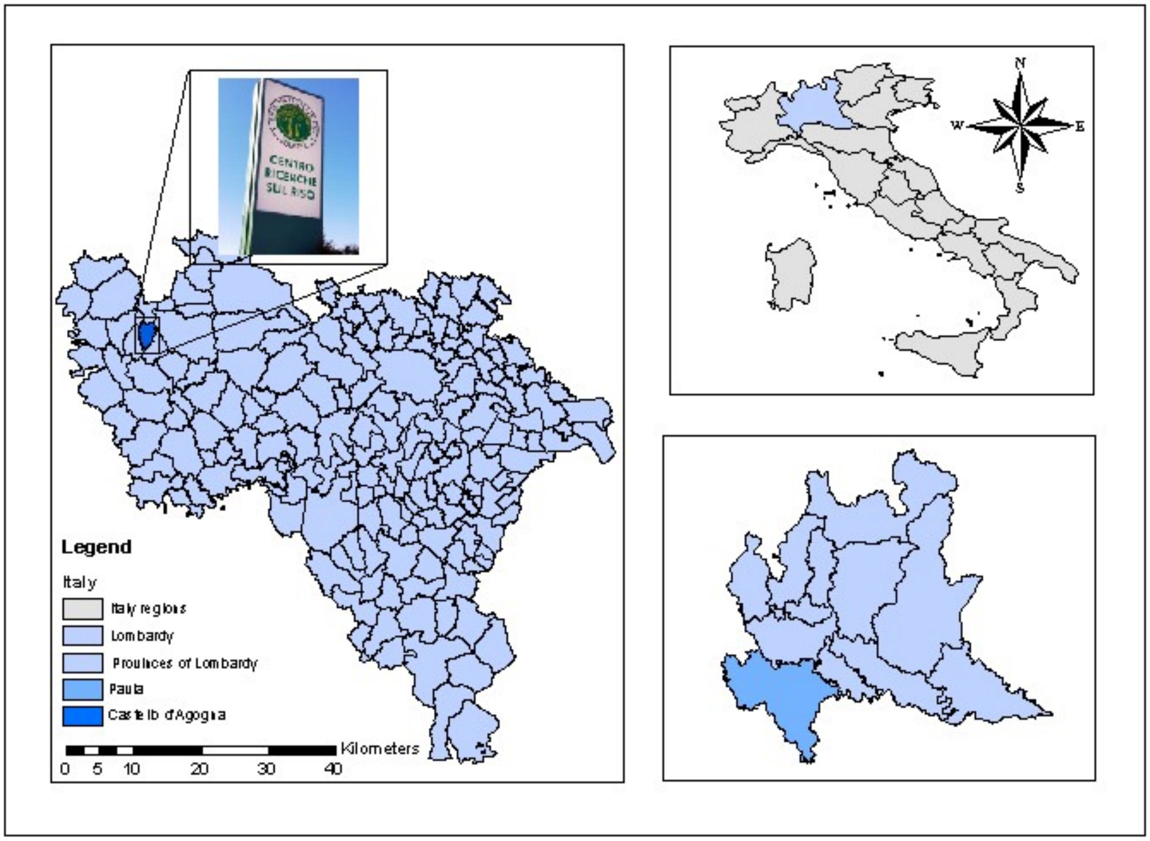


Figure 3.2. 1. Location of the experiment carried out at the Ricerche sul Riso Center, in Castelo D´Agogna, Pavia, Lombardy, Italy.

The site is situated in the low section of the river Po plain, which includes the distal part of the glacial alluvial Würmian flat and is characterized by the presence of bumps of Holocene fluvial dynamics and levelling due to the more recent agricultural processes. The climate is temperate, characterized by hot summers and two main rainy periods in spring and autumn. The mean annual precipitation was 1070 mm, respectively, while the mean value for the last 20 years was 704 mm. The mean annual temperature was 17.7 °C, in line with the 20 years mean (**Figure 3.2. 2**).

The topsoil (0-30 cm) was characterized by a sandy loam texture, with a pH in H_2O (1:2.5 w/v) of 5.0. Average organic C and total N contents were 12.0 and 1.0 g kg^{-1}, respectively, while cation exchange capacity (CEC) was 12.7 cmol$_{(+)}$ kg^{-1}.

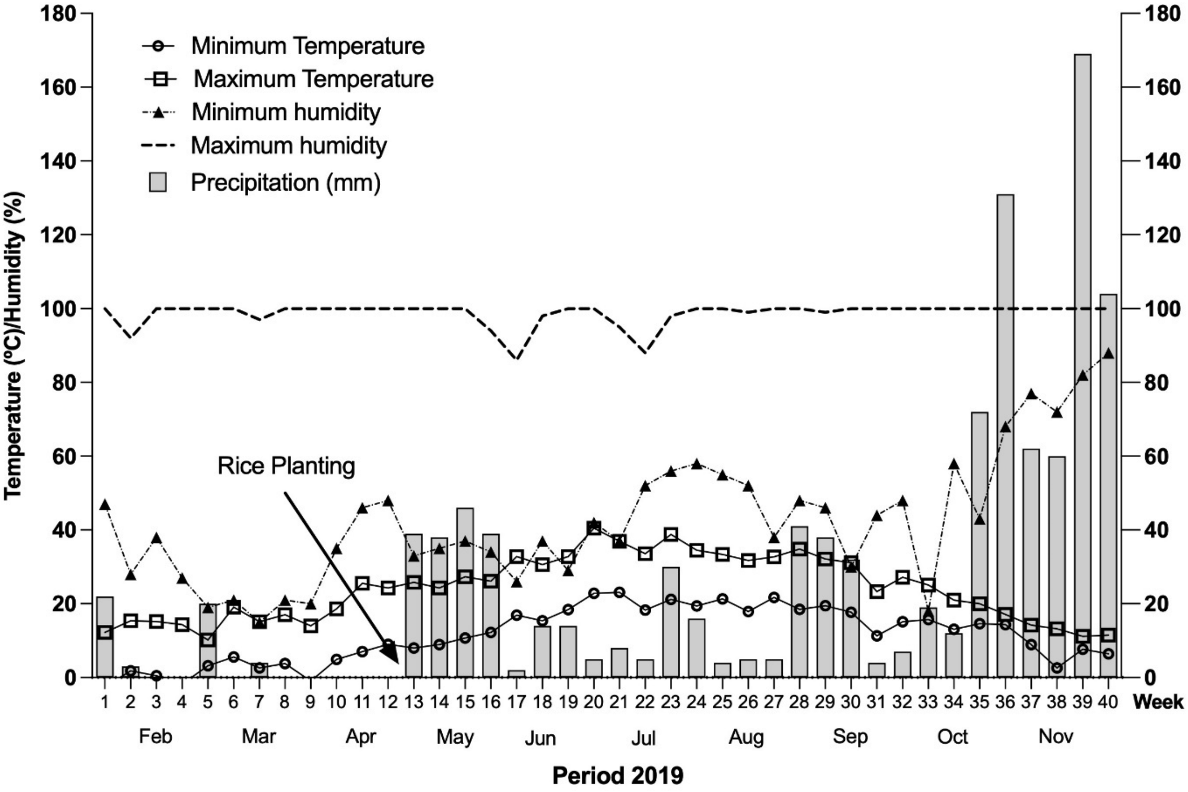


Figure 3.2. 2. Environmental conditions of the study area of the experiment.

3.2.2. Experimental design

The experimental site was divided into eight 20×80 m plots, randomly assigned and the design comprised a split-plot 2×4 factorial arrangement representing: (i) with and without hairy vetch and

(ii) four levels of N fertilization. These agronomic practices have been applied for 10 years, from 2009 to 2019, and the analyses were carried out over the last year (October 2018 – October 2019).

Each October, the species *Vicia villosa* var. *Villano* was sod seeded directly on the remaining rice straw residues at a density of 50 kg ha^{-1}. The plants were let to grow until the beginning of May and then incorporated into soil.

Water seeding was carried out immediately later and then the SOLE CL variety was drilled seeded into dry soil (2-3 cm deep with a 12 cm row spacing) at a seed density of 160 kg ha^{1}. Water management in the plots involved maintenance of dry conditions after dry seeding for approximately one month until tillering stage, towards the second half of June. The plots were subsequently flooded except for a short period in the second half of July for fertilizer and herbicide application. Drainage was allowed at the ripening stage (grain moisture between 26-28%), around 20–30 days before harvest.

For each treatment, four levels of N fertilization (as urea) were tested (0, 80, 120 e 160 kg N ha^{-1}), and split in three times during the cropping season (basal, tillering and panicle differentiation). All fertilizer applications were top-dressed except for the basal fertilization that was incorporated. Phosphorus and potassium were applied before sowing at a dose of 18 kg P ha^{-1} and 70 kg K ha^{-1}. Harvest was carried out between the end of September and the first 15 days of October depending on the year.

3.2.3. Crop yields, yield components and nutrient contents

Crop yields, yield components and N, P and K contents at harvest were determined on rice in the last year. Grain yields were determined by sampling a 25 m^2 area in each plot and expressing results on the basis of a 14% moisture content. Yield components, i.e., number of panicles m^{-2}, spikelets $panicle^{-1}$, sterility and 10^3 grain weight were determined. The panicle density and the harvest index (i.e. the ratio of dry grain yield to dry aboveground plant biomass at harvest), were calculated on three replicated 0.25 m^2 sampling areas within each plot. The tillering rate was calculated as ratio between the panicle density and the number of plants. The number of spikelets per panicle and the percentage sterility were determined on a sample of 20 panicles for each plot, while the weight of 10^3 grains was determined on two replicates per plot.

The head milled rice and the milled rice yield were obtained using a G390/R dehuller (Colombini & Co. Srl, Abbiategrasso, Milano, Italy) and a TM-05 grain testing mill (Satake Engineering Co., Tokyo, Japan), respectively, through a working range of 11.25-12.75%.

The broken kernels were separated by a rice length grader (TRG, Satake Engineering Co., Tokyo, Japan) with the appropriate size cylinders for each variety. Total N content in the grain and straw was determined by elemental analysis (NA 2500, Carlo Erba Instruments, Milano, Italy) and expressed on a dry weight basis. Apparent N recovery was calculated as the difference between aboveground plant N uptake in fertilized and control plots, normalized to the amount of applied fertilizer N. Total P and K were also determined in the grain and straw after acid digestion and determination by UV-vis colorimetric method (Ohno, 1992) and atomic absorption spectrometry, respectively.

3.2.4. Soil and vetch sampling and analyses

Soil samples were collected from those plots, with and without vetch, that were fertilized with 120 kg N ha^{-1}. Three soil samples were randomly collected and pooled into a composite sample on a monthly basis, from February to September 2019, with a hand corer at two depths (0-20 and 20-30 cm). The composite samples were then immediately sieved at 2 mm and partly used as such for N analyses while an aliquot was air dried for P analyses.

Nitrate and ammonium contents were determined on fresh sieved soil samples collected during the cropping season. Nitrate and ammonium were extracted from soil with 1 M KCl for 1 h and determined spectrophotometrically by the Berthelot method (Crooke & Simpson, 1971) and ion chromatography (AS50, Dionex, California, USA), respectively.

Vetch and rice straw were also collected from three replicated 0.25 m^2 sampling areas only once, just before soil incorporation. The plant roots were carefully separated from soil, washed with deionized water until soil particle free and then roots and shoots separated and air dried. Fresh and air-dry biomass was determined as well as C, N and P contents as described above.

Potential Mineralizable Nitrogen (PMN) was also measured in soil in April 2019 before vetch incorporation to estimate the effect of vetch on potential availability of residues derived organic N for the next rice crop. Soil samples were collected from the 0-30 cm layers in the plots with and without vetch. The soils with and without vetch were added with i) hairy vetch and rice straw and ii) only straw, respectively, with amounts comparable to those estimated in the field, and then incubated under both oxic and anaerobic conditions for 100 days at a temperature of 25 °C (modified by Sahrawat & Ponnamperuma, 1978; Waring & Brenmer, 1964). The soils under oxic conditions were maintained at 50% of moisture. The kinetics of organic N net mineralization were assessed by determination of available inorganic N at different times.

On air dried soils, bicarbonate extractable P (P_{Olsen}), assumed to represent readily bioavailable phosphate, was determined according to Olsen *et al*. (1954). An extraction with 0.1 M NaOH + 1 M NaCl (1:1) (P_{NaOH}) was also performed to determine labile P adsorbed on mineral surfaces, precipitated as Fe and Al phosphates (Compton & Cole, 1998), and P associated to humic substances. The phosphate content was determined in both extracts by molybdate colorimetry (Murphy & Riley 1962). SPAD analyzes (Soil Plant Analysis Development) were also performed using a chlorophyllometer.

3.2.5. Statical analysis

The applied statistical model was a two-way ANOVA accounting for vetch presence and N fertilization levels, and their two-way interactions. When F test was significant ($p < 0.05$), the means were compared using Bonferroni test. Statistical analysis was performed using SPSS version 26.

3.3. Results

3.3.1. Rice yield and yield components

The introduction of vetch strongly affected grain yield ($p < 0.05$), with more enhanced effects at low N fertilization levels (**Table 3.3. 1**) ($p < 0.01$). Total and straw biomass increased in the presence of vetch as well ($p < 0.01$ and $p < 0.05$, respectively), following fertilization levels with significant differences at all levels ($p < 0.05$ and $p < 0.01$ for total and straw biomass, respectively). The harvest index was hence lower in the presence of vetch at the highest N levels, with significant interaction cover crop x fertilization ($p < 0.05$).

Table 3.3. 1 Performance of the cover crop management (+Vetch or -Vetch) alone or in interaction with the four levels of N fertilization in terms of grain yield, total and straw biomass, and harvest index, and significance of the different analyzed effects.

	N Fertilization level	Cover crop management	Grain yield *($t\ ha^{-1}$)*	Total biomass *($t\ ha^{-1}$)*	Straw *($t\ ha^{-1}$)*	Harvest Index *(%)*
Average		+ Vetch	8.5 a	17.2a	8.7a	50a
		- Vetch	7.6 b	15.1b	7.6b	50a
	0		6.5c	12.8c	6.3c	51a
	80		8.3b	16.4b	8.2b	50ab
	120		8.8a	17.7a	9.0a	49ab
	160		8.7a	17.8a	9.1a	49b
	0	+ Vetch	7.6b	14.5c	6.9e	52a
		- Vetch	5.5c	11.0d	5.5f	50ab
	80	+ Vetch	8.8a	17.4ab	8.7bc	50ab
		- Vetch	7.8b	15.5c	7.7de	50ab
	120	+ Vetch	8.9a	18.4a	9.5ab	48bc
		- Vetch	8.6a	17.1b	8.5cd	50ab
	160	+ Vetch	8.9a	18.6a	9.7a	48c
		- Vetch	8.5a	17.0b	8.5cd	50abc

Sources	P(F) values			
cover crop manag.	0.050	0.010	0.050	ns
Fertilization level	0.010	0.050	0.010	ns
cover crop. × fertlization	0.010	ns	ns	0.050

Within a column for each cover crop management or for each fertilization level, means followed by different letters are significantly different according to Bonferroni's post hoc test.

The presence of vetch also influenced yield components (**Table 3.3. 2**). The different grain yields were related to the number of spikelets on average, which showed higher values in the presence of vetch ($p < 0.05$), with no significant differences due to fertilization levels. An increase in the 10^3 grain weight was also observed with vetch ($p < 0.05$), whereas a progressive decrease was caused by increasing N levels ($p < 0.01$). Conversely, panicle density did not show any effect determined by vetch, but increasing values from 426 to 562 panicles m^{-2} were due to the different levels of N fertilization ($p < 0.01$). This was reflected on tillering rate with no differences with or without vetch, but increasing values with N fertilization levels ($p < 0.01$). On the other hand, increasing values of applied N caused a higher sterility reaching 19.5% in the highest fertilized plot ($p < 0.01$).

Conversely to grain yield, the derived milled rice yield was not affected by vetch and N fertilization level, except for the control plots (0 kg N ha^{-1}, $p < 0.05$) (**Table 3.3. 2**). Vetch did not affect either the quality of rice in terms of damaged kernels and chalkiness. Notwithstanding the low observed variability, the highest percentage of damaged kernels was observed at the lowest N fertilization levels ($p < 0.05$), whereas the highest percentage of chalkiness was at the highest N fertilization level, although not statistically significant.

Table 3.3. 2. Performance of the cover crop management (+Vetch or -Vetch) alone or in interaction with the four levels of N fertilization in terms of yield components (panicle density, spikelets, 10^3 seeds weight and sterility) and tillering rate and significance of thedifferent analysed effects.

	N Fertilization level	Cover crop management	Spikelets *(panicle^{-1})*	10^3 seeds weight *(g)*	Panicle density *(m^{-2})*	Sterility *(%)*	Tillering rate	Milled rice yield *(%)*	Damaged kernels *(%)*	Chalkiness *(%)*
Average		+ Vetch	142a	25.1a	509a	16.0a	2.3a	71.4a	0.48a	1.3a
		- Vetch	129b	24.3b	490a	14.2a	1.9a	71.5a	0.54a	1.0a
	0		134a	25.5a	426c	10.1c	1.7c	71.6a	0.61ab	0.9b
	80		132a	24.8b	483b	14.2b	2.0bc	71.5ab	0.63a	1.2ab
	120		133a	24.4c	528ab	16.7b	2.3ab	71.3b	0.42bc	1.2ab
	160		145a	24.1d	562a	19.5a	2.4a	71.3b	0.39c	1.3a
	0	+ Vetch	143ab	25.0bc	432de	11.0d	1.8bc	71.6a	0.69ab	1.1ab
		- Vetch	124b	26.0a	420e	9.1d	1.5c	71.6a	0.53abc	0.7b
	80	+ Vetch	133ab	24.6cd	497bcd	15.7bc	2.1ab	71.5a	0.52abc	1.2ab
		- Vetch	131b	25.0b	469cde	12.7cd	1.9bc	71.5a	0.74a	1.2ab
	120	+ Vetch	141ab	24.0e	530abc	18.6ab	2.4a	71.1b	0.40bc	1.3a
		- Vetch	124b	24.9bc	527abc	14.7c	2.2ab	71.5a	0.41bc	1.1ab
	160	+ Vetch	151a	23.8e	579a	19.0ab	2.6a	71.3ab	0.31c	1.5a
		- Vetch	138ab	24.4d	545ab	20.0a	2.2ab	71.3ab	0.47abc	1.1ab
		Sources	*P*(F) values							
		Cover crop manag.	0.050	0.050	ns	ns	ns	ns	ns	ns
		Fertilization level	ns	0.010	0.010	0.010	0.010	0.050	0.050	ns
		Cover crop. × fertlization	ns	ns	ns	ns	ns	ns	ns	ns

Within a column for each cover management or for each fertilization level, means followed by different letters are significantly different acordeing to Bonferroni’s post hoc test.

3.3.2 Rice nutrient uptake and N apparent recovery

Grain N content was significantly affected by the presence of vetch ($p < 0.01$) as well as by the level of fertilization ($p < 0.01$) with increasing values up to 120 kg N ha^{-1} (**Table 3.3. 3**). However, significant differences between the plots with and without vetch were observed even at the highest N fertilization level. In general straw N content followed the same trend with significant differences due to cover crop management ($p < 0.05$) and N fertilization levels ($p < 0.01$), although slighter differences were evidenced at 0 or 80 kg N ha^{-1}. In terms of total N uptake higher values were always obtained with vetch, being on average 45% more than the plots without vetch ($p < 0.01$) at all N fertilization levels ($p < 0.01$). Nevertheless, the apparent fertilizer N recovery was not affected by treatments and, although the values were in general higher with vetch than without, the differences were not statistically different.

Table 3.3. 3. Performance of the cover crop management (+Vetch or -Vetch) alone or in interaction with the four levels of N fertilization in terms of grain N, straw N, total N uptake in fertilized and control plots, and apparent N recovery, and significance of the different analyzed effects.

N Fertilization level	Cover crop management	Grain N *(%)*	Straw N *(%)*	Total N uptake *(kg ha^{-1})*	Apparent N recovery *(%)*	Grain P *(%)*	Straw P *(%)*	Grain K *(%)*	Straw K *(%)*
Average	+ Vetch	1.40a	0.84a	195.0a	67.3a	0.26a	0.16a	0.28a	2,21a
	- Vetch	1.21b	0.74b	149.6b	62.0a	0.28a	0.16a	0.27a	1,69b
0		1.11c	0.65c	114.1c	-	0.24c	0,13b	0.23c	1.77d
80		1.26b	0.75bc	166.4b	63.3a	0,27b	0,14b	0.28b	1.87c
120		1.40a	0.85ab	199.5a	71.2a	0,29a	0,17a	0.30a	2.04b
160		1.45a	0.92a	209.3a	59.5a	0,27b	0,18a	0.28b	2.11a
0	+ Vetch	1.17cd	0.66c	134.3c	-	0,27ab	0,13bc	0.28ab	1.97b
	- Vetch	1.05d	0.65c	93.9d	-	0,21b	0,14bc	0.18b	1.58c
80	+ Vetch	1.38b	0.80abc	190.0b	65.7a	0,28a	0,15b	0.28ab	2.12b
	- Vetch	1.13d	0.70c	142.7c	60.9a	0,28a	0,14bc	0.30a	1.63c
120	+ Vetch	1.52a	0.96a	226.7a	77.1a	0,29a	0,19a	0.30a	2.33a
	- Vetch	1.27bc	0.74bc	172.3b	65.3a	0,30a	0,16b	0.31a	1.76b
160	+ Vetch	1.53a	0.96a	228.8a	59.1a	0,29a	0,18ab	0.28ab	2.42a
	- Vetch	1.36b	0.88ab	189.7b	59.9a	0,26bc	0,18ab	0.27ab	1.81b
	Sources			*P*(F) values					
	cover crop manag.	0.010	0.050	0.010	ns	ns	ns	ns	0.010
	Fertilization level	0.010	0.010	0.010	ns	0.050	0.050	0.050	0.050
	cover crop. × fertilization	ns	ns	ns	ns	ns	ns	ns	ns

Within a column for each cover crop management or for each fertilization level, means followed by different letters are significantly different according to Bonferroni's post hoc test.

The highest differences were recorded in the second phase of the crop cycle. The P and K content was measured only on grain and straw of the plots treated with and without vetch and fertilized with 120 kg N ha^{-1} and no differences occurred (**Table 3.3. 3**).

The different N uptake was confirmed by chlorophyll measurements in leaves during the growing season (**Table 3.3. 4** and **Figure 3.3. 1**), which values were increasingly higher with vetch than without starting from tillering to harvesting with a progressively more enhanced effect of fertilization level. The interaction cover crop management x N fertilization level was also significant at DAS 42 (p <0.05).

Table 3.3. 4. Chlorophyll content (SPAD) in the rice leaves at different time during the cropping season as affected by cover crop management (+Vetch or -Vetch) alone or in interaction with the four levels of N fertilization, and significance of the different analyzed effects.

	N Fertilization level	Cover crop management	31	42	55	67	81	94	109
Average		+ Vetch	38.60a	42.99a	37.11a	34.5a	38.0a	40.2a	39.20a
		- Vetch	35.00b	41.99a	36.03a	31.8b	35.3b	37.5b	36.10b
	0		36.40a	36.03c	32.05d	30.90c	32.10d	34.7c	33.30d
	80		37.10a	43.63b	35.71c	32.80b	35.70c	38.4b	36.60c
	120		36.90a	45.03a	38.39b	33.90b	38.10b	40.6a	39.20b
	160		36.90a	45.29a	40.11a	35.10a	40.60a	41.6a	41.50a
	0	+ Vetch	38.20a	37.40a	32.88a	32.20a	33.70a	36.10a	34.30a
		- Vetch	34.70b	34.65b	31.23a	29.60b	30.60b	33.30b	32.20b
	80	+ Vetch	39.10a	44.43a	36.50a	34.60a	36.80a	39.70a	38.10a
		- Vetch	35.10b	42.83b	34.93b	31.00b	34.70b	37.10b	35.10b
	120	+ Vetch	38.70a	45.00a	38.70a	35.40a	39.50a	42.10a	40.90a
		- Vetch	35.20b	45.05a	38.08a	32.50b	36.70b	39.10b	37.50b
	160	+ Vetch	38.60a	45.13a	40.35a	36.00a	42.10a	42.80a	43.50a
		- Vetch	35.20b	45.45a	39.88a	34.30b	39.20b	40.40b	39.50b
		Sources	*P*(F) values						
		cover crop manag.	0.050	ns	ns	0.010	0.010	0.010	0.010
		Fertilization level	ns	0.010	0.010	0.010	0.010	0.010	0.010
		cover crop. × fertilization	ns	0.050	ns	ns	ns	ns	ns

Within a column for each cover crop management or for each fertilization level, means followed by different letters are significantly different according to Bonferroni's post hoc test.

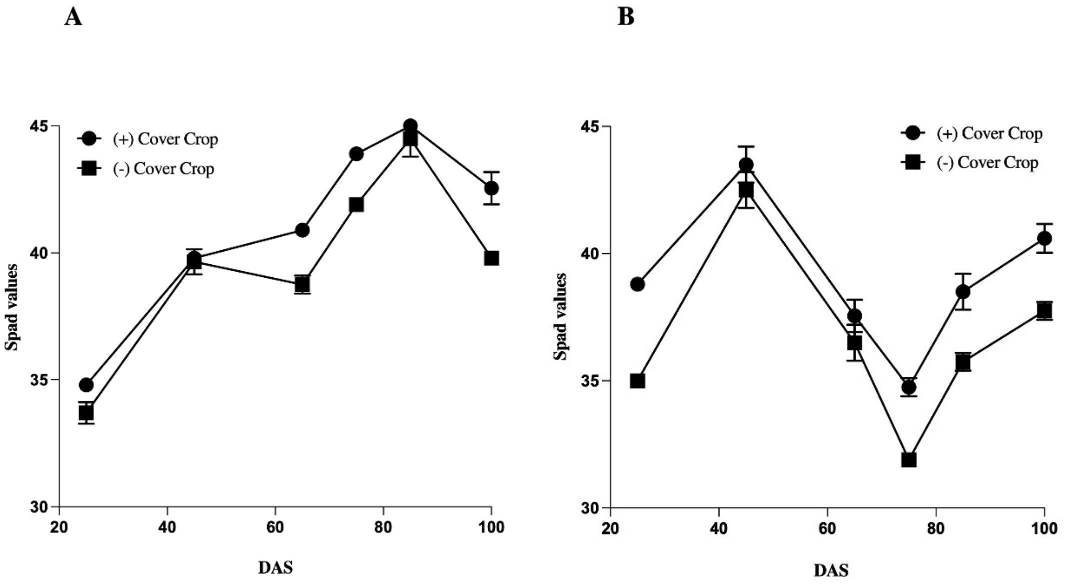


Figure 3.3. 1 Measurement of spad values in rice. **A)** In the presence (+ Cover crop) or absence (- cover crop) in submerged (100 % Water-filled pore space - WFPS); and **B)** dry conditions (50 % WFPS), for the period 2018-2019.

3.3.3 Vetch biomass and nutrient uptake

Vetch total biomass increased from 0.6 to 1.5 g DW kg^{-1} before paddy ploughing, corresponding to a final value of 3.2 kg DW m^{-2} (**Figure 3.3. 2**). This increase was mostly related to shoot development leading to double the shoot:root ratio after 3 months. Both N and P concentration decreased in shoot and roots with growth, while C increased. N reached 36 and 42 mg g^{-1} in shoot and root, respectively, corresponding to 11.4 g N m^{-2}. Phosphorus reached 2.1 and 1.3 mg g^{-1} in shoot and root, respectively, corresponding to 0.578 g P m^{-2}.

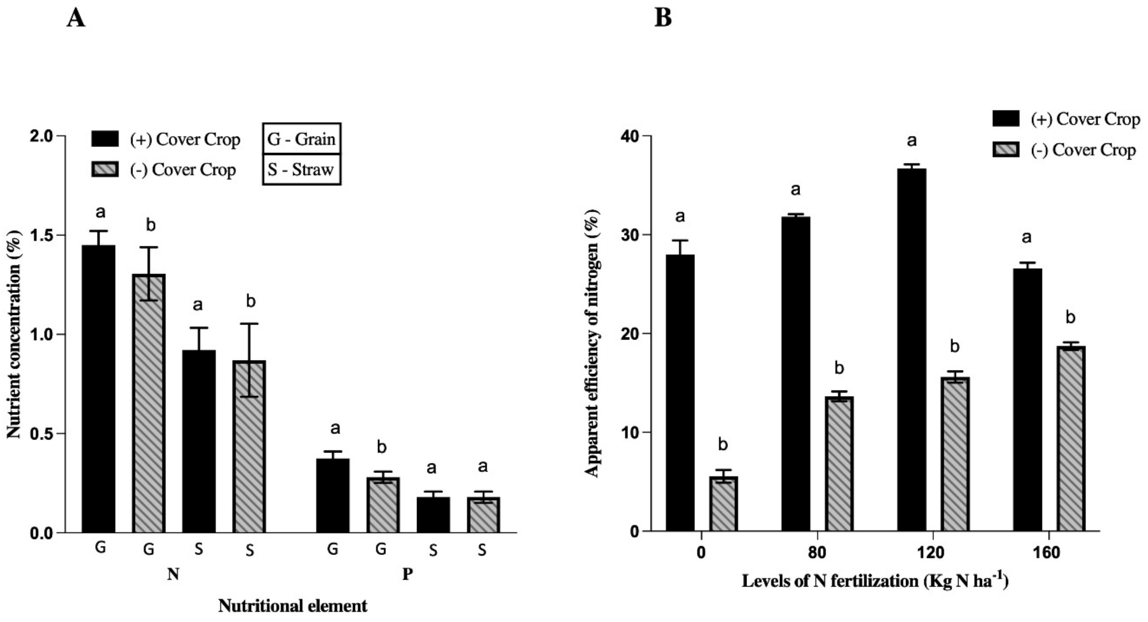


Figure 3.3. 2 Efficiency in the absorption of macronutrients in rice: **A)** Concentration of nitrogen (%), phosphorus (%) and potassium (%) in grain and rice straw in 2019, under the effect of the presence and absence of the cover crop (*Vicia villosa*); **B)** Apparent efficiency of nitrogen added with Cover crop (*Vicia villosa*) for the different levels of inorganic fertilization considered.

3.3.4 Soil nitrogen and phosphorus forms and temporal dynamics

Cover crops affected soil nutrient availability. Extractable nitrate concentrations were relatively low with values <1 mg kg^{-1} soil until vetch incorporation (**Figure 3.3. 3**). Afterwards a pick to 13 and 7 mg kg^{-1} was recorded in soil with and without vetch, respectively, corresponding to the first fertilization and then the values decreased again, around 1-2 mg kg^{-1}. Ammonium showed slightly higher values during the whole crop season with a pick at corresponding to the tillering stage. Although in this period NH_4^+ concentration was higher with vetch than without, in general the values were not different between the two treatments.

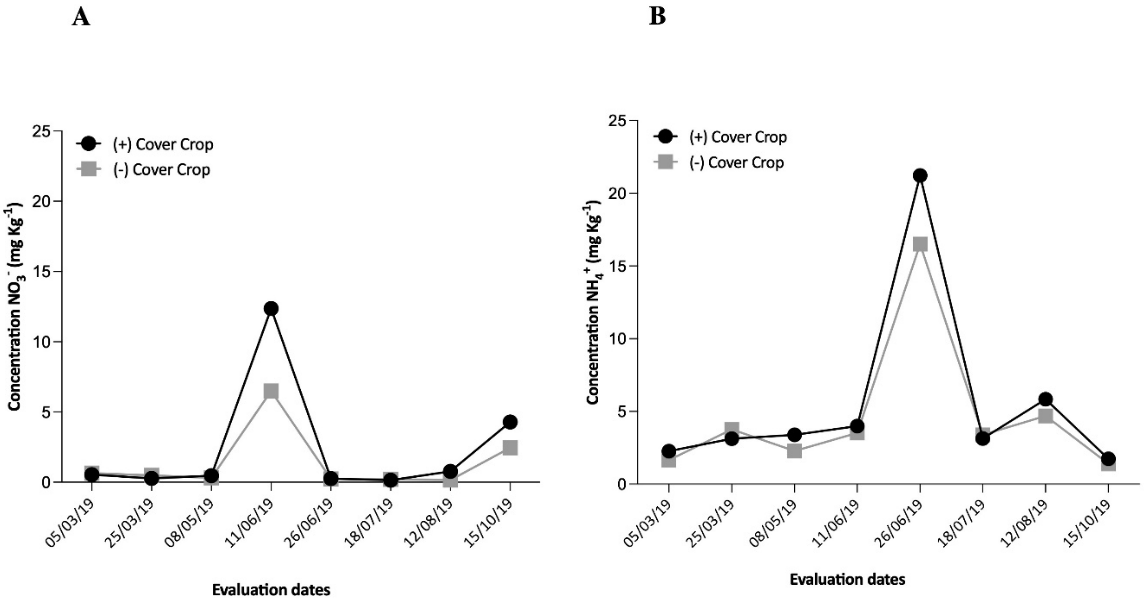
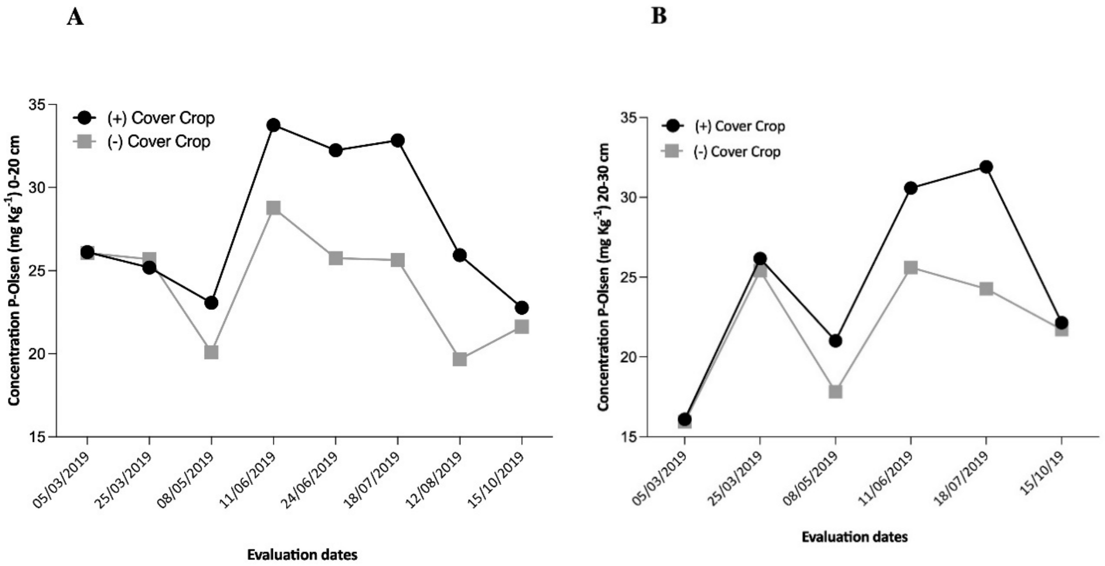


Figure 3.3. 3. A) Distribution of the concentration of NO_3^- and **B)** NH_4^+ from 0-20 cm depth at different evaluation times.

Phosphorus was instead more affected by cover crop management. Both readily available and moderately available forms were higher with vetch than without with a strong increase corresponding to the tillering stage. The values thereafter progressively decreased maintaining, however, strong differences between the two treatments (**Figure 3.3. 4**).



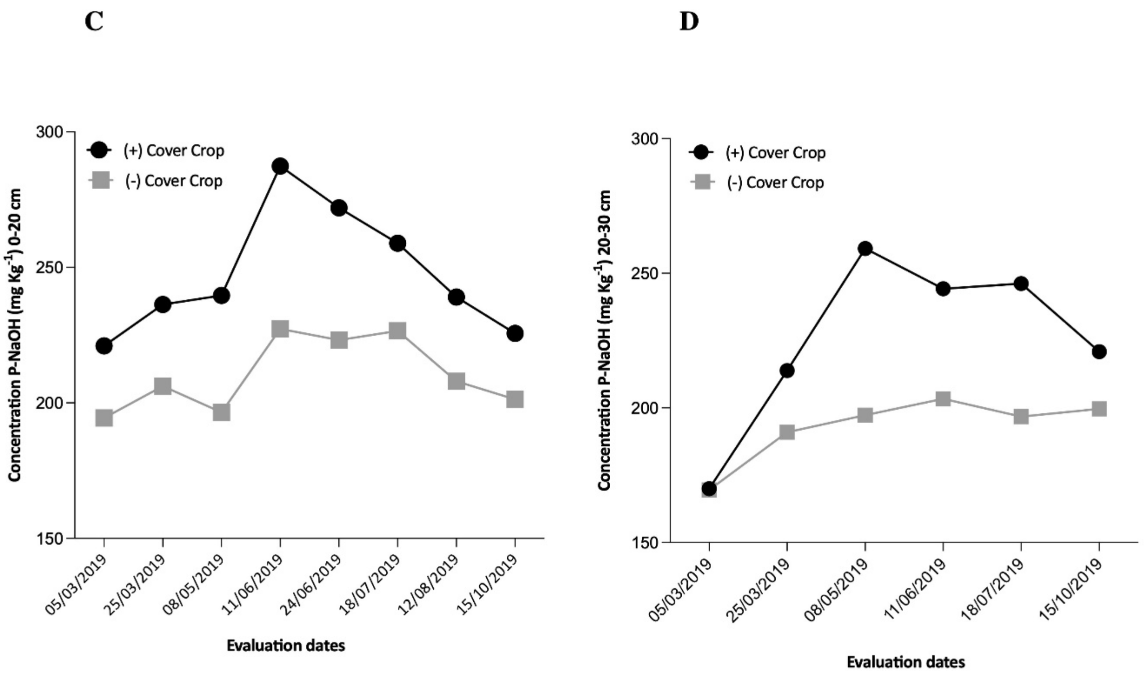


Figure 3.3. 4. A) Distribution of the concentration of Phosphorus-Olsen in the depths of 0-20; **B)** 20-30 cm at different evaluation times; **C)** Distribution of the concentration of Phosphorus-NaOH in the depths of 0-20 and **D)** 20-30 cm at different evaluation times.

To better evaluate the capacity of cover crops to increase nutrient availability during the cropping season a parallel mesocosm experiment was carried out to determine the pool of N that can be potentially mineralizable, while assessing the N release from decomposition of both vetch and rice straw as affected by anaerobic vs aerobic conditions (**Figure 3.3. 5**). Nitrogen input to soil due to straw residues and vetch was 52 and 114 kg N ha^{-1}, respectively, for a total of 166 kg N ha^{-1}. After their incorporation, the C:N ratio was 30.4 in the presence of straw and decreased to 24.2 when vetch was added. Under anaerobic conditions, a faster and more intense net N mineralization was determined by vetch, with greater values of potentially mineralizable N even in the last stages of the growing season, reaching 234 ± 21 kg N ha^{-1} with respect to 176 ± 24 kg N ha^{-1} without vetch. This was related to the prompt production of ammonium accompanied by a rapid disappearance of nitrate forms.

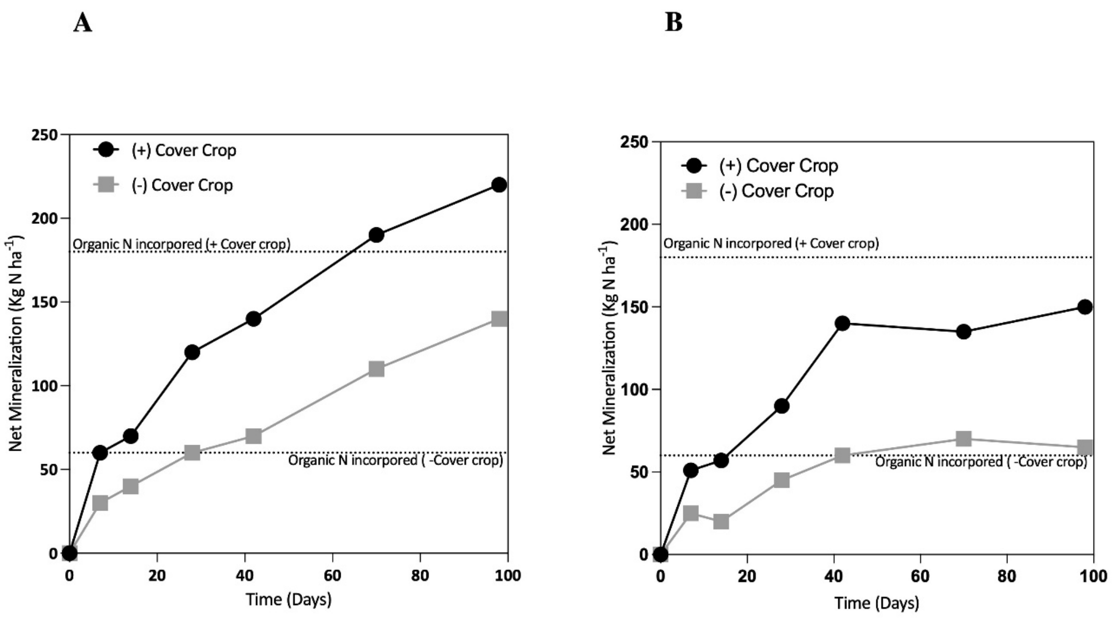


Figure 3.3. 5. Kinetics of net mineralization of organic nitrogen. **A)** In the presence (+ Cover crop) or absence (- cover crop) of *Vicia villosa* biomass in submerged (100 % Water-filled pore space - WFPS); **B)** or dry conditions (50 % WFPS).

By comparison, under aerobic conditions, net mineralization was slower, mainly due a lag time before formation of nitrate that was the only mineral N form present in the soil system (**Figure 3.3. 6**). The net mineralization increased with time as well, reaching, however, values lower than under anaerobic conditions, being 153 ± 12 and 84 ± 6 kg N ha^{-1} with and without vetch, respectively.

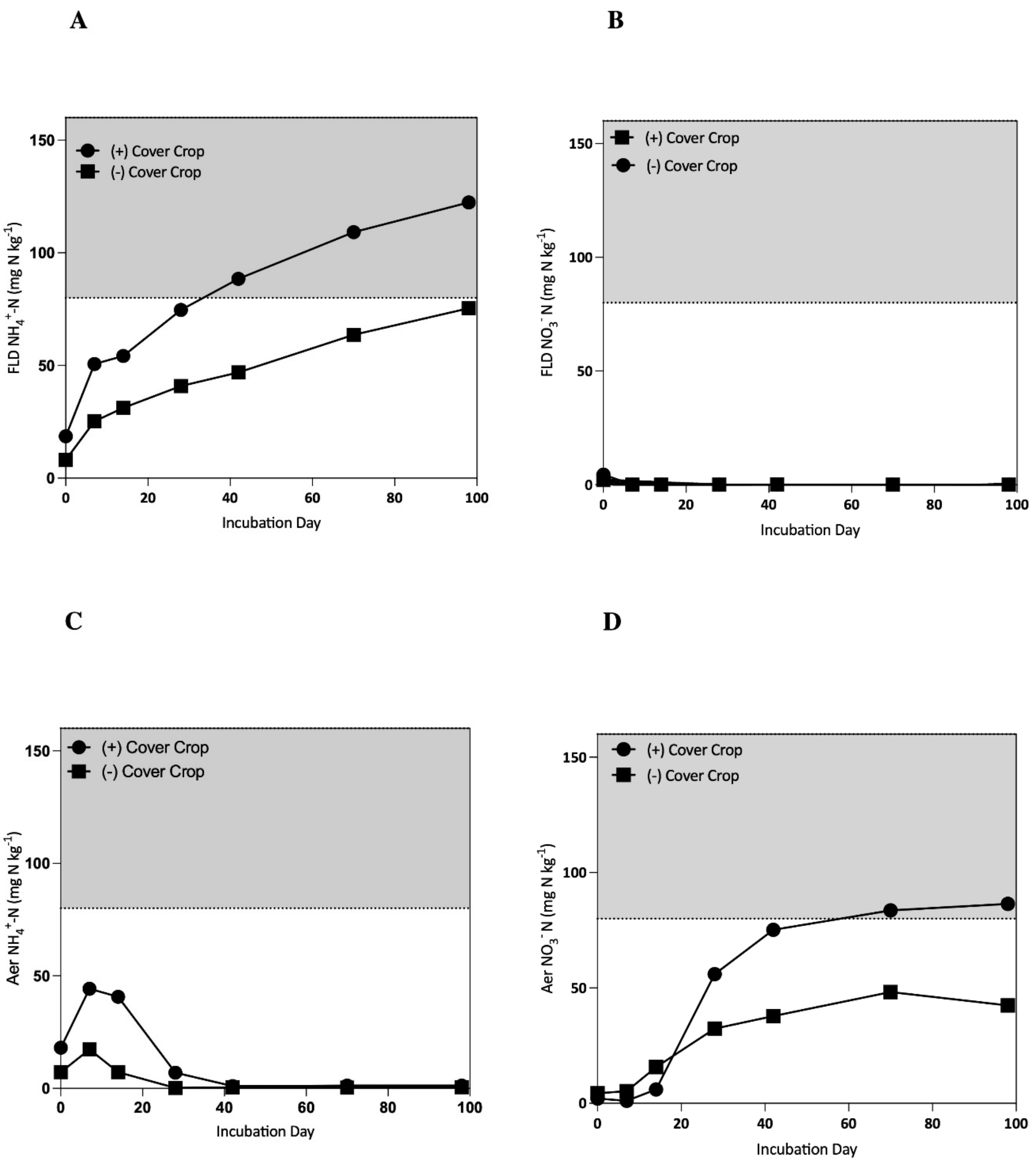


Figure 3.3. 6. Potentially mineralizable N (PMN) in rice soil. FLD conditions for NH_4^+ **(A)** and NO_3^- **(B)** and under aerobic conditions for NH_4^+ **(C)** and NO_3^- **(D).**

3.3.5. Concentration of P and N in Cover crop

In relation with the nutrient concentration, it can be said that for P there was a decrease in the shoot with the different sampling times, presenting significant differences ($p < 0.05$) reaching the pre-flowering and flowering stages (**Figure 3.3.** 7). For the root, the opposite occurred, since there was a significant increase in the concentration of P, which may be related to the increase in nodules for the nitrogen fixation process. In the case of N concentration, both in the shoot and in the root, there was a decrease, presenting at the end 3.01 % in the shoot and 2.3 % for the root, which could have occurred due to the fact that in flowering there is a translocation Nitrogen to meet the requirement of this stage for fertilization and previous fruit formation.

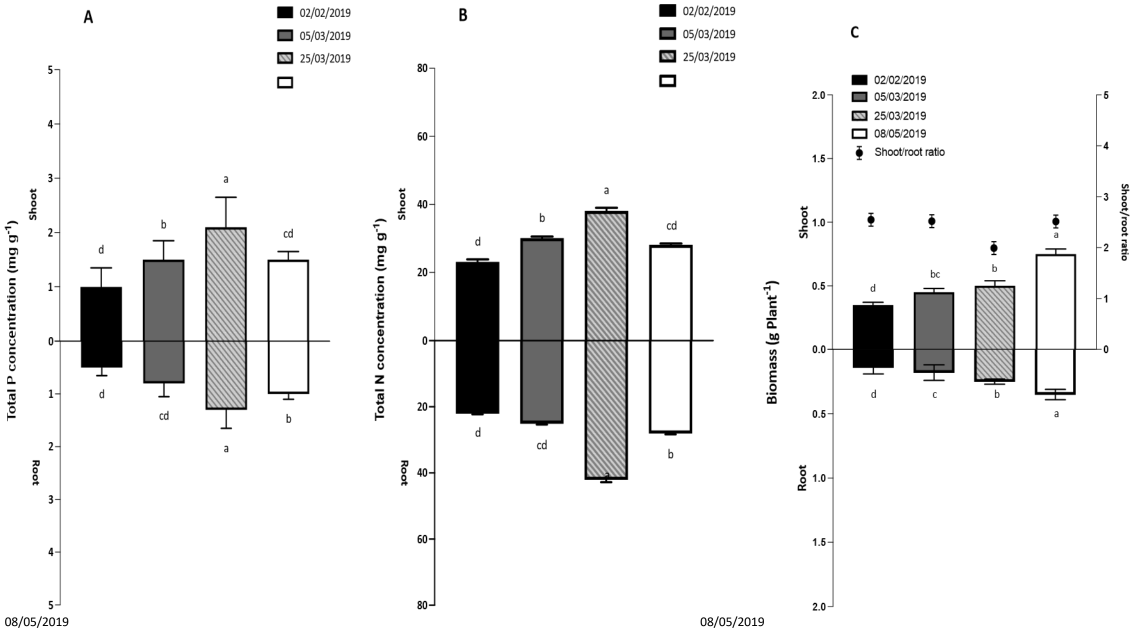


Figure 3.3. 7. Concentration of P (A), N (B) and Biomass (**C**) in Cover crop (*Vicia villosa*) in Shoot and root, for different sampling times.

3.4. Discussion

3.4.1 Hairy vetch determines rice yield performance as a function of N fertilizer levels

The seeding and cropping of leguminous cover crop during the cold fallow season strongly affected rice productivity. These results were in agreement with a number of reports (Haque *et al.,* 2013; Zhu *et al*., 2014; Xia *et al*., 2018), who found that cover crops significantly stimulate rice growth and plant yield. Interestingly, the benefit of cover crop was more evident at low N fertilization levels with a cover crop x fertilizer interaction. With 80 kg N ha^{-1}, rice crop associated to vetch reached indeed a great performance, being 12% higher than in the plot without vetch and similar to that obtained with 120 and 160g N ha^{-1}. Similarly, Yang *et al.* (2019) found that the incorporation of green manure improved early and late rice yields in northern China allowing a reduction of 20 % of the recommended amount of chemical fertilizer.

Apart from a higher grain productivity, the plots with vetch showed also higher yields of straw biomass, producing on average 1.1 t ha^{-1} more than without vetch, with significant differences at all N levels. The highest straw biomass was reached combining vetch with a fertilization of 120 kg N ha^{-1}, probably due to a greater allocation of N in the grain, requiring higher N input for maintaining adequate availability to build the straw N budget (Chen *et al.*, 2020; Guopeng *et al.*, 2020).

Within the components of rice yield the only factors affected by vetch were the number of panicles and, to a lower extent, the weight of 1000 seeds as found by Kaewpradit *et al.* (2009), Yang *et al.* (2019) and Nie *et al.* (2019) following different combinations of legume residues and grasses. This implies that the increase of rice grain yield determined by vetch was a result of more vigorous growth of rice plants mainly at or before tillering stage (Shi *et al.*, 2021). Cover crop, rather than N fertilization levels, seemed indeed favouring the first stages of rice growth, as confirmed by the higher chlorophyll content observed after 31 DAS. However, at mid tillering the positive effect of vetch on SPAD values was negligible, and at panicle initiation, when rice growth rate increased, the effect was reversed with greater differences due to N fertilization levels. Thereafter, vetch incorporation combined with N levels again affected chlorophyll content, probably suggesting the positive effect of the legume residues on maintaining an appropriate soil N availability even at the late growth stages. The lower values observed at the reproductive stage in both treatments, with and without vetch, could be attributed to a higher extraction of N from leaves for filling the grain (Shi *et al.*, 2019).

In general, the higher SPAD values were mirrored by a greater total N uptake with vetch at all N fertilizer levels, indicating that under integrative techniques, increased yield sink capacity and N use efficiency were closely related to improvements in the physiological characteristics of rice, such as increases in the percentage of productive tillers and leaf photosynthetic rate (Zhang *et al.*, 2018).

Vetch did not significantly affect grain sterility, which was instead strongly influenced by N fertilization levels (Hirzel & Rodriguez, 2017), increasing consistently from 10.1 up to 19.5%. This suggests that vetch could indirectly prevent the disease, by reducing the amount of chemical fertilizer required for obtaining optimal productivity performances. Combining vetch with 80 kg N ha^{-1}, grain sterility was indeed reduced by 15 and 27%, with respect to 120 and 160 kg N ha^{-1}, respectively. This, combined with a reduced percentage of chalkiness, may guarantee a higher grain quality while maintaining a sustainable productivity.

3.4.2 Hairy vetch increases N and P availability

The positive abovementioned results were first related to vetch biomass input. In fact, hairy vetch showed a great capacity to accumulate aerial biomass and N (Campiglia *et al.*, 2010), corresponding to 150 kg ha^{-1} biomass and 166 kg N ha^{-1}. Aside from increasing the total soil N pool, vetch biomass appeared to rapidly release NH_4^+ forms in soil, promptly available in the first vegetative phases of rice, even encompassing the effect of N fertilizer. Net N mineralization measured in microcosms reached indeed in 40 days values comparable to the input derived from the highest fertilization level (160 kg N ha^{-1}) under both anaerobic and aerobic conditions. This was related not only to the intrinsic decomposability of vetch, as deduced by its relatively low C:N ratio (Kautsar *et al.*, 2022), but also to the synergic effect of co-decomposing rice straw residues. If rice straw alone, due to its high C:N ratio and consequent slow decomposition , can cause N immobilization (Eagle *et al.*, 2000; Said-Pullicino *et al.*, 2014), when incorporated together with vetch, underwent a positive priming effect (Mortensen *et al.*, 2021; Shi *et al.*, 2019), leading to net N mineralization increase from 66 to 150 kg N ha^{-1}, corresponding to 80% of total N input.

The increasing N availability can explain the greater tillering rate observed in the field and the higher SPAD values found at the vegetative stage in the presence of vetch (Choi *et al.*, 2014). However, more interestingly was the fact that, under anaerobic conditions, the incorporation of vetch continuously fed the N available pool even in the 60-100 days span, favouring net N mineralization beyond the amount of organic N incorporated into soil. This may lead to a continuous source of NH_4^+ during the periods of major needs required by rice, corresponding to flowering and grain filling stage (Lowry *et al.*, 2021; Haque *et al.*, 2013). The benefit of recycling pre-rice available plant residues on N availability was thus evident not only at the early stages of the crop, but also at the late phenological stages, although this was not mirrored by a consequent trend in the soil extractable NH_4^+ observed in the field, constantly low throughout the whole cropping season. One possible reason for this dynamic is that N plant uptake occurring in the field masked the positive effect of vetch observed in controlled microcosms.

Regarding P, although no differences were found in its content in both grain and straw, the total P uptake was significantly affected by the presence of vetch, as a consequence of the greater biomass. This was followed by a higher soil P availability in the plots where hairy vetch was incorporated. We found significant changes in the $NaHCO_3$-Pi content starting from seeding date and reaching values 25% higher with vetch than without at the tillering stage. This P fraction is considered as labile P and its changes are largely influenced by biocycling favoured in the presence of a highly degradable residue, such as vetch (Cropping, 2005; Wang *et al*., 2016), with fast recycling of P immobilized in both vetch and straw biomass.

The fraction of P extracted with NaOH was in general ten times higher than that with $NaHCO_3$. The NaOH-Pi fraction, which represents P that is strongly adsorbed on the Fe and Al (hydr)oxide surfaces in the soil, is generally reported to be the highest in paddy environments (i.e. Xiao *et al*., 2017). The large values found could be related to the alternating redox conditions following water management, which cause reductive dissolution of Fe (hydr)oxides, transport of soluble Fe^{2+}, and redistribution of pedogenetic Fe (hydr)oxides (Hanke *et al*., 2013; Said-Pullicino *et al*., 2016; 2021), facilitating the release of P associated to their surfaces. This was particularly evident between 32 and 93 DAS, during the flooding period, when reducing conditions enhanced Fe(II) formation.

The presence of vetch emphasized P release, since NaOH-P content significantly increased during the whole year. This was evident even in the fallow period, during vetch growth. Vetch can exude organic acids and protons that can compete with phosphate for the same sorption sites (Celi & Barberis, 2005; Celi *et al*., 2020), releasing also sparingly accessible P (Lambers *et al*., 2015; Tarui *et al*., 2013). This can create in the root surrounding, the conditions for making the rice more able to acquire P from weakly available forms (Solangi *et al*., 2019). On the other hand, hairy vetch can favour the formation of microbial niches that can increase P cycling enhancing dissolution of P-bearing salts or indirect P release via Fe reduction (Srivastava & Ngullie, 2009). This could turn in an increase of N_2 biological fixation, a high ATP demanding process strongly controlled by P availability (Jakobsen, 1985), highlithing that hairy vetch can have important effects on nutrient co-regulation.

The increased values of NaOH-Pi found after 32 DAS can be related to the adittional effect related to the presence of easily degradable compounds produced by vetch residues that can either weak the bonding of phospahte with minerals and enhance microbial activity.

CHAPTER 4

Phosphorus availability from different fertilizers in tropical rice agrosystems

4.1. Introduction

In tropical soils, 1: 1 clays and iron and aluminum (hydr)oxides predominance govern P surface adsorption. In rice systems, under anaerobic conditions, the reductive dissolution of Fe oxides and changes in the electrochemical properties of their surfaces with which orthophosphate reacts, strongly affect its solubility and dynamics, resulting in increased P availability. Neverthless, P availability remains low in tropical rice systems and require a higher addition of nutrients via fertilizer (Gomes *et al*., 2020; Teixeira *et al*., 2014), compared to temperate regions.

The selection of the phosphorus source has to do with the efficiency of supplying the right doses needed by crops during their phenological phases and the degree of fertilizer solubility (water, neutral ammonium citrate and citric acid). Other aspects can be considered in the selection of phosphate fertilizers, such as supply of other nutrients (synergisms), pH, granulometry, mechanical resistance, segregation, density, hygroscopicity, saline index and physical compatibility and chemistry in mixtures (Sousa *et al*., 2014; Alvarez *et al*., 2018). With the addition of P fertilizers, there is an accumulation of P in inorganic and organic forms with different degree of binding energy, although the accumulation is more pronounced in inorganic forms (Daroub *et al*., 2000). The redistribution of P in various forms is dependent on the source used (Jin *et al*., 2014; Jiang *et al*., 2014).

After the application of a phosphate fertilizer to the soil, there is a sequence of physicalchemical events that transform this phosphate into a complex phosphate substance, which then govern the availability of this nutrient in the soil. If P is in solution or poorly adsorbed, this is considered to be the labile form, as it is available. However, when adsorption occurs through stronger bonds, this degree of interaction makes P difficult to desorb, feeding the non-labile forms (Ker *et al*., 2016; Daly *et al*., 2015).

The chemical fractionation of soil P is one of the main analyzes used to obtain information about the solubility of the different forms of P in and their availability to plants (Condron *et al*., 1985; Condron & Newman, 2011). Although, the fractionation methods have some limitations related to be operationally defined, complex, time consuming, there is hippermanipulation of samples and the methods do not determine the soil P species, several methods with different modifications have been proposed over the years (Condron & Newman, 2011; Cross & Schlesinger, 1995; Gatiboni *et al*., 2013). Soil chemical fractionation techniques are especially important when comparing the influence of soil management practices on P lability (Cherubin *et al*., 2016; Gatiboni *et al*., 2013; Pavinato *et al*., 2009; Rodrigues *et al*., 2016; Teles *et al*., 2017).

The Hedley *et al*. (1982) method modified by Condron *et al*. (1985) uses, sequentially, reagents with progressively increasing extracting capacity and allows for the separation of P forms according to their nature (organic or inorganic) and sorption strength (Cross & Schlesinger, 1995; Gu & Margenot, 2021). The forms of P extracted by anion exchange resin and by sodium bicarbonate ($NaHCO_3$) are considered labile forms, with greater availability for plants; those extracted with 0.1 mol L^{-1} NaOH, moderately labile forms; and the P fractions extracted by NaOH 0.5 mol L^{-1} (Condron *et al*., 1985), by HCl 1 mol L^{-1} and the residual fraction, non-labile forms.

The contents of P extracted with resins and $NaHCO_3$ represent the dissolved P of the solid phase in equilibrium with the soil solution. The fraction Pi NaOH 0.1 mol L^{-1} represents the moderately labile inorganic P, chemosorbed through monodentate and bidentate bonds to oxides and kaolinite (Hedley *et al*., 1982; Sharpley & Smith, 1985; Costa *et al*., 2016), while the organic fraction obtained by this extractant represents the moderately labile organic P bound to humic acids. The Pi and Po fractions extracted with NaOH 0.5 mol L^{-1} represent the physically protected inorganic and organic P inside microaggregates. The 1 mol L^{-1} HCl fraction represents the inorganic forms of P associated with Ca. The residual fraction represents the P in humic substances, as well as inorganic forms of insoluble P. Total P represents all forms, including structural P (Cross & Schlesinger, 1995; Cui *et al*., 2019).

Another interesting technique to study the dynamics of P in the soil-plant system is the use of P isotopes (Nagy *et al*., 2019; Siegenthaler *et al*., 2020). These techniques, used to study the P cycle in soils for agricultural and forestry use, can help to better determine the distribution and bioavailability of P in various organic and inorganic forms. They can help to understand the components that affect the dissolution of mineral phosphates, the retention of P by inorganic constituents, decomposition of organic P and the efficiency of phosphate fertilizers and its absorption by crops (Chen *et al*., 2019). Numerous studies have been carried out to understand and quantify these processes, and many of these studies involve the use of the two most important isotopes, ^{32}P and ^{33}P (Menzel & Smith, 1984; Helfenstein *et al*., 2018). ^{32}P has been used extensively in soil fertility studies and P cycles since it became available in the early 1900s. The Beta radiation emitted by ^{32}P with its high energy (1.71 MeV) can be easily detected and counted using a liquid scintillation counter (L'Annunziata, 2012). The use of ^{33}P has increased in recent years. Its longer half-life (24.4 days) allows for longer experiments. The beta radiation emitted by ^{33}P has a lower energy level (0.248 MeV) than that of ^{32}P, and is therefore less dangerous for external exposure.

The objective of this work is to determine the dynamics of P in two tropical acid soils added with different P fertilizers and subjected to two water management techniques to evaluate the different availability as affected by redox changes and Fe related dynamics.

4.2. Materials and methods

4.2.1 Experimental setup

The experiment was conducted from October 2019 to January 2020 in a greenhouse at the Nuclear Energy Center for Agriculture of the University of São Paulo (CENA-USP), located Piracicaba country, São Paulo State, Brazil (**Figure 4.2. 1**).

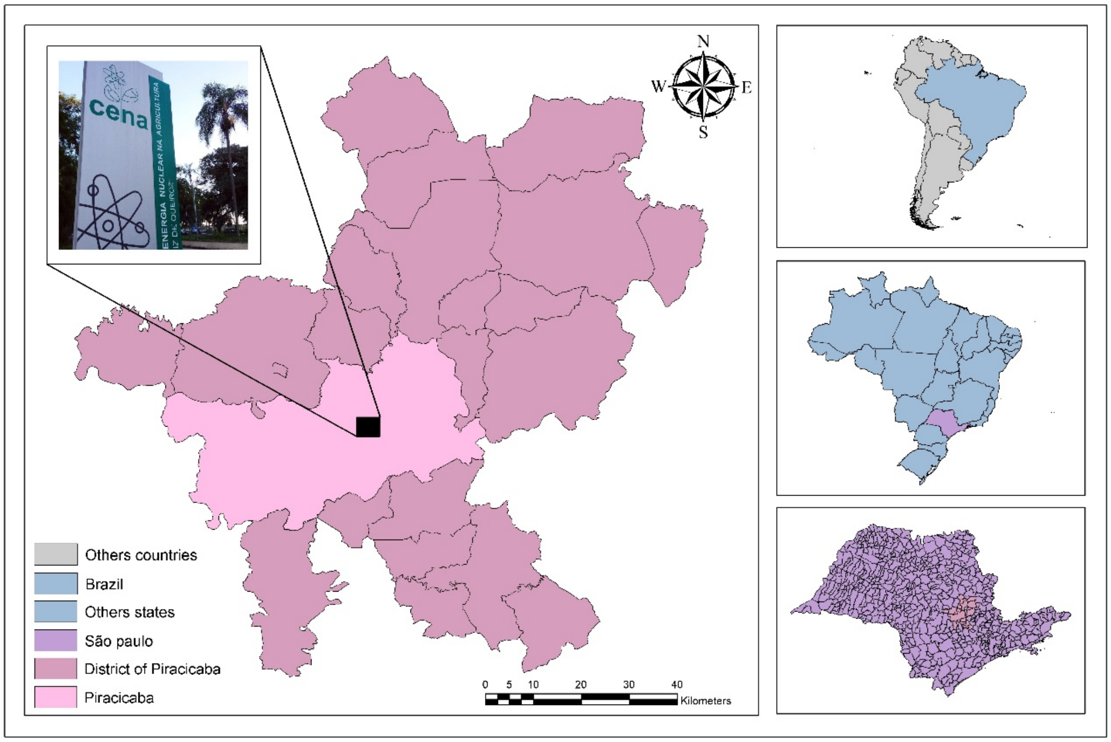


Figure 4.2. 1 Localition of the experiment carried out in São Paulo, Brazil.

Local minimum, mean, and maximum air temperature and relative air humidity were derived from the meteorological station (record from Professor Jesus Marden dos Santos) approximately 300 meters from the greenhouse experimental station for the months of October, November and December of 2019 and January of 2020

Figure 4.2. 2).

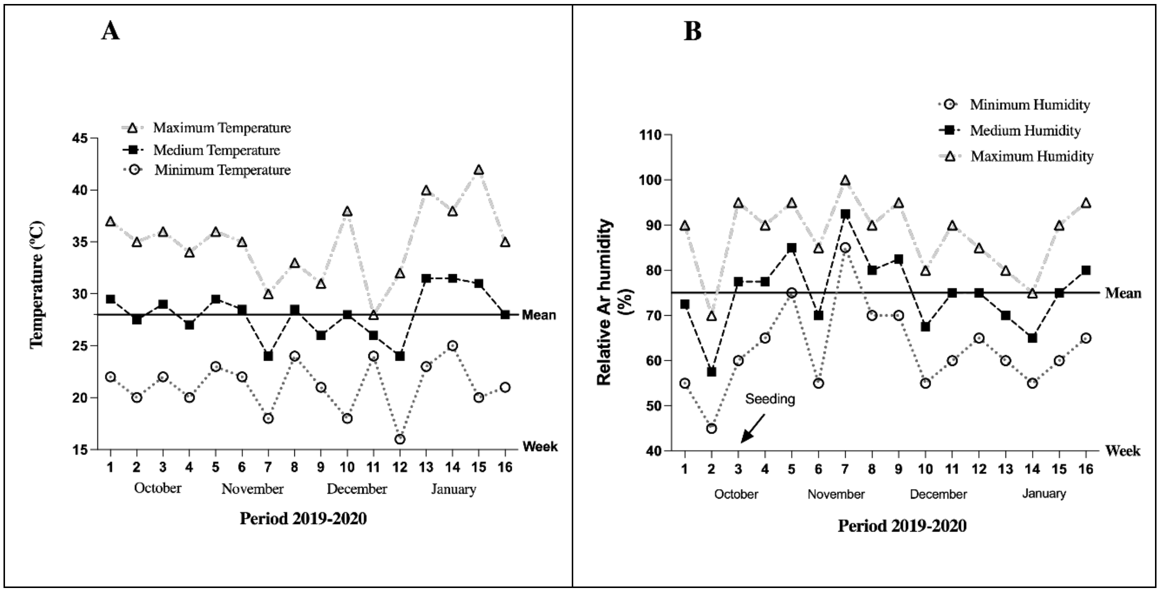


Figure 4.2. 2. Minimum, mean and maximum values for air temperature (a) and relative air humidity (b) Piracicaba country, São Paulo State, Brazil, during the development of the experiment.

An initial trial experiment with 5 kg soil (dry weight) of the superface soil horizon (0-20 cm), with plastic pots line polyethene bags. Soil acidity was corrected with $CaCO_3$ and $MgCO_3$ to achieve a base saturation of 70% (Van Raij *et al.*, 1997) by incubating the soil for 30 days at 70% water-holding capacity (WHC). To quantify accurately the P taken up by the plants from the fertilizers, the isotopic dilution method with ^{32}P (indirect method) was used. For this procedure, soils were uniformly labelled with 1.76 MBq kg^{-1} of carrier-free ^{32}P-orthophosphate (halflife of 14.3 days) by incorporating 20 g of dry sand previously labelled with ^{32}P, and then the soil was thoroughly mixed and incubated for 10 days. The soil moisture was adjusted daily to 70% WHC.

After homogenizing the fertilizers with the soil with a single dose of 200 mg P dm^{-3} of soil. A background fertilization at planting across all treatments was performed with 356 mg of NH_4NO_3, 104 mg of KCl, 11.8 mg of H_3BO_3, 20 mg of $CuSO_4$, and 14.8 mg of $ZnSO_4$. After planting, topdressing fertilization with nitrogen and potassium was carried out, providing 150 mg of each element per pot. Then, the rice cultivar BRS Pampa (irrigated rice) was sown (Seeds from Embrapa - Brazilian Agricultural Research Corporation) using five seeds per pot. Two plants per pot was kept after seedings emergence. The pots were irrigated 2 to 3 times a day with deionized water, maintaining the water level, according to the treatments (field capacity or flooded). At 75 days of cultivation the plants were cut at 1 cm from the soil surface. The determination of the oxidation-reduction processes was carried out by taking samples of three replicates of each of the treatments on days 0, 0,5, 1, 2, 5, 10, 20, 35, 50, 75. Day 0 is defined as the start day of the experiment; Data on day 0 were collected at 12 and 24 h.

4.2.2 Experimetal Design

The experimental was conducted as a completely randomized blocks, in a 4x2x2 factorial arrangement, with four replicates (n= 4) for each experimental unit. The treatmens included four (4) types of phosphate fertilizers as follows: MAP (Monoammonium Phosphate, TSP (Triple Super Phosphate), Struvite and the control (No phosphate fertilizer); two (2) types of soil moisture: field capacity and water-saturated (flooded soil); two (2) acid and highly weathered soils: an Oxisol (Typic Hapludox) from the Piedade region (São Paulo, Brazil), and an Ultisol (Humic Hapudult) soil from the Guarapuava region (Paraná, Brazil), classified according to the USDA.

4.2.3 Plant and soil sampling and analyses

Two soil samples, one Oxisol and one Ultisol, were collected from vergin fields (GU-UC, PD-UC) at a depth of 0 - 20 cm, air-dried, passed through a 4-mm sieve, and stored. After homogenization, subsamples were collected and passed through a 2 mm sieve (air-dried soil sample - ADSS), the Oxisol soil presented the following physicalchemical characteristics: pH of 4.0 (in $CaCl_2$ 10 mmol L1, 1:2.5 soil: solution ratio), a clay and sand content of 58.7 and 41%, respectively, corresponding to a clay agricultural soil. -Acid sandy soil, with an available P content (P-Resin) of 6 mg dm^{-3}, an organic matter content of 5.9% and a cation exchange capacity of 6.5 $cmol_{(+)}$ kg^{-1}. The Ultisol soil presented a pH of 4.1, a clay and sand content of 44 and 60 %, respectively, corresponding to an acid Clay-Sandy Loam agricultural soil, with an available P content (P-Resin) 5 mg dm^{-3}, an organic matter content of 2.5% and a cation exchange capacity of 3.2 $cmol_{(+)}$ kg^{-1}.The sequential soil P fractionation was performed according to the methodology proposed by Hedley *et al*. (1982), modified by Condron *et al*. (1985). which is the most commonly used methodology in tropical soils (Gatiboni *et al*., 2013; Rocha *et al*., 2019; Rodrigues *et al*., 2016; Teles *et al*., 2017).

The determination of soil maximum phosphorus adsorption capacity (MPAC) was estimated values of MPAC were consistent with the PBC of the soil, estimated by clay content and P-rem. The MPAC ranged from 357.1 μg P g^{-1} in the PD-UC (Ultisol) soil to 1890.5 μg P g^{-1} in GU-UC (Oxisol), which are, respectively, lower and higher buffered soil (**Figure 4.2. 3**).

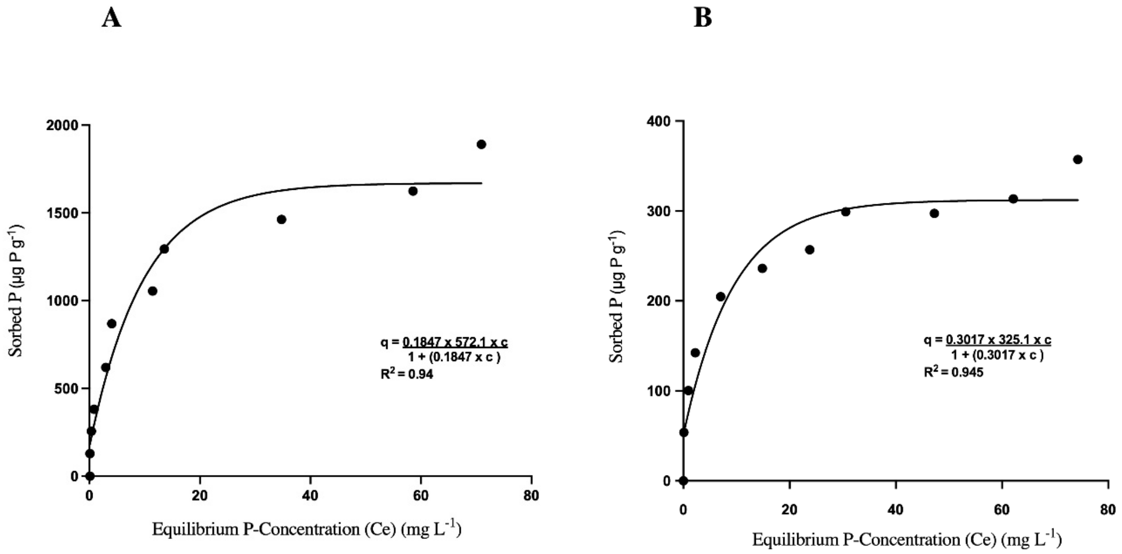


Figure 4.2. 3 Maximum P adsorption capacity of Oxisol (A) and Ultisol (B) by fitting the Langmuir model.

The mineralogical analysis was performed in the clay fraction separated by sedimentation, according to Teixeira *et al.* (2017), (**Figure 4.2. 4**).

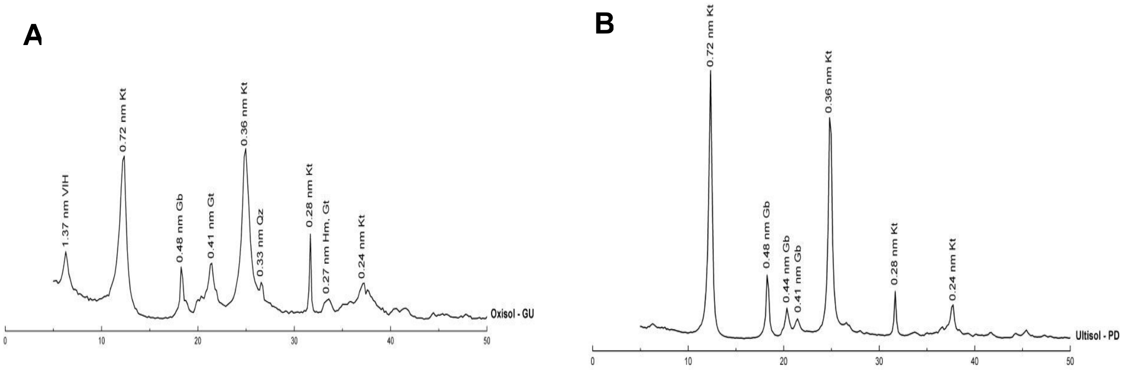


Figure 4.2. 4. X-ray diffractograms of oriented clay fraction of soil samples collected for (**A**) Oxisol – GU and (**B**) Ultisol – PD.

Systematic redox oscillations were imposed on the soil over 75 days in a 1:10 soil: water in suspension. Each experimental unit (vessel) consisted of 4.5 g (dry-weight) of soil suspended in a 2 mM KCl and 10 mM MES (2-N-morpholino-ethanesulfonic acid), buffered at pH 5.5 (Gimsing & Borggaard 2001; Melamed & Boas 1998). Samples were taken for two vessels within each of the treatments (Fertilizers), to which four platinum electrodes (Paleo Terra Inc., Amsterdam, The Netherlands) adjacent to the plots measuring the redox potential (Eh) every minute and storing the data every hour through the CR23X Campbell dataloggers. On the other hand, Galvanic Apogee O_2 sensors encapsulated in polyvinyl chloride (PVC) chambers were used to track soil O_2 concentrations (Liptzin *et al.*, 2011).

Ferrous (Fe^{II}) and ferric (Fe^{III}) iron were determined according to the methodology of according to Barcellos *et al.* (2018) and Thompson *et al.* (2006) with the 562-nm and 500-nm intensities measured in 96-well microplates (Huang & Hall, 2017). Moist soils were used for citrate-ascorbate (Fe_{ca}) extractions since soil drying can improve Fe crystallization (Hall and Silver, 2015). Water-extractable dissolved organic carbon (DOC) was obtained by extraction at 1:5 soil: deionized (DI) water ratio (2 g of soil) and shaking for 1 h in a horizontal shaker at room temperature (Haynes *et al.*,1993; Boyer & Groffman, 1996; Ghani, 2003).

The determination of biomass was carried in the mature stage, the plants from 4 pots of each treatment were harvested 75 days after sowing, including shoots and roots of rice. Taking the average of the readings in the last expanded leaf, fully developed, from the apex, which is considered the diagnosis leaf of rice plants (Malavolta *et al.*, 1997; Freitas *et al.*, 2011). The analysis of the Carbon, nitrogen, and phosphorus concentrations and their respective ratios (C:N:P) was carried out according to the methodology mentioned in the previous chapters.

4.2.4 ^{32}P application and analysis

One day before seeds were transplanted, a carrier free solution of $KH_2{}^{32}PO4$ was added to each pot. The $KH_2{}^{32}PO_4$ solution had a specific activity (s.a) of 300 µCi/20 ml. To each pot, 10 ml of the solution was added. The ^{32}P solution was mixed thoroughly for maximum of ^{32}P homogeneiteis. The harvested plants were separated between aerial and root parts. Afterwards, ashing and further steps were undertaken to create a clear solution. This solution was then counted by liquid scintillation counter (LSC) which used Cherenkov counting (Spinelli *et al.*, 2011) since ^{32}P is a strong β emitter. Data from P content and ^{32}P activity were used to determine the specific activity and the proportion (%Pdff) and amount (mg pot^{-1}), in plants, of P derived from MAP, TSP, Estruvite or from soil (%PdfS), by calculations according to the isotopic dilution method (Vose, 1980; Hocking *et al.*, 1997), using the following equations:

Method (Vose, 1980; Hocking *et al*., 1997), using the following equations:

(a)
$$\%\boldsymbol{Pdff} = \frac{\text{SAshoot}}{\text{SAfertilizer}}$$

(b)
$$\%\boldsymbol{Pdf}\text{S} = 100 - \%\text{Pdff}$$

4.2.5 Chemical characterization of fertilizers

The total P content of the three fertilizers (MAP, TSP, Struvite), the chemical fractions of P, as well as the concentrations of N, Ca, Mg and S, were determined according to the Brazilian national guidelines (Embrapa, 1997). The P fractions included measurements of (i) P soluble in citric acid, (ii) neutral ammonium citrate and P soluble in water (NAC + H_2O), and (iii) P (**Table 4.2. 1**).

Table 4.2. 1 Nutrient content of fertilizers used as sources.

Fertilizer	pH	N	P_2O_5				%			
			Total	CNA[1]+ H_2O	H_2O	[2]CA (2%)	Ca	Mg	S	[3]OC
MAP	5.14	10	48	4	44	0	0	0	10	2
TSP	5.74	0	41	4	37	0	10	0	0	3
Estruvite	6.44	5	28.7	0.3	0	28.4	0	8.3	0	75

[1]NAC: Neutral Ammonium Citrate; [2]CA: Citric Acid 2%; [3]OC: Organic carbon

4.2.6 Microbial activity

Rhizospheric soil (soil adhered to the root system, 3 mm) was collected for determination of acid and alkaline phosphatase activity (Tabatabai, 1994) and of microbial carbon (Vance *et al*., 1987), was also determined FDA (Fluorescein diacetate) (Green *et al*., 2006), also Dehydrogenase activity (DHA) was estimated by monitoring the rate of production of triphenyl formazon (TPF) from tri-phenyl tetrazolium chloride (TTC) (Klein *et al*., 1971).

4.2.7 Statistical analysis

Prior to analysis of variance (ANOVA) the data sets were tested for normality and homogeneity of variance by Shapiro-Wilk (p >0.05) and Levene-test (p >0.05), respectively. One-way ANOVA was used to evaluate the effects of phosphate fertilizers, acid soil type, and irrigation management on all parameters measured separately for each sampling time.

When F test was significant (p <0.05), the means were compared using Bonferroni test. Statistical analysis was performed using SPSS version 26.0 (SPSS Inc., USA).

4.3. Results

4.3.1 Fate of ^{32}P and phosphorus uptake by plants

The fate of P from fertilizer and soils varied according to the treatment and soil condition **Figure 4.3. 1**. In the control treatment (no fertilizers added), 100% of the P originated from the soil; whereas in the treatments cointaining soluble fertilizers, such as MAP and TSP, almost 50% of the P was recovered from fertilizers for plant development. In the treatment with struvite amendment, we observed a recovery of 65% of the P added (**Figure 4.3. 1A**).

Comparandig the effects of the two irrigation treatments, in irrigation by field capacity, we observed a recovered 50% of P (from fertilizer), while the treatment with flood irrigation recovered only 35% of the P from fertilizers, so that the remaining (65%) came from the soil (**Figure 4.3. 1B).**

By comparing the two soil types used, we observed greater recovery of P from fertilizers (48%) in for the Ultisol treatments (from Piedade) comparted to a P recovery of 38% for the Oxisol treatment (from Guarapuava) (**Figure 4.3. 1C**).

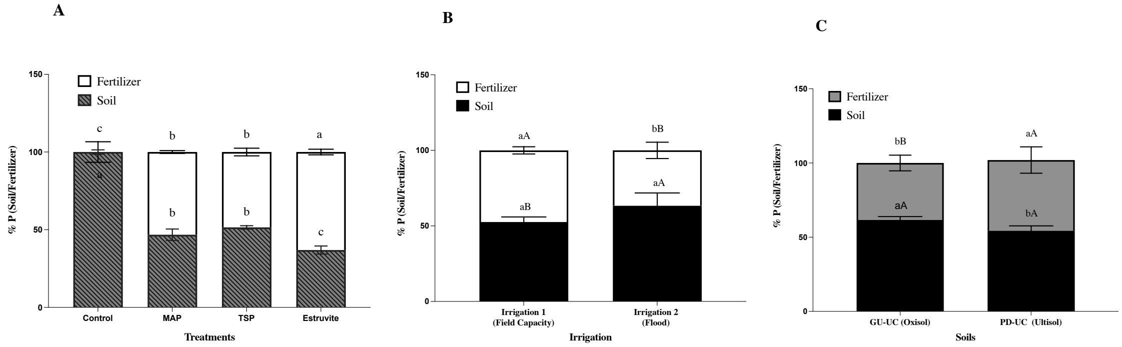


Figure 4.3. 1 A) percentage of P marked with unstable isotope ^{32}P that comes from the soil or fertilizers evaluated; B) P marked in both types of irrigation (field capacity (Irrigation 1) and Submersion irrigation (Irrigation 2) C) P marked in the effect of the two types of acid soils with high P fixation. The mean values followed by the same letter did not differ by test (p <0.05).

This study shows that in **Figure 4.3. 1** it can be observed the concentrations of C, N and P which were altered by the different irrigation conditions (p <0.05), presenting higher concentrations in flood irrigation; however, for C and N, there were no differences among the phosphate fertilizer treatments, only differences with the control. It is important to highlight the concentrations of P, which showed significant differences between the treatments (p <0.05) and also with the type of irrigation (p <0.01). Among the treatments, the highest concentrations of P in the plant were for the use of struvite, the highest concentrations were presented when rice was handled in flood environments. In the case of biomass, significant differences were also observed in irrigation management (p <0.01), with higher weights in flood irrigation.

Table 4.3. 1 Concentration of C, N P and Biomass of shoot and root in rice (*Orysa sativa*), in oxisol and ultisol under irrigation at field capacity and flooded.

Treatment	Irrigation	C	N	P	Biomass	
					Shoot	root
			g kg $^{-1}$		g Plant^{-1}	
	Field capacity	430b	27.63b	1.13b	22.34b	3.18
	Flood	550a	32.19a	1.53a	27.45a	4.25a
		Oxisol				
Control	Field capacity	710a	65.85a	0.76c	15.85c	2.06
MAP		460b	20.47b	1.41b	33.45a	3.85a
TSP		435b	21.25b	1.33b	28.75b	3.15b
Estruvite		430b	21.22b	1.88a	29.35b	3.18b
Control	Flood	780a	74.45a	0.52c	18.73b	3.15d
MAP		610b	21.59b	1.39b	35.45a	4.58b
TSP		580b	21.45b	1.22b	33.05a	4.15c
Estruvite		575b	19.64b	2.29a	34.79a	5.12a
		Ultisol				
Control	Field capacity	390a	55.45a	0.56c	13.45c	1.15
MAP		350b	15.78b	1.21b	30.45a	3.15a
TSP		345b	17.31b	0.953b	27.75b	2.99b
Estruvite		325b	17.5b	1.88a	22.35c	3.05b
Control	Flood	610a	62.45a	0.87c	16.65b	2.35c
MAP		460b	16.91b	1.53b	28.45a	4.05b
TSP		435b	17.51b	1.43b	26.35a	3.88b
Estruvite		430b	15.85b	1.93a	25.75a	4.55a
	Sources		*P*(F) values			
	Fertilizer x irrigation	0.050	0.010	0.010	0.010	0.010
	Fertilizer x Soil	0.050	0.050	0.050	0.050	0.050

Within a column for each irrigation management or for each type of phosphate fertilizer, means followed by different letters are significantly different according to Bonferroni´s post hoc test. Test. p <0.05 are also shown.

The results for the two acid soils used in the experiment demonstrated significant differences in the availability of nutrients (C, N, P) and biomass showed significant differences. All the evaluated parameters presented significant differences ($p < 0.05$), with higher concentrations in the Oxisol soil compared to the Ultisol soil. The biomass presented the same sequence presenting higher weights for aerial part and root in Ultisol soil.

4.3.2 Microbiological activity

All the microbiological variables in **Table 4.3. 1** showed highly significant differences ($p < 0.01$) for irrigation management. All variables presented higher concentrations in flood irrigation in rice cultivation. It is important to mention that there were also significant differences under the effect of acid soils, in this case the trend was that there were higher concentrations in the soil Oxisol than Ultisol, in addition, between the treatments, there were higher concentrations of DHA and MBC for MAP under conditions of field capacity, but higher concentrations for Struvite in flood conditions, this same trend was presented under the effect of the two soils ($p < 0.05$). For the FDA, the highest activities were presented in the MAP treatment, under all effects, including the types of irrigation and the two types of soils ($p < 0.05$). In the case of both acidic and alkaline APA, the activities were presented for the Estruvite fertilizer, these concentrations were higher than the other phosphate fertilizers, both for the effects of irrigation systems, as well as for the case of the effect of the types of acid soils ($p < 0.05$).

Table 4.3. 2 Microbial and enzymatic activity in different humidity conditions in Oxisol and Ultisol soil under the effect of phosphate fertilizers of high and low solubility.

Treatment	Irrigation	DHA	MBC	FDA	APA	
					Acid	Alkaline
		µg TPF g–1 day–1	µg g–1	µg Fluorescein g–1 hr–1	µg p-nitrophenol g–1 h–1	
	Field capacity	145,65b	198,45b	1,25b	75,25b	92,35b
	Flood	255,95a	248,35a	1,75a	110,55a	135,45a
			Oxisol			
Control	Field capacity	85,45c	91.65d	1,21b	48,45	65,85c
MAP		145,55a	135.65a	1.45a	65.45	85.45b
SPT		125,35b	116.95c	147a	57.35	69.45c
Estruvite		115,25b	125.15b	151a	75.55a	105,65a
Control	Flood	117,45d	115.75d	1.31c	55.65d	95.45d
MAP		225,35b	237.45b	1.75a	105.65b	125.55c
SPT		185,45c	165.45c	1.55b	85.65c	135.95b
Estruvite		265,75a	255.45a	1.51b	125.55a	145.65a
			Ultisol			
Control	Field capacity	65,85d	78.45c	1.15c	35.65d	45.75d
MAP		118,55a	126.35a	1.35a	55.45b	65.35b
SPT		95,85b	111.25b	1.21b	48.55c	56.45c
Estruvite		87,45c	107.85b	1.25b	65.95a	78.55a
Control	Flood	131,55d	97.15d	1.23c	75.15d	87.15d
MAP		178,95b	195.45b	1.49a	115.15b	124.85b
SPT		155,45c	135.45c	1.34b	95.45c	105.25c
Estruvite		212,55a	235.45a	1.29b	125.11a	137.75a
	Sources			*P*(F) values		
	Fertilizer x irrigation	0.010	0.010	0.010	0.010	0.010
	Fertilizer x Soil	0.050	0.050	ns	0.050	0.050

Within a column for each irrigation management or for each type of phosphate fertilizer, means followed by different letters are significantly different according to Bonferroni´s post hoc test. Test. *p* <0.05 are also shown.

4.3.3 Sequential soil P fractionation

In the present experiment, it is important to have an overview and a final summary for the results across treatments for the total P, labile P, and non-labile P is provided in the **Figure 4.3. 2**. The total soil labile P (obtained by the sum of all fractions), was higher in the treatment with Struvite (p <0.05) comparated to the other fertilizers, in box Oxisol and Ultisol. In the case of the moderately labile P fraction, the P concentrations were higher in the treatment with struvite, which also presented significant differences with the other treatments (p <0.05), followed by the treatment with MAP. In the non-labile fraction, the highest concentration was for MAP, with higher concentrations of non-labile P in the Oxisol compared to the Ultisol.

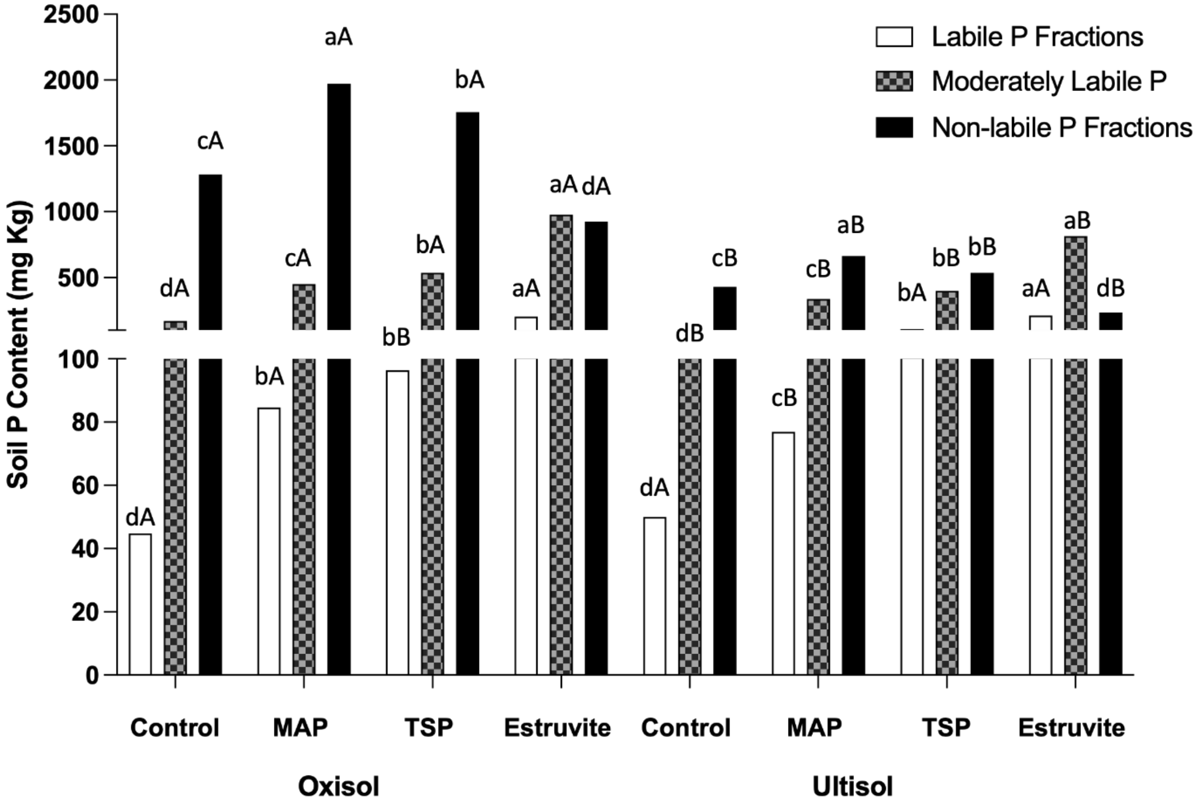


Figure 4.3. 2. Determination of the sequential fractionation of P, of its forms, labile, moderately labile and nonlabile, in acid soils (Oxisol and Ultisol), under different types of phosphate fertilizers. The mean values followed by the same letter did not differ by test (p <0.05). The first letter represents the difference between treatments (Fertilizers). The second letter represents the same treatment with different types of soils (Oxisol and Ultisol).

4.3.4 Redox Processes

The application of the fertilizer treatments linearly decreased the concentration of O_2 in the soil, when they were subjected to flooding (**Figure 4.3. 3B** and D); even the values of the treatments with phosphate fertilization were lower compared to the control. In the two soils subjected to field capacity, the values increased up to 35 days and subsequently had a slight decrease (**Figure 4.3. 3A** and **Figure 4.3. 3C**) with the exception of MAP and struvite.

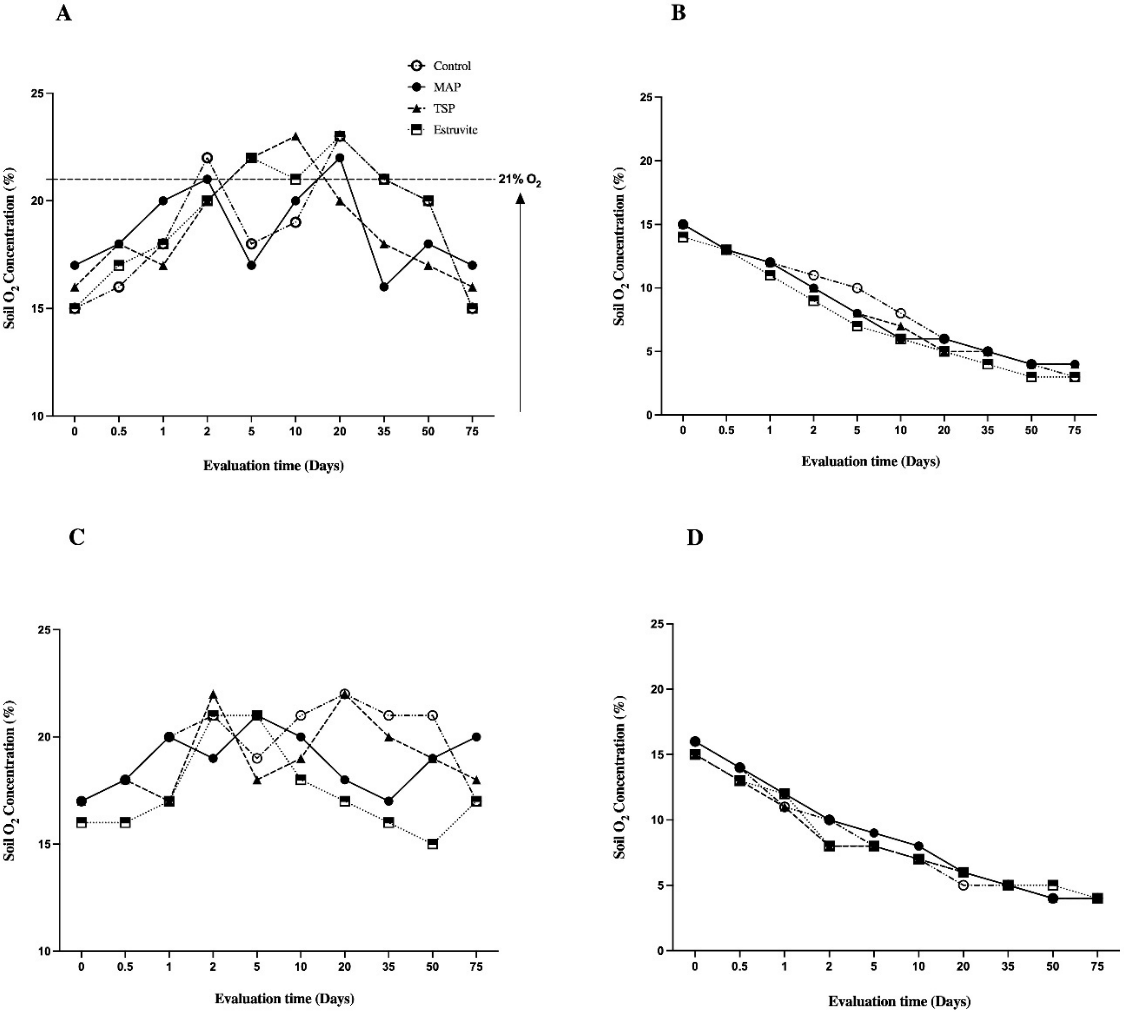


Figure 4.3. 3 Soil O_2 concentration for different evaluation times. a) Treatments at field capacity in Oxisol; b) Treatments with flood irrigation in Oxisol; c) Treatments at field capacity in Ultisol; d) Treatments with flood irrigation in Ultisol.

Similarry, the application of the fertilizer treatments linearly decreased the E_h of the soil, when they were subjected to flooding (**Figure 4.3. 3**); even the values were lower compared to the control. In the two soils subjected to field capacity, the values increased up to 35 days and subsequently had a slight decrease (**Figure 4.3. 3**). Under anaerobic conditions (<E_h 100),

E_h dropped sharply from 200 mV to -580 mV during the 75 days of incubation for Oxisol soil and from 200 mV to 500 mV for the Ultisol soil (**Figure 4.3. 4**).

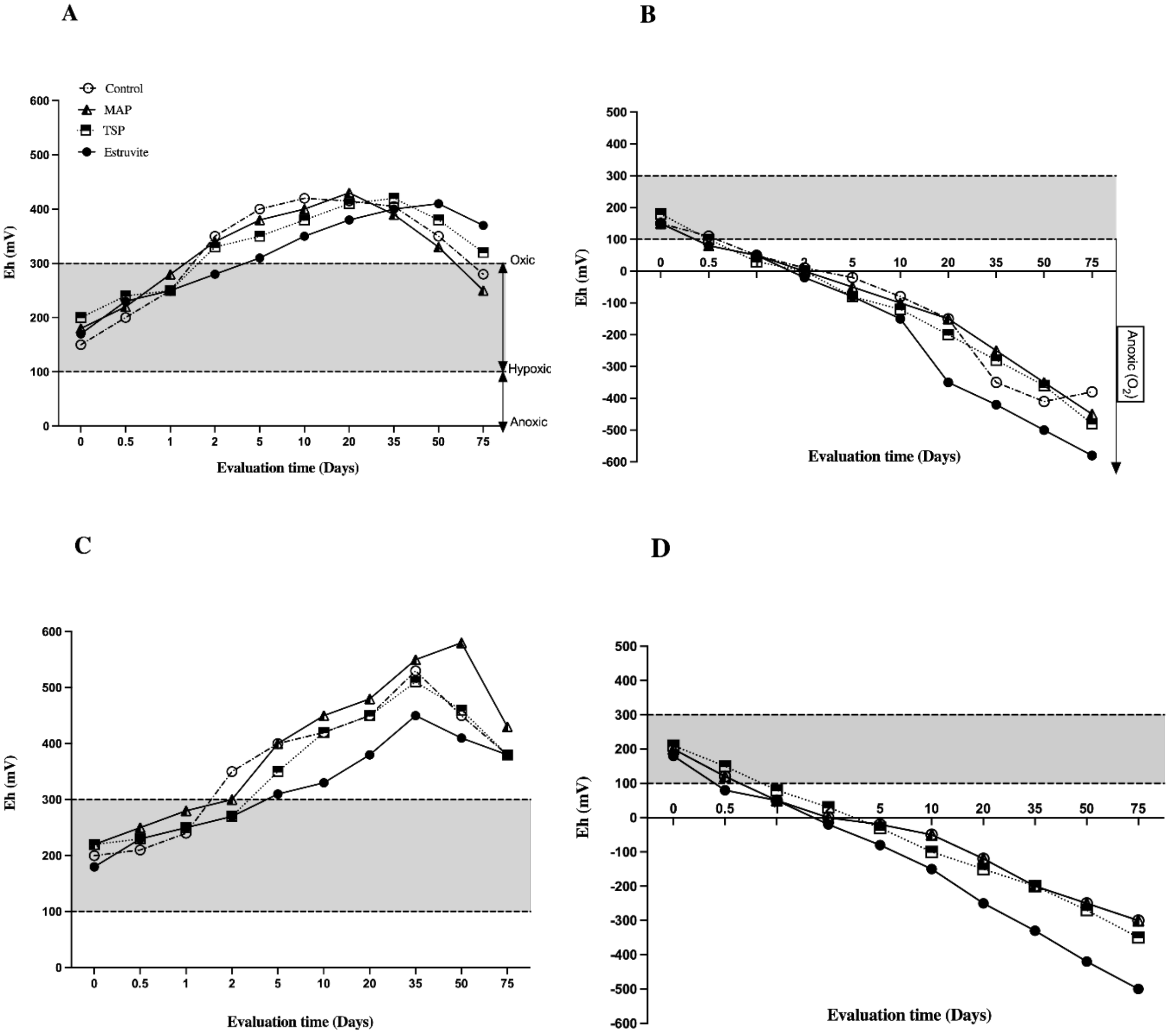


Figure 4.3. 4 Redox potential (E_h) using platinum electrodes for different evaluation times. a) Treatments at field capacity in Oxisol; b) Treatments with flood irrigation in Oxisol; c) Treatments at field capacity in Ultisol; d) Treatments with flood irrigation in Ultisol.

Furthermore, the addition of phosphate fertilizers modified the initial pH conditions in both Oxisol and Ultisol (**Figure 4.3. 4**). The magnitude of the gradual increments in pH on the first day, depended on the irrigation conditions, which for field capacity conditions were: in the order of Control> TSP> MAP> Estruvite in both soils and for flood conditions: Control > MAP> TSP> Estruvite (**Figure 4.3. 5**), for Oxisol soil; and for the Ultisol soil under field capacity conditions, the sequence was: Control> TSP> Estruvite> MAP (**Figure 4.3. 5**A and

Figure 4.3. 5C); and in flood conditions it was: Control> MAP> TSP> Estruvite (**Figure 4.3. 5B** and **Figure 4.3. 5D**). In both soils there was a gradual increase in pH under the two irrigation conditions during the 75 days of incubation with phosphate fertilizers, however there were higher pH values in flood conditions for both the Oxisol and Ultisol soil.

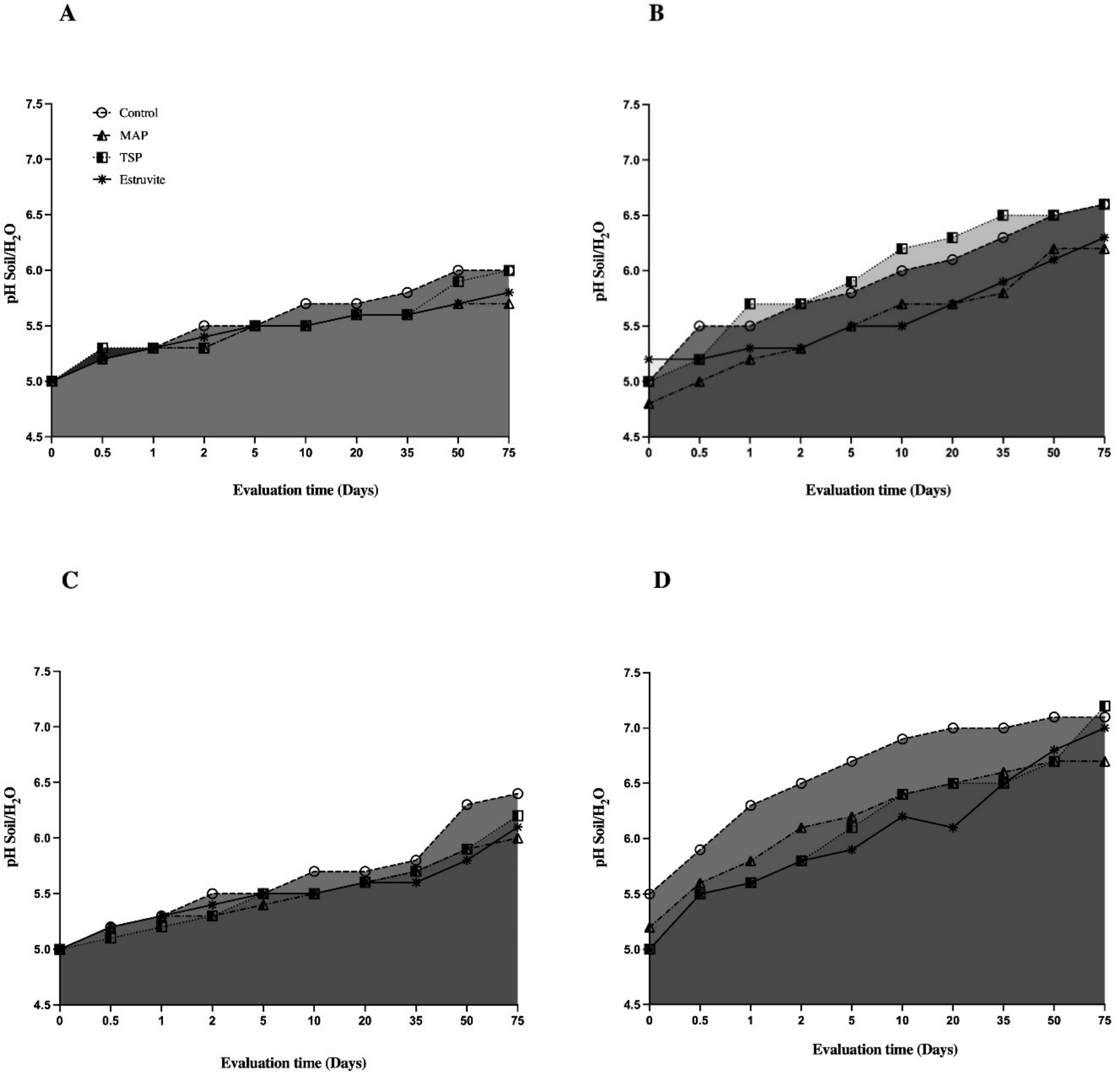


Figure 4.3. 5. pH for different evaluation times. a) Treatments at field capacity in Oxisol; b) Treatments with flood irrigation in Oxisol; c) Treatments at field capacity in Ultisol; d) Treatments with flood irrigation in Ultisol.

During the experiment, elevate concentrations of Fe^{II} accumulated in the two soils under floodint irrigation, due to the reduction of Fe^{III}-minerals as well as the mineralization of the fertilizer inputs in the labile states, comparted the controls (**Figure 4.3. 6B** and **Figure 4.3. 6D**) ($p < 0.05$). In both soils under flooding irrigation, the fertilizers MAP and struvite induced to a greater production of Fe^{II}, than the control and TSP treatments. The Oxisol and Ultisol under flooding irrigation conditions, we observed an acumulation of Fe^{II} more rapidly during the first 35 days, followed by a platea in Fe^{II} production. On the contrary, under irrigation conditions at field capacity, there was a decrease in Fe^{II} concentration from day 0 to 35, due to the presence oxygen in soils at field capacity (**Figure 4.3. 6A** and **Figure 4.3. 6C**). However, after 50 of the experiment Fe^{III} reduction started in both Oxisol and Ultisol at field capacity condition.

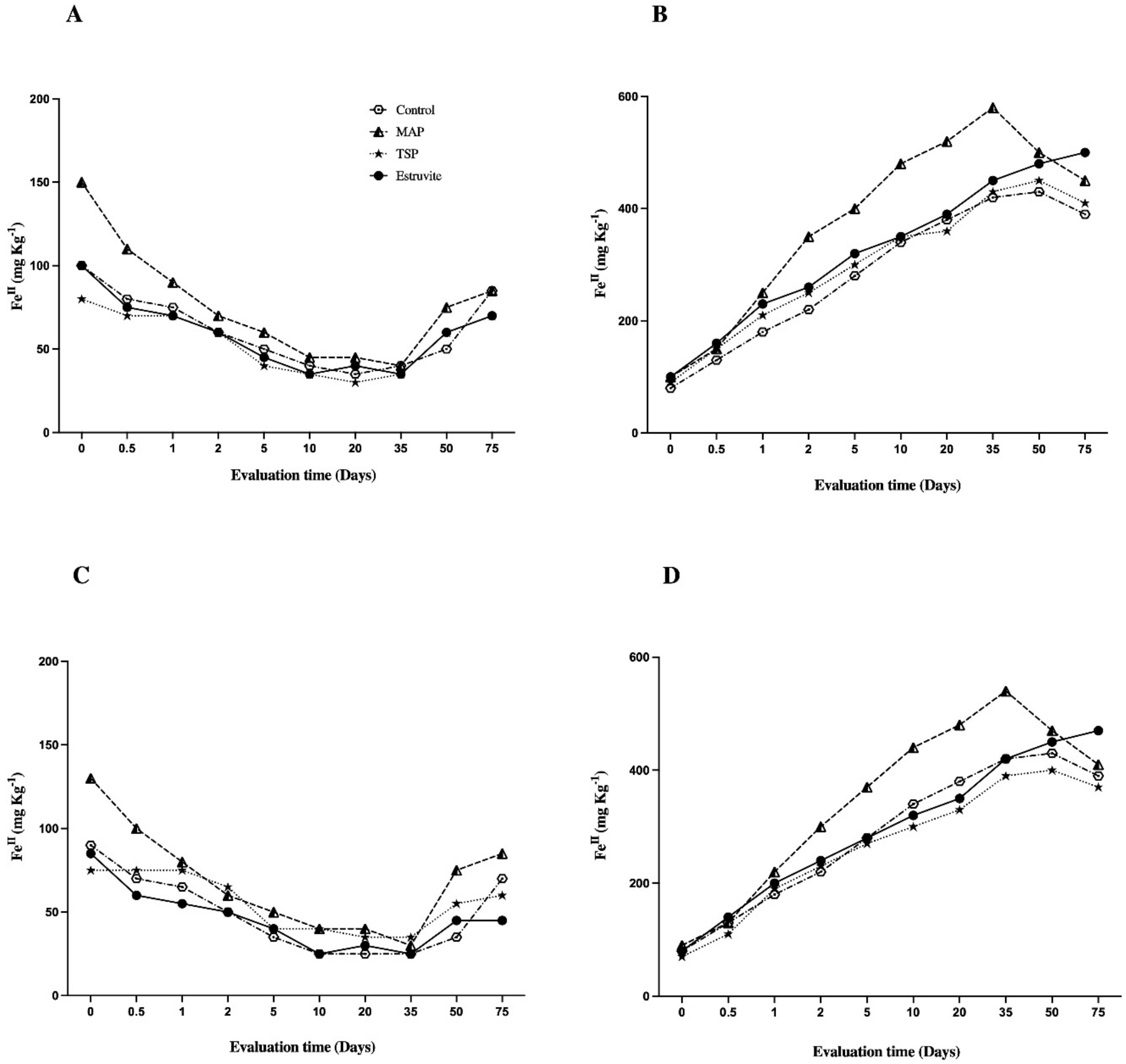


Figure 4.3. 6 Fe^{II} concentration for different evaluation times. a) Treatments at field capacity in Oxisol; b) Treatments with flood irrigation in Oxisol; c) Treatments at field capacity in Ultisol; d) Treatments with flood irrigation in Ultisol.

The effect of the different treatments in our experiment also strongly affected the $Fe^{III}_{(HCl)}$ content for soils under different types of irrigation. In the case of the flooding irrigation, it was observed in the $Fe^{III}_{(HCl)}$ content from 250 mg kg^{-1} to 25 mg kg^{-1} in both studied soil types (**Figure 4.3. 7B** and **Figure 4.3. 7D**). On the other hand, with the irrigation leading the soil to the field capacity, it was observed a minor decrease of the $Fe^{III}_{(HCl)}$ content, from 400 to ~ 200 mg kg^{-1} (**Figure 4.3. 7A** and **Figure 4.3. 7C**).

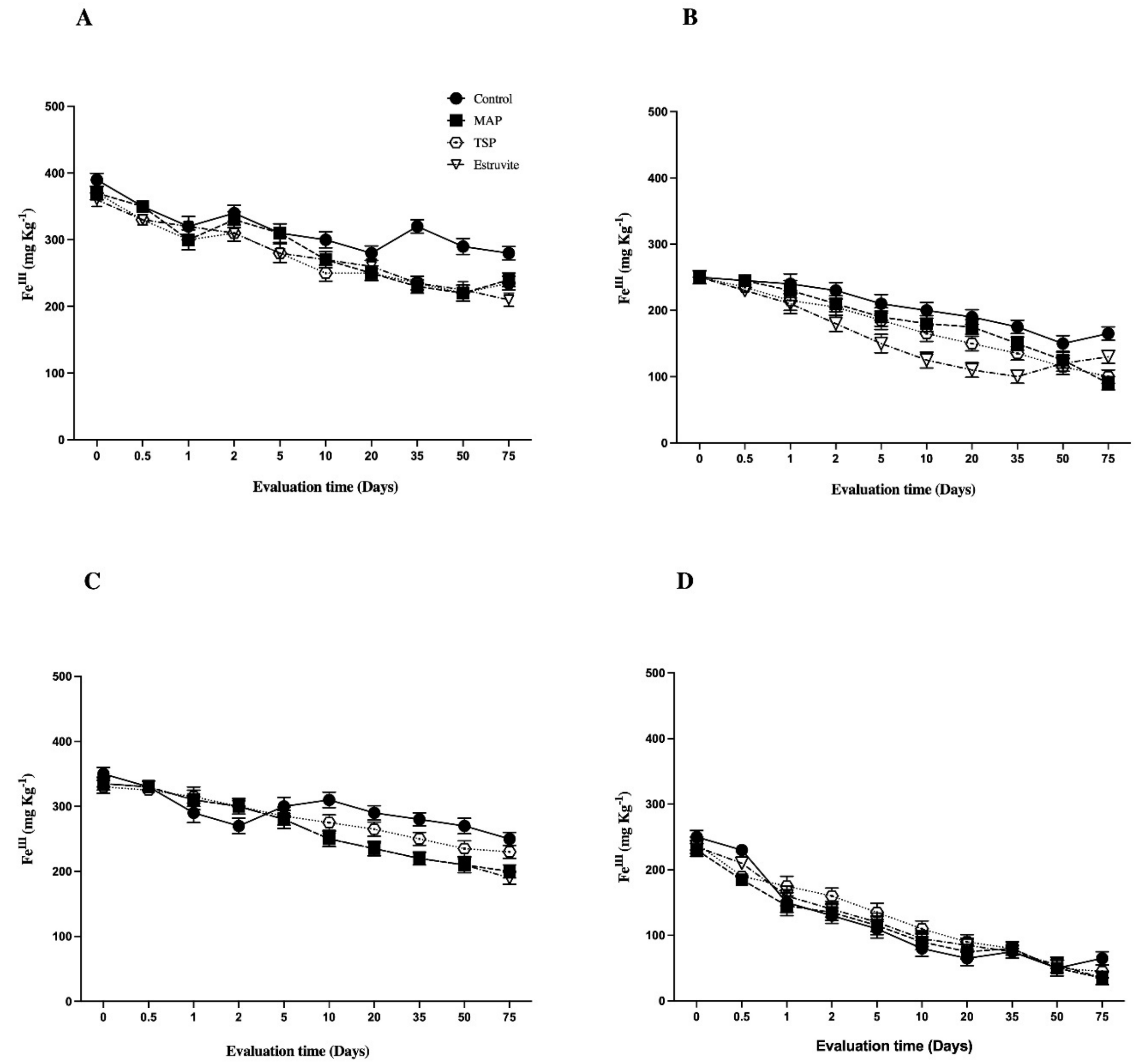


Figure 4.3. 7 Fe^{III} concentration for different evaluation times. a) Treatments at field capacity in Oxisol; b) Treatments with flood irrigation in Oxisol; c) Treatments at field capacity in Ultisol; d) Treatments with flood irrigation in Ultisol. Values represent the means of the three replicates and the error bars represent the standard error of the means.

The concentration of low-crystallinity Fe^{III} (hydr)oxides (obtained by citrate-ascorbate extraction: Fe$^{III}_{CA}$) in both Oxisol and Ultisol, and for both types of irrigation, generally decreased throughout the experiment (from day 0 to 75). The decrease in Fe$^{III}_{CA}$ content were faster in the Oxisol (**Figure 4.3. 8A** and **Figure 4.3. 8B**) than in the Ultisol (**Figure 4.3. 8C** and **Figure 4.3. 8D**); However, compared to Oxisol, Ultisol soil presented a higher rate of reduction of Fe^{III}, in any case, the greatest reductions were presented in flood conditions in rice, reaching almost 200 mg kg^{-1}. The period of Fe^{III} and Fe$^{III}_{CA}$ decrease in both rice fields coincides with the Fe^{II} accumulation period (**Figure 4.3. 6B** and **Figure 4.3. 6D**). However, the addition of MAP, TSP and Struvite notably increased Fe^{III}, with high reduction rates in both Oxisol and Ultisol.

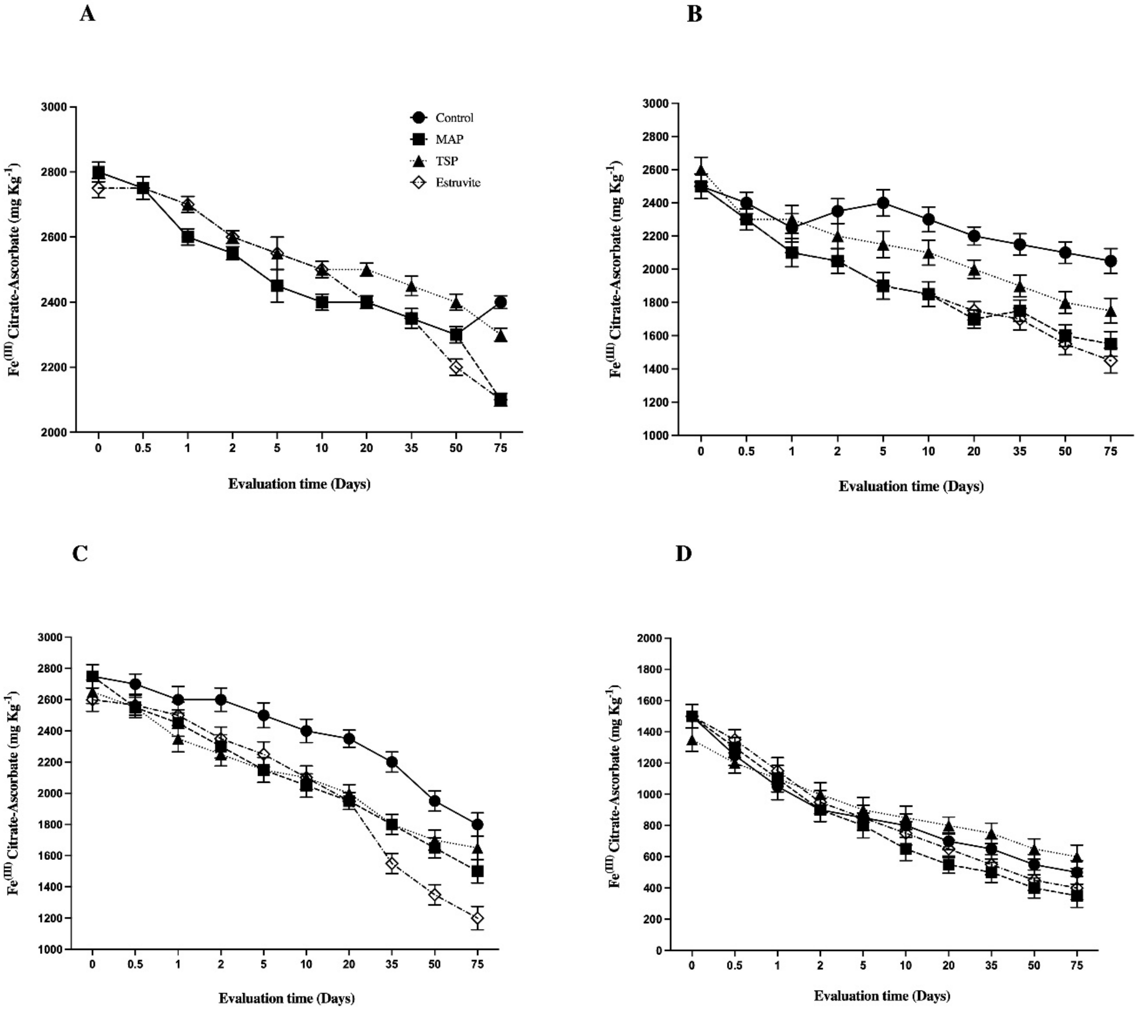


Figure 4.3. 8. Low-crystallinity Fe^{III} (hydr)oxides, by extraction Citrate-Ascorbate (Fe$^{III}_{CA}$). Concentration for different evaluation times. a) Treatments at field capacity in Oxisol; b) Treatments with flood irrigation in Oxisol;

c) Treatments at field capacity in Ultisol; d) Treatments with flood irrigation in Ultisol. Values represent the means of the three replicates and the error bars represent the standard error of the means.

Over the course of the experiment, the addition of phosphate fertilizers, considerably increased the soil dissolved organic carbon (DOC) contents in both soils compared to the control, within the flood irrigation treatments (**Figure 4.3. 9B** and **Figure 4.3. 9D**). The Oxisol soil presented higher concentrations of DOC than the Ultisol, which reached a concentration of 580 mg kg^{-1}. In the case of soil aireation (i.e., soil at field capacity), the two soils presented linear decrease in DOC contents during the 75 days. Once the experiment was established (**Figure 4.3. 9A** and **Figure 4.3. 9C**), the same condition was also presented, with higher concentrations in the soil Oxisol reaching a concentration of 180 mg kg^{-1} corresponding to struvite.

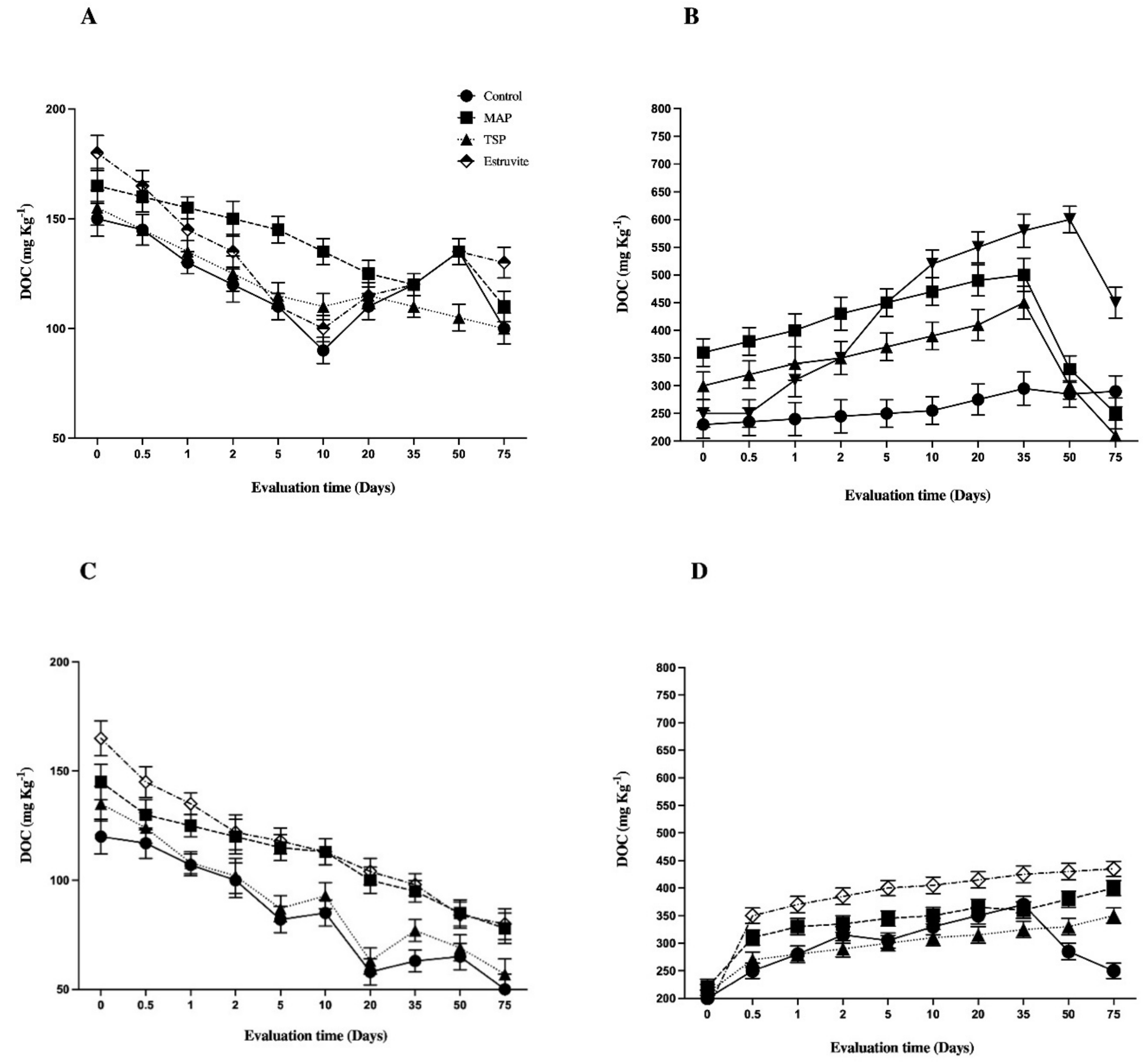


Figure 4.3. 9. Dissolved organic carbon (DOC). Concentration for different evaluation times. a) Treatments at field capacity in Oxisol; b) Treatments with flood irrigation in Oxisol; c) Treatments at field capacity in Ultisol; d) Treatments with flood irrigation in Ultisol. Values represent the means of the three replicates and the error bars represent the standard error of the means.

The concentrations of $NaHCO_3$-P demonstrated a linear increase in the two studied soils and within the two soil moisture/irrigation regimes. The greatest $NaHCO_3$-P concentrations occurred under flood conditions in rice (**Figure 4.3. 10B** and **Figure 4.3. 10D**) where there were differences significant (p <0.05). In the case of concentrations in humidity conditions at field capacity, they were much lower, which were below 100 mg kg^{-1} (**Figure 4.3. 10A** and **Figure 4.3. 10C**).

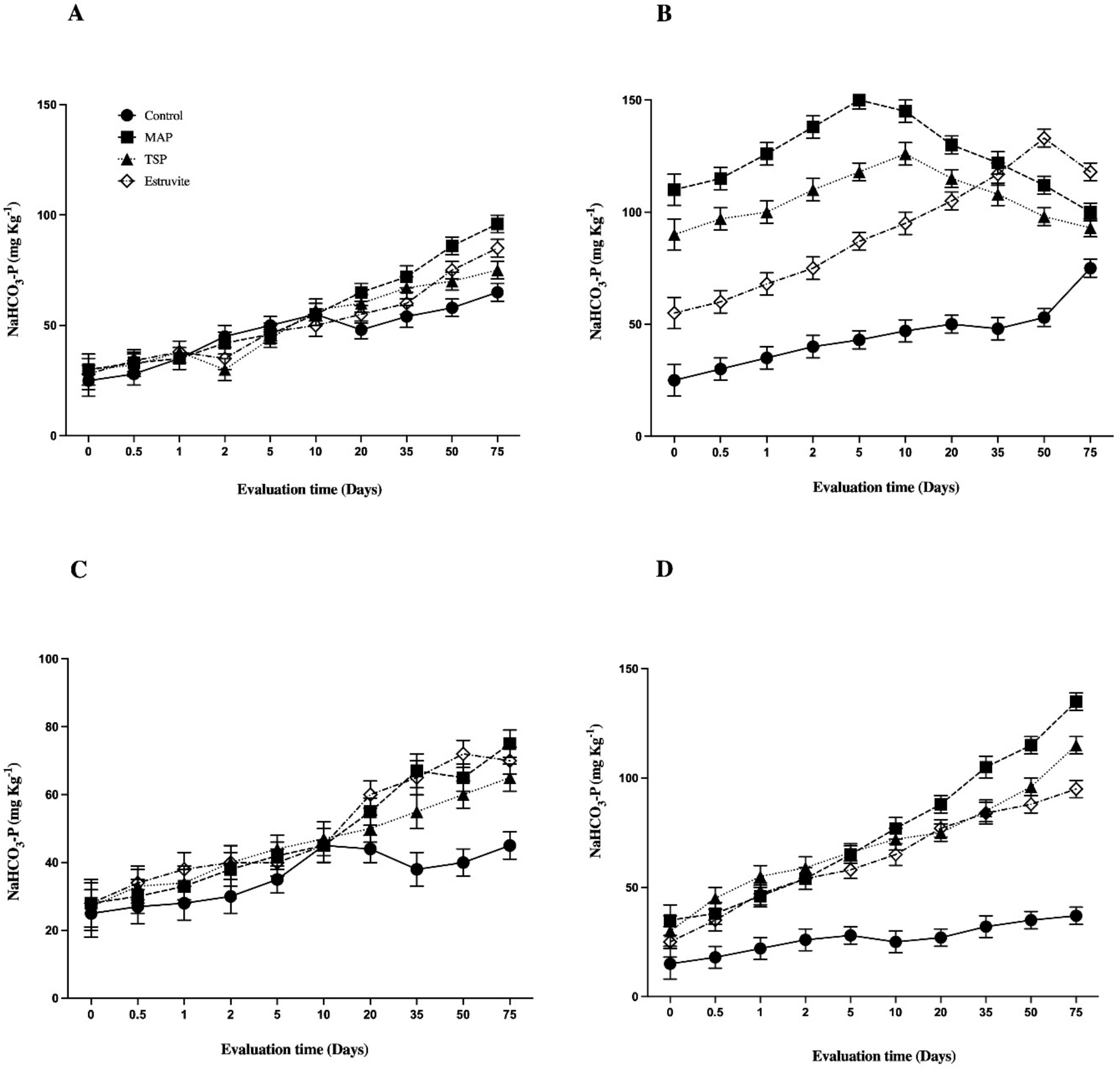


Figure 4.3. 10 $NaHCO_3$-P. concentration for different evaluation times. a) Treatments at field capacity in Oxisol; b) Treatments with flood irrigation in Oxisol; c) Treatments at field capacity in Ultisol; d) Treatments with flood irrigation in Ultisol.

For Fe-P concentrations, there was a gradual and slight increase for humidity management at field capacity (**Figure 4.3. 11A** and **Figure 4.3. 11C**), however the concentrations did not exceed 60 mg kg^{-1}, much lower than humidity management by flooding in rice (p <0.05) (**Figure 4.3. 11B** and **Figure 4.3. 11D**). It is important to highlight that in the soils managed with flood irrigation they showed a negative linear regression trend, reaching concentrations up to 250 mg kg^{-1} and decreasing until the end of the evaluation period 50 mg kg^{-1}.

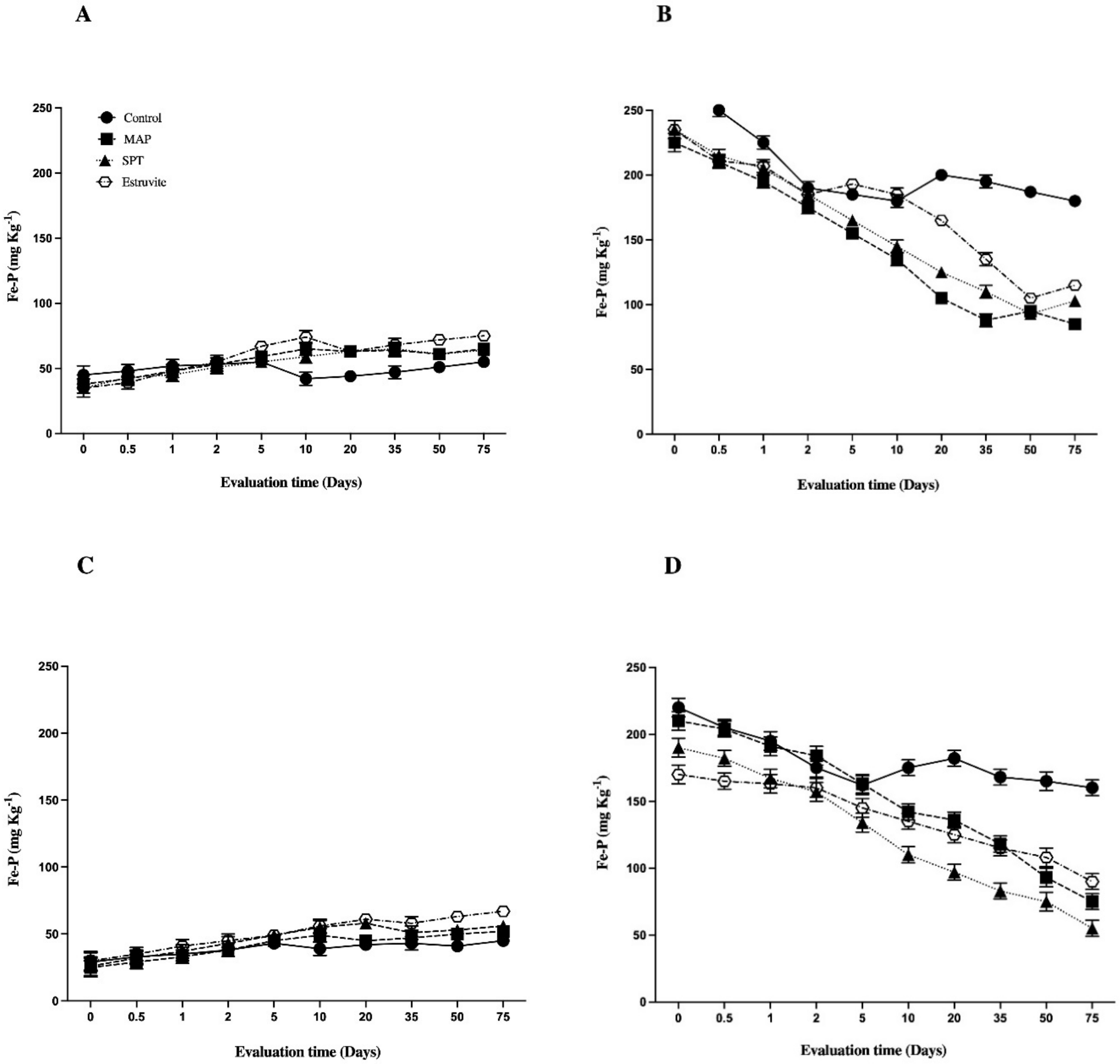


Figure 4.3. 11. Fe-P. concentration for different evaluation times. a) Treatments at field capacity in Oxisol; b) Treatments with flood irrigation in Oxisol; c) Treatments at field capacity in Ultisol; d) Treatments with flood irrigation in Ultisol.

All treatments presented a reduction in SPAD values and a quadratic trend; In addition, there were significant differences between the treatments ($p < 0.05$), the control being the treatment that presented the lowest SPAD values, both in the condition of the two soils and in the types of irrigation. For the case of flood irrigation (**Figure 4.3. 12B** and **Figure 4.3. 12D**), this effect presented the highest SPAD values compared to the treatments managed in irrigation at field capacity (**Figure 4.3. 12A** and **Figure 4.3. 12C**). On the other hand, it is important to highlight that according to the two soils, the Oxisol soil presented higher values than the Ultisol soil.

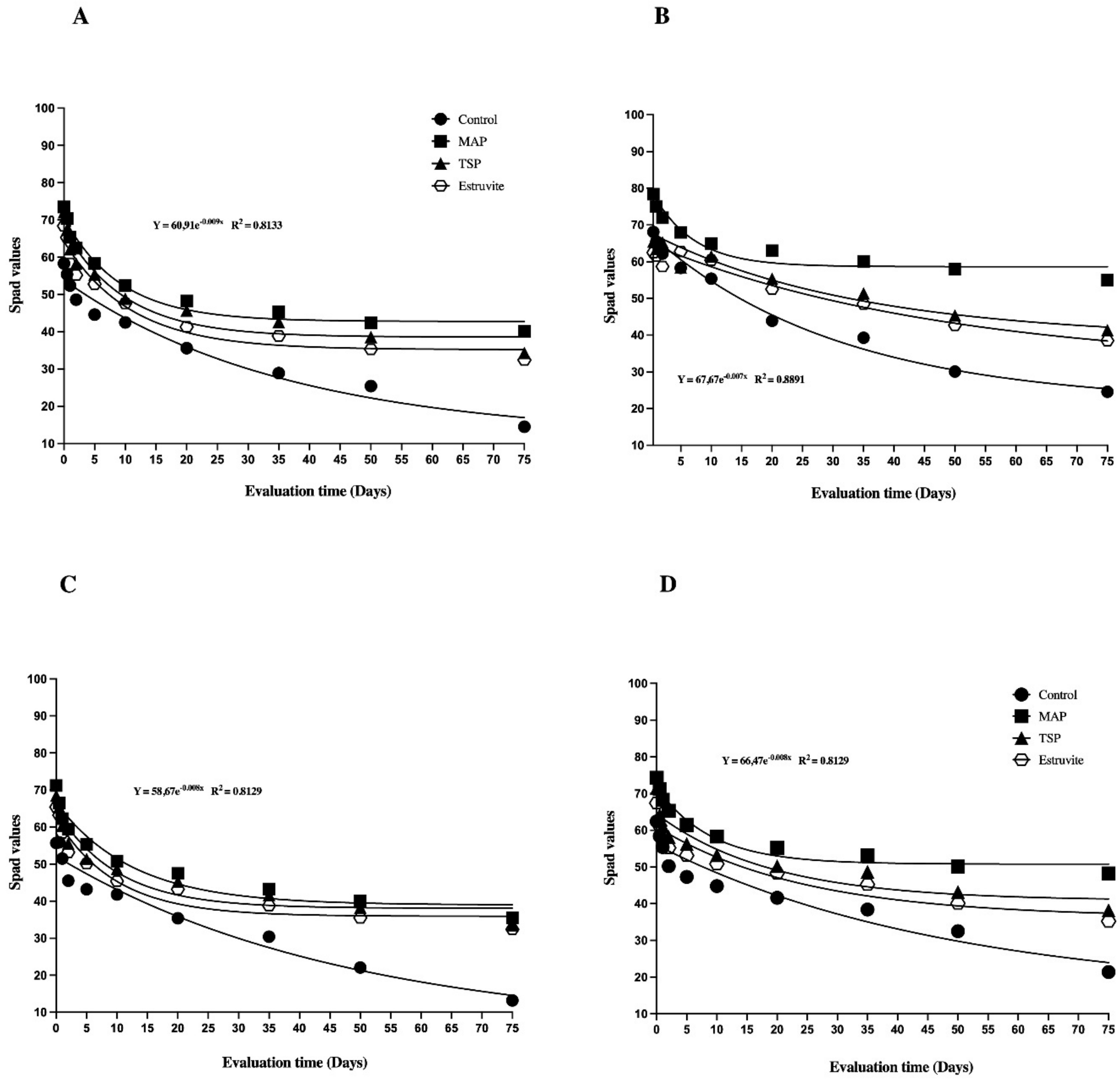
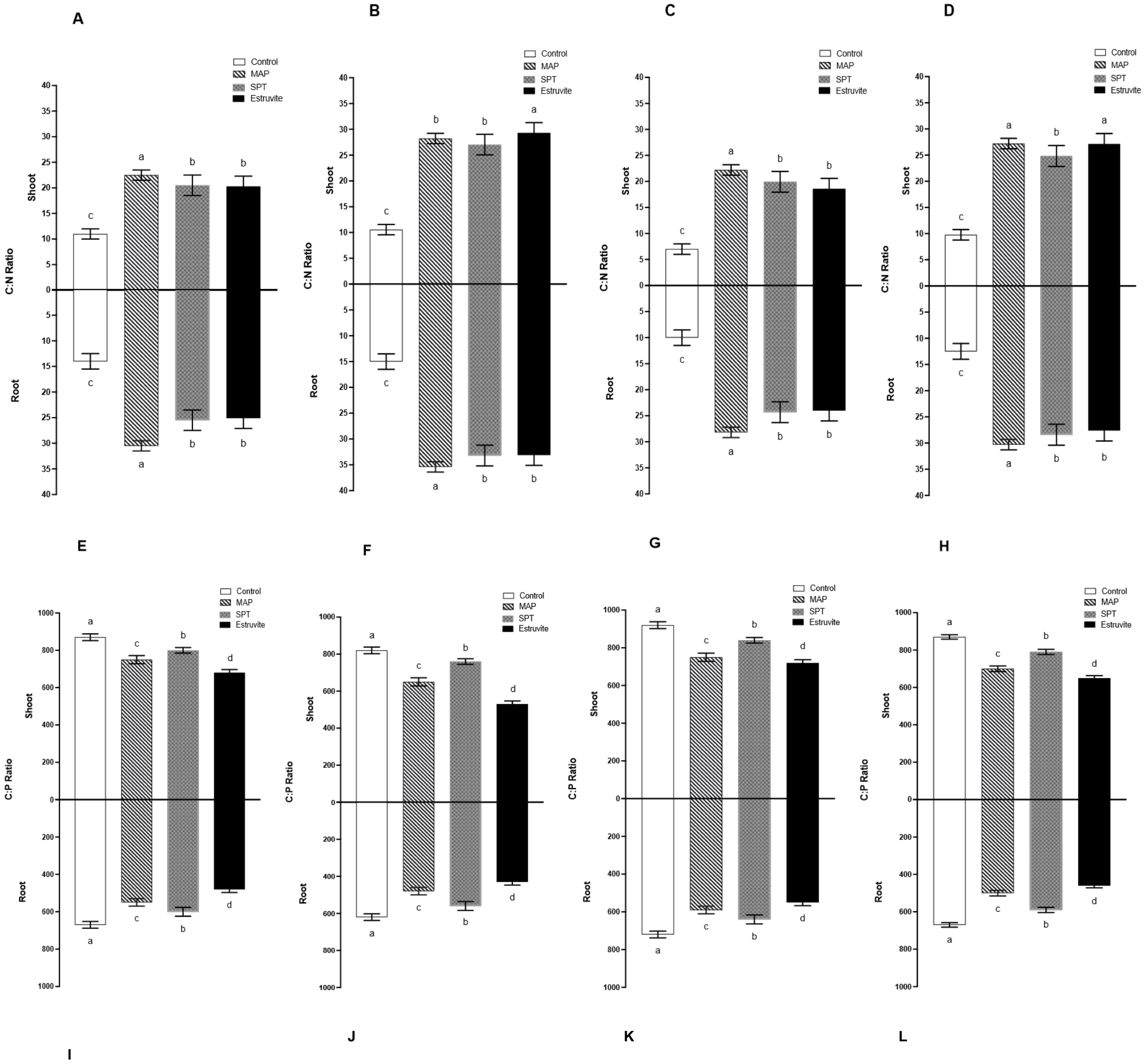


Figure 4.3. 12 SPAF values for different evaluation times. a) Treatments at field capacity in Oxisol; b) Treatments with flood irrigation in Oxisol; c) Treatments at field capacity in Ultisol; d) Treatments with flood irrigation in Ultisol.

According to the calculated stoichiometric ratio, for the C:N ratio the lowest ratios were presented by the control for both the shoot and root, which presented significant differences (*p* <0.05). In addition, there were significant differences between the irrigation effect, which presented higher ratios in flood irrigation (**Figure 4.3. 13B** and **Figure 4.3. 13D**) compared to irrigation at field capacity (**Figure 4.3. 13A** and **Figure 4.3. 13C**).

For the C:P ratio, among the treatments, the one who presented the highest ratio was the control and the lowest ratio was presented by Struvite (*p* <0.05).



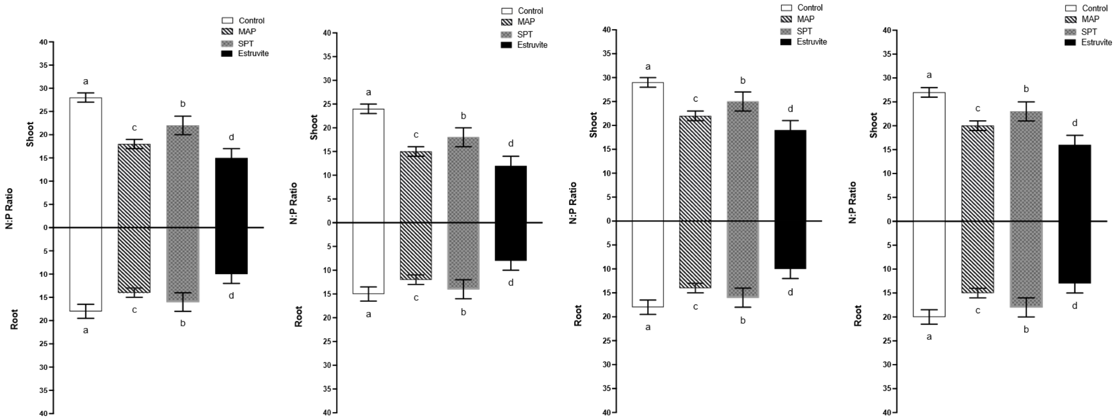


Figure 4.3. 13. Changes in the stoichiometric ratio in shoot and root in *Oryza sativa* managed under different sources of phosphate fertilizers. C: N ratio in Oxisol to field capacity (A), flood (B), Ultisol to field capacity (C), flood (D). C: P ratio in Oxisol at field capacity (E), flood (F), Ultisol at field capacity (G), flood (H). N: P ratio in Oxisol to field capacity (I), flood (J), Ultisol to field capacity (K), flood (L). Different letters indicate significant differences between the treatments $p \leq 0.05$. The stack bars show the standard error based on the average of four replicates.

The lower C:P ratio for all treatments under the irrigation effect, in this case for flood irrigation in rice (**Figure 4.3. 13F** and **Figure 4.3. 13H**), compared to treatments under irrigation at field capacity (**Figure 4.3. 13E** and **Figure 4.3. 13G**). Regarding the effect of the soil, the treatments under the management with Ultisol soil, presented higher C:N ratios, than those managed under the Oxisol soil.

Finally, the N:P ratio established a similar trend to the C:P ratio, where the control presented the highest concentrations compared to the other treatments, it should be noted that struvite presented the lowest N: P ratios under soil and irrigation effects. Higher ratios were presented in the Ultisol soil (**Figure 4.3. 13**K and **Figure 4.3. 13**L), compared to the treatments managed in the Oxisol soil (**Figure 4.3. 13**I and **Figure 4.3. 13**J).

4.4.Discussion

4.4.1. Soil mineralogy, pH, Eh and P availability

Clay content and mineralogical composition are key factors in determining adsorption processes in weathered soils, such as the studied Oxisol (58.7 % of clay) and the ultisol (44 % of clay) (Jalali & Jalali, 2016; Fink *et al.*, 2016). Kaolinite (kt) is the predominant clay mineral occurring in both soils (**Figure 4.2. 4**). The Oxisol contained the following minerals: Kt, gibbsite (Gb), goethite (Gt), hematite (Hm), and vermiculite with aluminum hydroxyl intercalated (HIV). The models for lability and speciation of P in both studied soils showed that P adsorbed or coprecipitated with Al and Fe (hydr)oxides are the least labile forms

(Hesterberg, 2010; Xu *et al.,* 2014). Therefore, due to the predominance of Fe and Al (hydr)oxides in these soils, low P content in labile fractions is expected, unless lime additions may promote the precipitation of more soluble calcium phosphates in soils (Zavaschi *et al*., 2020).

The studied soils were extremely acidity (pH <4.5) being considered extremely acidic, with a low Cation Exchange Capacity and with high concentrations of Fe >200 mg kg^{-1}, characteristic weathered of tropical soils (Aprile & Lorandi, 2012; Gurmessa, 2021). Both pH and E_h play a role in the release of P in environments under reducing conditions (Huang *et al*., 2018; Khan *et al*., 2019). For both Oxisol and Ultisol, it was observed a strong increase in pH as the flooding take place in rice cultivation (**Figure 4.3. 5B** and **Figure 4.3. 5D**), which probably induce a depletion in O_2 concentrations by anaerobic microbial activity. Most of the reactions occurring in flooding environments consume H^+, which causes an increase in soil pH (Narteh & Sahrawat 1999; Huguenin-Elie *et al*., 2009). In fact, it is the CO_2 in the reduced soils that buffers the pH in the ranges of 6.5 and 7.5, by the H_2CO_3-HCO_3^- reactions (McBride, 1994; Amery & Smolders, 2012). In the case of studied acid soils (Oxisol and Ultisol) during the flooding irrigation, the pH increased until reaching the neutrality (Huguenin-Elie *et al*., 2009; Wei *et al*., 2019). The intensity of the reduction is greater in the presence of organic matter and its active state, which is OC, since it is oxidized and the soil components are reduced due to anaerobic microbial respiration (Ponnamperuma, 1972; Keiluweit *et al*., 2017; Wei *et al*., 2019; Wang *et al*., 2021). This may be the reason for the low E_h values in the soils with considerable concentrations of OC, this was observed with the dissolved OC values (**Figure 4.3. 9**), which was an increase in the two soils, but with significant differences ($p < 0.05$), where struvite presented higher increases in pH (**Figure 4.3. 5**) and lower E_h values (**Figure 4.3. 4**), which leads to an increase in pH values compared to soils with lower OM content**.** It seems that more flooding time was required beyond 75 days to reach more stable values of E_h and pH (Chacon *et al*., 2006; Grybos *et al*., 2009; Huang *et al*., 2018).

Regarding P, flood irrigation management increased the availability of P, and this is related to the reduction of Ferric phosphates (Fe^{+3}) to ferrous phosphates, closely related to the mineralogical component of clays, in the case Oxisol soil, presented greater variability and mineralogical richness (**Figure 4.2. 4**), presenting Aluminum hydroxides (HIV) and minerals such as Goethite, rich in iron oxides (**Figure 4.2. 4**A), Thus the Oxisol presented a greater capacity of binding to the minerals (**Figure 4.2. 4**), which can be observed with a higher phosphate adsorption capacity, compared to Ultisol soil, which can also be correlated in the percentage of clays in which it was higher for Oxisol soil than Ultisol. The release of P from insoluble components of Fe and Al (Higashi, 1983; Tassano *et al.*, 2021).

4.4.2 Iron dynamics

With active microorganisms (Fe-reducers), labile carbon sources, and reducible Fe (hydr)oxides forms, the reduction of Fe^{III} may take place in soils under reducing conditions. As the low-crystallinity Fe^{III} minerals would undergo reductive dissolution, we were capable to observe an increase in pH in the soils under reduction conditions, which favored a mobilization of P in the two acid soils, showing a Negative correlation with Fe (II), this facility to make P available in acid soils and facilitate P availability in acid soils. The solubilization of Fe and Al phosphates also favored an increase in soil pH, which was also observed by (Sah *et al.*, 1989; Khan *et al.*, 2019).

Furthermore, the observed decrease in Eh, followed by the increase in pH and the reduction of Fe^{III} phases, all promoted the release of occluded P, which led to a strong decrease in the adsorption force of Fe-OP, which represents a increased P availability, possibly due to fluctuations in E_h during C mineralization, representing in this case the dynamics of OC (**Figure 4.3. 9**), which alters the Fe (III) reduction rate (Khan *et al.*, 2019; Wang *et al.*, 2021).

In Oxisol, the Fe (III) reductions decayed after a vertiginous start, this is possibly due to an almost neutral pH (6.5) at the end of the experimental stage, which is related to the fact that a more acidic pH produces a greater reduction of Fe (Johnson & Loeppert; 2006; Huang & Hall, 2017). The increase in Fe (II) concentration in the two soils under flood conditions (**Figure 4.3. 6**B and **Figure 4.3. 6**D), produced not only a decrease in Fe (III), but also a decrease in Fe (III) concentrations Fe_{ca}, which is Fe-Recalcitrant, these decreases control the formation of Fe (II) in acid soils such as those evaluated in this experiment. Some authors have established that the amount of Fe (II) produced in periods of anoxia (Flood), is predicted through selective methods such as the extraction of Fe-Citrate-Ascorbate, pointing to ordered phases of Short-range-order Fe (SRO), these Fe labiles are rapidly used as electron acceptors by Fe reducing bacteria (Gross *et al*., 2018). The resulting lability or reactivity of these phases (SRO) is based on the initial crystallinity of these phases, but also on the amount of aqueous Fe (Boland *et al*., 2014; Barcellos *et al*., 2018).

The most labile forms of Fe increased with these treatments, particularly in soils that contained more organic matter, given this is the case of the soils studied that presented high concentrations of OM (Sousa & Lobato, 2004; Matus *et al*., 2019). Therefore, higher concentrations of OC and Fe^{II} in soils Oxisols than Ultisols (**Figure 4.3. 6**) which are recorded, which leads to a rapid decrease in E_h (**Figure 4.3. 4**), after immersion, coinciding with current investigations (Zhao *et al*., 2018; Ginn *et al*., 2017; Khan *et al*., 2019), which leads to a faster oxygen consumption due to microbial activity and consequently a faster reduction of Fe^{III} to Fe^{II}, under these reducing conditions, considerable amounts of P are easily released with low binding energy (Handler *et al*., 2009; Couture *et al*., 2015; Ginn *et al*., 2017; Khan *et al*., 2019). It is important to emphasize that this release of P (**Figure 4.3. 10** B and **Figure 4.3. 10** D) occurs because Fe^{II} has a lower binding force than Fe^{III} (Lin *et al*., 2020). The reducing dissolution of crystalline Fe can be catalyzed by a high concentration of Fe^{II}, (Huang & Hall, 2017), resulting in a greater availability of P in Oxisol (Zhu & Whelan, 2018; Lizcano-Toledo, 2021); to this it should be mentioned that microbial mineralization is faster when there is greater OC availability (Greater in Oxisol), which reduced P through a specific adsorption analogous to phosphate ions (Narang *et al*., 2000; de Sousa Campos et al; 2019); or also by chelation of Fe and Al (Khan *et al*., 2019), which leads to an early availability P. On the other hand, the Fe-P concentration decreased in a pattern that was similar to an increase in available forms (P -$NaHCO_3$) for the plant, which suggests that the reduction of little available forms (Fe-P) (**Figure 4.3. 11**), which facilitated the mobilization of P which increased with flood management (**Figure 4.3. 10** B and **Figure 4.3. 10** D). However, after a dynamic until day 35 of Fe-P, it gradually stabilized under flood conditions for Oxisol (**Figure 4.3. 11**B). This could be due to the reabsorption of part of the P in crystalline oxides precipitating in complexes of Fe^{II}-P in Oxisols (Ajmone-Marsan, 2006; Amery & Smolders, 2012; Gou *et al*., 2020).

4.4.3 Dynamics of Phosphates fertilizers

Taking into account the use of the ^{32}P isotopic as shown in **Figure 4.3.1**, where the treatment with the highest P absorbed by the rice plant came from the Struvite fertilizer when evaluating the absorption of the plants at the end of the experiment; This effect may be associated with the dynamics of P through fractionation in the summary graph (**Figure 4.3.2**); Increases in P concentration were observed, which were higher in the Oxisol soil than in the Ultisol, possibly because the Oxisol soil presented higher Fe-P fixation characteristics (as discussed above), also higher concentrations. of organic matter (dissolved organic carbon) and CEC promoted by microbial activation (Esberg *et al*., 2010; Wei *et al*., 2019); These characteristics lead to greater adsorption and desorption processes of P in the soil (Brenner *et al*., 2018), being part of a dynamic and constant transformation where mineralogical, chemical and biological processes intervene, generating long-term transformations of P. and conjunctural changes due to P extraction by crops, which make it move from one pool to another (Alvarado, 2012; Frazão *et al*., 2019). In the final fractionation stage (75 days), there were higher concentrations of labile struvite P, followed by high-solubility fertilizers. This could have occurred due to the slow solubility of P characteristic of struvite (Struvite), which allows for a slower and more gradual release of P and less adsorption by the less available pool; Thus, the TSP and MAP fertilizers presented higher accumulations in the concentrations of P with lower availability (non-labile P) observed in **Figure 4.3.2.** Similar results were observed by Raniro *et al*., 2022, in which he worked with high solubility phosphate fertilizers, these promoted a high release of P during the first 60 days, however, within the fertilizers with which he worked, struvite showed a late release, although lower total leached P than TSP and MAP; in addition, the struvite promoted the highest concentration of P-labile in the soil.

4.4.4 Microbial activity in Redox processes

Microbial biomass has been assigned an important role in rice soils as a nutrient reservoir, a driving force for nutrient turnover, and an early indicator of soil/crop management (Shibahara & Inubushi 1997). More dynamic features such as microbial biomass, soil enzyme activity, and soil respiration respond very quickly. to changes in crop management practices or environmental conditions (Das *et al*., 2011). MBC and enzyme activity were higher under flooded conditions in rice and could be due to higher microbial activity under flooded conditions (Liesack *et al*. 2000). There was significant positive correlation of MBC with ADF, DHA, β-glucosidase (**Table 4.3. 2**) indicating that microbial activities and decomposition are the main driving forces of MBC content of soils. DHA is considered as an indicator of oxidative metabolism in soils and therefore of microbiological activity (Wlodarczyk *et al*., 2002), because it is exclusively intracellular and, theoretically, can only function within viable cells. Soil DHA increased significantly (p <0.01) as a result of constant moisture regimes in the two soils.

4.4.5 Plant biomass yield and SPAD Values

Leaf chlorophyll content readings (SPAD values) in plants can be affected by treatments, where higher values can be indicators of higher yield (Parthasarathi *et al*., 2012) and photosynthetic rate (Anjum *et al*., 2011), this happened with fertilizers high solubility phosphates that presented higher values than struvite and control; This significant increase may be due to the improvement in the nutritional condition of the soil, which may reflect plant growth and chlorophyll content, in addition to the role played by P in microbial activation and degradation of organic compounds, with the consequent release of N (Panhwar *et al*., 2014, Cao *et al*., 2017). It is also one of the most important biochemical indicators for plants (Liu *et al*., 2014; Halim *et al*., 2018). During this study, an initial increase in chlorophyll was observed in the early growth stage (10-35 DAT) for a subsequent decrease in SPAD values.

CHAPTER 5

General discussion (spanish version)

El P es un macronutriente requerido en altas concentraciones en los cultivos comerciales, no obstante, existe limitaciones en la física-química del P, debido a la ocurrencia de reacciones simultaneas e instantes como la solubilización, precipitación, adsorción (retención) y óxido-reducción (Abel *et al*., 2002; Wang *et al*., 2018; Zhang *et al*., 2019). A esto hay que sumarle que el P se debe adicionar en planes de fertilización sostenible, para lo cual hay que tener en cuenta, todas las reacciones que se pueden presentar como la alta fijación en suelos tropicales y subtropicales con elementos problema como Al y Fe en condiciones ácidas, así como también de Ca en pH alcalino (Bai *et al*. 2017; Qin *et al*., 2010). Se ha establecido que las reservas mundiales de P, publicado por la International Fertilizer Development Center – IFDC apuntan a reservas de cerca de 47 billones de toneladas de P_2O_5, basado en estos números y considerando los niveles actuales de producción, las reservas mundiales estarían disponibles por lo menos de 300 a 400 años (Everaert *et al*., 2018). Sin embargo, las reservas de excelente calidad ya se habrían agotado o estarían próximo de esto, lo que indica que el sector de fertilizantes depende cada día más de fuentes alternativas de P y/o prácticas agrícolas sostenibles (Schindler *et al*., 2016).

Entro los cultivos con una gran demanda de P se encuentra el arroz (*Oryza sativa* L.), cultivo esencial para la seguridad alimentaria mundial, pero que genera graves problemas debido a la aplicación excesiva de fertilizantes fosfatados acidulados, lo que conduce a altos impactos ambientales, además de la poca eficiencia agronómica (Reetz, 2016). De igual manera, el uso cada vez más insostenible del agua y pobres prácticas con nuevos enfoques agronómicos hacen que el P se vea limitado en los suelos arroceros (Yan *et al*., 2016). Es importante tener en cuenta que el P tiene importante relación con otros nutrientes, entre ellos el N, no solamente por ser aniones, sino también porque las gramíneas extraen en altas concentraciones entre estos dos macronutrientes (Eghball *et al*., 1990). La recuperación de fertilizante nitrogenados por parte de los cultivos suele oscilar entre 30 hasta el 70 %. Es bien sabido que cualquier factor que limite el potencial de rendimiento, reducirá la recuperación y el beneficio de los nutrientes aplicados (Cassman & Doberman, 2022). Por ejemplo, en suelos deficientes de P, la fertilización fosfatada eficiente, aumenta la respuesta en la absorción de N, lo que evidencia el efecto sinérgico entre el P y N como lo relata algunos autores (Ameloot *et al*., 2015).

Una forma de implementar prácticas agronómicas sostenibles con un gran potencial para mejorar la fertilidad del suelo y el ciclo de N y P, es el establecimiento de cultivos de cobertura (Delgado & Gantzer 2015). Estos pueden incrementar el rendimiento de los cultivos y la calidad de las cosechas (Ren *et al*., 2019). Entre los cultivos de coberturas usados en la agricultura se encuentra la Veza (*Vicia villosa*), la cual puede proporcionar cantidades significativas de N a los suelos arroceros (Cassman & Doberman 2022). El manejo de la veza también limita las pérdidas de NO_3^- durante el invierno europeo (Girondé *et al*., 2015). La incorporación de residuos de veza puede influir en la disponibilidad de N en el suelo. Además, estos residuos presentan relación C:N relativamente baja, siendo una fuente directa de N (Said-Pullicino *et al*., 2014). En este contexto, los cultivos de cobertura pueden aumentar también la disponibilidad de P, debido a la mayor actividad microbiana que aprovecha el aumento de residuos vegetales (energía), lo que puede favorecer la liberación de P debido a la producción de ácidos orgánicos que pueden disolverse o competir con el fosfato por los mismos sitios de sorción de Fe y Al (Oberson *et al*., 2015; Celi *et al*., 2020).

Para entender la dinámica de P y su sinergia con el N, se cultivaron plantas de veza inoculadas con rizobios en un suelo con P limitado y evaluando el crecimiento de la planta, la absorción de N y P y la capacidad BNF (Por dilución de isótopos), en función de las entradas de Pi y Po en forma de ortofosfatos y ácido fítico respectivamente. Para esto, nos planteamos la hipótesis de que bajo una limitación severa de P, (a) mejorar el suministro de P a través de entradas de Pi, lo que puede favorecer el crecimiento de la planta y la asignación de C a los nódulos de la raíz y, en consecuencia, mejorar la capacidad de BNF de las plantas de veza, mientras que (b) aumentar Po como producto que puede mejorar parcialmente el efecto de limitación de P en la fijación de N_2, gracias a la capacidad de las plantas de veza para acceder a fuentes de Po. A continuación, los objetivos del capítulo 2 fueron los siguientes:

1. Comprender cómo la disponibilidad y las formas de P del suelo pueden afectar la producción de biomasa y la eficiencia de BNF de un cultivo de cobertura típico (*Vicia villosa*).
2. Determinar la disponibilidad de Po y su capacidad de aumentar la eficiencia de la BNF.

A continuación, los resultados y la discusión más relevante del capítulo 2 establece como primer lugar la relación que hay entre el crecimiento de la leguminosa y la disponibilidad de P en el suelo, que puede afectar directamente las tasas de fijación de N_2, esto puede ocurrir por varios factores como la alta actividad microbiana (energía), la capacidad fotosintética de la planta y el C almacenado del suelo a las raíces y nódulos (Divito & Sadras, 2014). En nuestro caso, hubo un aumento del efecto del Pi sobre las variables de crecimiento, lo que condujo a la veza a invertir menos energía en el desarrollo de las raíces (menores relaciones Raíz:Brote). En segundo lugar, el aumento de la absorción de N se vinculó previamente con una respuesta de crecimiento al suministro de P en varias otras especies de leguminosas (P. ej., *Trifolium repens* L. por Almeida *et al*. 2000 and Høgh-Jensen *et al*. 2002, *Medicago* spp. por Püschel *et al*. 2017). En tercer lugar, hubo una correlación entre el aumento de la biomasa de los nódulos de la veza con la disponibilidad de P, lo que da lugar, como cuarto aspecto relevante de este estudio que mientras la nodulación estaba fuertemente limitada por el P, las plantas distribuían más P a la simbiosis con las bacterias fijadoras de N_2, cuando el P se encontraba altamente disponible. Como quinto punto, en este trabajo observamos aumentos en la absorción de P, como resultado de la adición de Po, sobretodo en la dosis más alta, aunque menor que con Pi, lo que sugiere que la veza puede, hasta cierto punto, acceder a fuentes de P orgánico para sus requerimientos de P, y esto puede mejorar parcialmente el efecto de limitación de P en la fijación de N_2, no obstante el Po no afectó significativamente el crecimiento y nodulación de las plantas, estos hallazgos están en la línea con Adams & Pate (1992), quienes demostraron que, aunque el fosfato mioinositol es una fuente potencial de P para el crecimiento de *Lupinus* spp. Y que son capaces de regular al alza las enzimas fosfohidrolazas de la raíz, que representa una fuente de P mucho más pobre en el suelo, donde la disponibilidad de la susceptibilidad a las fosfatasas del suelo y la interacción con los minerales del suelo (Celi *et al*., 2004; 2020; Giavento *et al*., 2010).

Teniendo en cuenta la dinámica del Pi y Po en la veza y en la disponibilidad de N a través del proceso de BNF, se llevó a cabo una segunda investigación (Capítulo 3) en condiciones de campo. En este sentido, los cultivos de cobertura como la veza, puede aumentar la disponibilidad del P a lo largo de la fenología de cultivos como el arroz, a través de la acción microbiana por el efecto de los residuos vegetales, lo que pueden favorecer la liberación de P, por la acción de los ácidos orgánicos (Oberson *et al*., 2015; Celi *et al.,* 2020).

Con esta investigación se estableció como objetivos, investigar los efectos de los cultivos de cobertura (*Vicia villosa*) sembrados en estación invernal antes del cultivo principal (Arroz), en la estación primavera-verano, en un agroecosistema templado (noroeste de Italia) sobre: (i) el rendimiento de los cultivos y los componentes de producción, (ii) la recuperación aparente de N del fertilizante y el nivel óptimo de fertilización nitrogenada con la veza; y, (iii) la variación temporal de las formas de N y P durante la temporada de cultivo de la leguminosa y el arroz.

De acuerdo con nuestros resultados, la veza condicionó las variables de rendimiento, en función a los niveles de fertilización nitrogenada, resultados similares fueron descritos por otros autores por (Haque *et al*., 2013, Zhu *et al*., 2014 and Xia *et al*., 2018), donde los autores encontraron que los cultivos de cobertura estimulan significativamente el crecimiento del arroz y el rendimiento de las plantas, como se observó en el capítulo 2, que la veza puede acumular N y P dependiendo del tipo de crecimiento y la disponibilidad de P a lo largo del tiempo (Yang *et al*., 2019). Fue interesante observa que el efecto de la veza fue más evidente con niveles bajos de N (80 Kg N ha^{-1}), siendo superior en un 12 % al manejo del cultivo de arroz sin cultivo de cobertura, y similar a los rendimientos obtenidos con el mayor nivel de fertilización que fue de 160 Kg N ha^{-1}. Yang *et al.* (2019) observaron resultados similares, donde el cultivo de cobertura mejoró los rendimientos en cultivos de arroz de ciclo corto y largo en el norte de china, en el que se concluyó que se podría reducir hasta el 20 % de la aplicación de N en la fertilización, sin comprometer la productividad. También la veza incrementó la disponibilidad de N y P, mostrando una gran capacidad para acumular biomasa aérea y aporte de N (Campiglia *et al*., 2010). Alcanzando una biomasa aérea de 150 Kg N ha^{-1} y 166 Kg N ha^{-1}. Otro aspecto importante al incorporar la veza al suelo, fue la liberación de NH_4^+ en el suelo, rápidamente disponible en las primeras etapas del cultivo, incluso abarcando el efecto de la Urea. La mineralización neta de N, evidenció que llegando a los 40 días, alcanzó valores comparables al aparte derivado del nivel más alto de fertilización N, tanto en condiciones aeróbicas como anaeróbicas, lo que lleva a que la veza presente relaciones C:N relativamente bajas (Anougroho *et al*., 2010); además se puede incorporar la veza con residuos de barbecho de cosechas anteriores, pueden tener un efecto positivo (Liang *et al*., 2017; Zhou *et al*., 2019), lo que lleva a un aumento de la mineralización neta, lo que corresponde al 80 % del aparte total de N. Sin embargo, lo más interesante fue el hecho de que, en condiciones anaeróbicas, la incorporación de veza alimentó continuamente la reserva de N disponible incluso en el lapso de 60 a 100 días, lo que favoreció la mineralización neta de N más allá de la cantidad de N orgánico incorporado al suelo. Esto puede conducir a una fuente continua de NH_4^+ durante los períodos de mayores requerimientos nutricionales en el arroz, correspondientes a la etapa de floración y llenado del grano (Lowry *et al*., 2021; Haque *et al*., 2013).

En cuanto al P, aunque no se encontraron diferencias en su contenido tanto en grano como en paja, la absorción de P total se vio significativamente afectada por la presencia de veza, como consecuencia de la mayor biomasa. Esto fue seguido por una mayor disponibilidad de P en el suelo en las parcelas donde se incorporó la veza. Encontramos cambios significativos en el contenido de Pi-$NaHCO_3$, a partir de la fecha de siembra y alcanzando valores un 25% más altos con veza, que sin ella en la etapa de macollamiento. Esta fracción de P se considera P lábil y sus cambios están influenciados en gran medida por el biociclado favorecido en presencia de un residuo altamente degradable, como lo es la arveja (Kuo *et al*,. 2005; Wang *et al*., 2016), con un reciclaje rápido de P inmovilizado tanto en la veza, como en la biomasa de paja. La fracción de P extraída con NaOH fue en general diez veces mayor que con $NaHCO_3$. La fracción Pi-NaOH, que representa el P fuertemente adsorbido en las superficies de (hidr)óxido de Fe y Al en el suelo, generalmente se reporta como la más alta en ambientes de arrozales (Xiao *et al*., 2017).

Ya en el contexto global y frente a diferentes escenarios en el que se maneja el P, se realizó una tercera investigación cuyo objetivo principal fue determinar la dinámica del P en suelos ácidos tropicales (Oxisol y Ultisol), adicionando fuentes de fertilizantes fosfatados convencionales y alternativos, sometidos a diferentes formas de riego, evaluando los cambios Redox y la dinámica relacionada con el Fe. Para esto, se desarrolló en condiciones controladas en el centro de energía nuclear para la agricultura (CENA), ubicado en el estado de São Paulo (Brasil). Los resultados obtenidos sugieren que el tipo de arcilla es fundamental para determinar la adsorción de P en el suelo (Jalali & Jalali, 2016; Fink *et al*., 2016). A nivel mineralógico se pudo definir que la caolinita (kt) es el mineral arcilloso predominante que se presentó en los dos suelos ácidos evaluados. el suelo Oxisol, que además de la presencia de Kt, presentó Gibsita (Gb), Goethita (Gt) y Hematites (Hm + Gt), este suelo, también presentó Vermiculita con Hidroxilos de Aluminio Intercalado (HIV por sus siglas en inglés), lo que originó en una mayor retención de P, de acuerdo a la prueba de máxima capacidad de adsorción de P. El P adsorbido o coprecipitado con óxidos de Al y Fe son las formas menos lábiles (Esberg, 2010; Xu *et al*., 2014). Por tanto, debido al predominio de los óxidos de Fe y Al en los suelos, es posible esperar un bajo contenido de P en las fracciones lábiles, a menos que el encalado promueva la precipitación de fosfatos de calcio más solubles en los suelos donde se ha aplicado fertilizantes fosfatados solubles y poco solubles como es el caso de este experimento (Zavaschi *et al*., 2020). En el caso de los suelos ácidos estudiados, durante el riego por inundación, el pH aumentó hasta alcanzar la neutralidad (Huguenin-Elie *et al*., 2009; Wei *et al*., 2019). La intensidad de la reducción fue mayor en presencia de materia orgánica y su estado activo, que es el OC (Carbono orgánico), ya que se oxida y se reducen los componentes del suelo debido a la respiración microbiana anaerobia (Ponnamperuma 1972; Keiluweit *et al*., 2017; Wei *et al*., 2019; Wang *et al*., 2021). Esta puede ser la razón de los bajos valores de E_h en los suelos con concentraciones altas de CO, esto se observó con los valores de CO disueltos (DOC).

Respecto a la dinámica del Fe, la disminución observada en el E_h, seguida por el aumento del pH y la reducción de las fases de Fe^{III}, promovieron la liberación de P ocluido, lo que condujo a una fuerte disminución en la fuerza de adsorción de Fe-OP, lo que representa una mayor disponibilidad de P, posiblemente debido a fluctuaciones del E_h durante la mineralización de C, representando en este caso la dinámica de OC, que altera la tasa de reducción de Fe (III) (Khan *et al*., 2019; Wang *et al*., 2021). Las formas más lábiles de Fe aumentaron con estos tratamientos, particularmente en los suelos que contenían más materia orgánica, como los suelos estudiados (Souza & Lobato, 2004; Matus *et al*., 2019). Se observaron mayores concentraciones de OC y Fe^{II} en el suelo Oxisol que en el ultisol. Esto puede conllevar a disminución del E_h. Esto se observó después de la inundación, coincidiendo con otras investigaciones (Joulina *et al*., 1997; Ginn *et al*., 2017; Khan *et al*., 2019). En estas condiciones reductoras, se liberan fácilmente cantidades considerables de P con baja energía de unión (Handler *et al*., 2009; Couture *et al*., 2015; Ginn *et al*., 2017; Khan *et al*., 2019).

Ya en escenarios globales, con miras a futuro, y dados los problemas de la agricultura actual, la cual tiene como principio la extracción y remoción de nutrientes, lo que implica que a que haya un desbalance ecológico de nutrientes como el P. Esto, por un lado, hace que aumente la desertificación y salinización de los suelos agrícolas, y, por otro lado, la escasez y aumento de los precios de los alimentos (Khan *et al*., 2018). Es importante destacar la importancia de no solo mejorar la cantidad de alimento, sino también, la calidad de los mismos, esto incluye calidad nutricional de las leguminosas y los cereales tienen un efecto directo en los seres humanos y son los alimentos más consumidos en el mundo (Jia *et al*, 2020). La gestión mejorada de los nutrientes, pueden potenciar la disponibilidad de los macronutrientes, requisito fundamental para el crecimiento y desarrollo de los cultivos y conseguir los rendimientos óptimos (Li *et al*., 2019). Además de recursos como el agua, el aire, la luz solar y las condiciones física de los suelos, los nutrientes deben ser esenciales para mejorar no solo la producción, sino también mejor la resiliencia de las plantas ante el cambio climática, y generar un impacto positivo en la salud humana a través de la calidad nutricional de los alimentos (Fankem *et al*., 2006).

La contribución de técnicas nucleares e isotópicas para elementos como el C, N y P permiten estimar el contenido de N procedente de fuentes alternativas fijado y agregado al suelo por las leguminosas, además de la cantidad de carbono fijado por las plantas y secuestrado en el suelo. Estas herramientas ayudan a rastrear el movimiento desde el suelo hasta la planta y la determinación de la eficiencia del fertilizante, así como también, las pérdidas que pueden ocurrir a partir de la percolación o escorrentía, terminando en cuerpos de agua (Eutrofización). Además, este tipo de técnicas permiten analizar la dinámica del P en la transición del suelo hacia la planta para mejorar su gestión en los ecosistemas agrícolas (Yan *et al*., 2016)

Estudiar a futuro la importancia de relaciones estequiométricas importantes de otros nutrientes con el P, como por ejemplo la relación P-Zn, ya que estudios han observado (Cakmak & Marschner, 1987) que cuando se aumenta las concentraciones de P, se incrementa también la biomasa de las plantas, sin embargo, el contenido en los tejidos es bajo. Los dos elementos cumplen un papel importante en la estimulación de biomasa radicular, lo que puede llevar a un mejor anclaje, pero también mayor absorción de nutrientes por parte de la planta (Hammond & White, 2008).

Por otro lado, esta tesis y sus resultados, pueden incentivar investigaciones locales o regionales para reducir el uso de Urea, un fertilizante altamente soluble, pero también, altamente contaminante y la reducción de fertilizantes fosfatados acidulados, que pueden alterar la microbiota del suelo por su propiedades para alterar el pH del suelo, por fuentes alternativas, algo necesario, ya que las reservas de roca fosfórica (Principal materia prima para la producción de fertilizantes fosfatados acidulados), está limitado con acceso a muy pocos países, con precios históricamente muy elevados, haciendo improductivo el sector agrario. Es por esto, que fuentes alternativas de materiales reutilizables como la estruvita, el Biochar, o el uso de tecnologías con portadores como el Grafeno, podrían definir una nueva era de fertilización alternativa y balanceada, con menos contaminación, disminuir los procesos de pérdidas y limitaciones de P (Antes no se hablaba de pérdidas de P) a mediano plazo. Por lo tanto, las investigaciones futuras deben ser dirigidas a: (i) Investigar la dinámica del P orgánico en los ciclos leguminosa-graminea; (ii) Evaluar la eficiencia agronómica de los nuevos fertilizantes fosfatados alternativos en condiciones del trópico y subtrópico y la posible combinación con fertilizantes fosfatados convencionales; (iii) Determinar como estos fertilizantes alternativos, puede impactar el flujo de C y su alteración en gases de efecto de invernadero; (iv) Evaluar el papel del Carbono orgánico y su relación con la dinámica de P en suelos inundados en el manejo de arrozales; (v) Evaluar la calidad nutricional de alimentos como el arroz y las leguminosas con aplicaciones de fertilizantes alternativos y el aporte de P orgánico y, (vi) Entender la importancia de otras relaciones estequiométricas como la relación P-Zn.

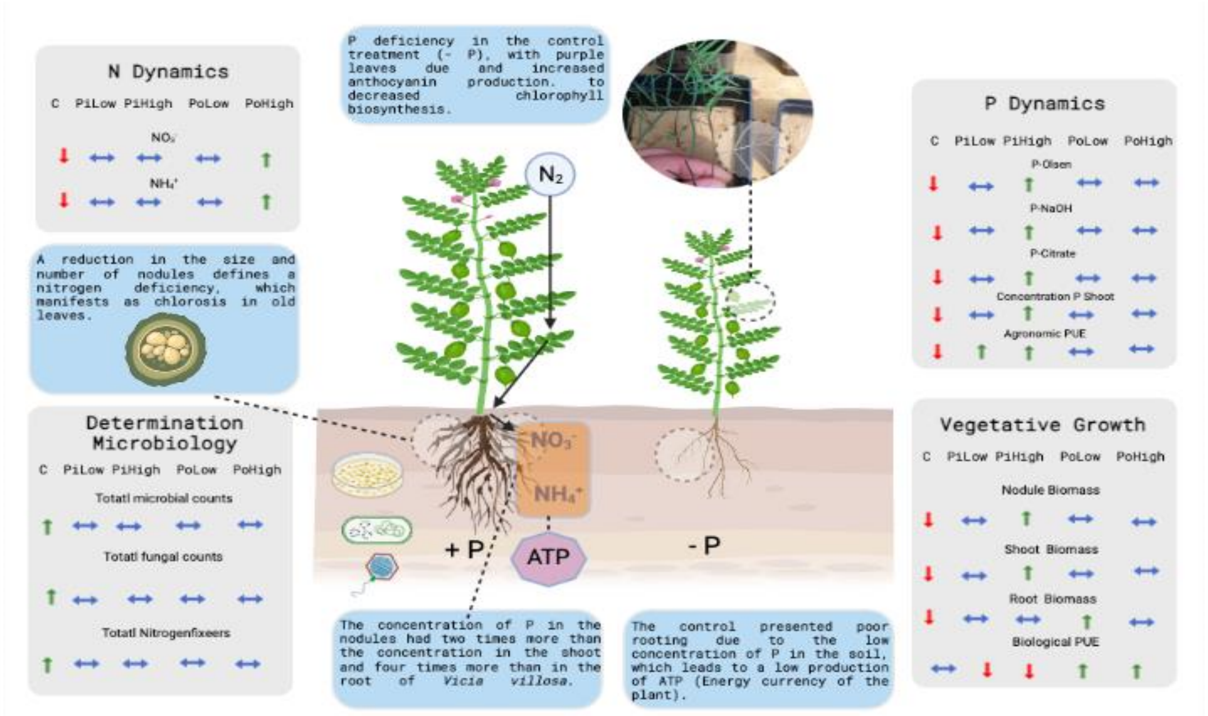
CHAPTER 6

General discussion and conclusions

6.1. Chapter 2

In general, plant growth is greatly influenced by soil mineral P availability, and in leguminous plants, an increase in P availability can positively affect BNF rates. This can be due to the high P demand of N_2-fixing bacteria as well as to the effect of P availability on the plant photosynthetic capacity and belowground C allocation to roots and nodules (Divito and Sadras 2014).

The effects of P availability on *Vicia villosa* growth and BNF capacity are biologically interesting and agronomically relevant, particularly when considering the importance of this leguminous cover crop for improving organic N inputs and reducing mineral N use in agroecosystems. When compared to P-deficient conditions where BNF was primarily limited by plant growth rather than directly due to the high P costs of symbiotic N fixation, increasing the soil P availability through the addition of Pi enhanced plant growth (3-fold), nodule formation (16-fold), P acquisition (6-fold) and BNF efficiency (7-fold). With the addition of Po, hairy vetch was able to access organic P sources that could partially alleviate the P limitation effect on N_2 fixation, albeit to a lesser extent with respect to Pi and without actually increasing plant growth or N acquisition through BNF. However, the strategies that could have been activated by hairy vetch to increase nutrient availability under P deficient conditions remain elusive as no notable effects of P addition on rhizosphere soil phosphatase activities were observed.



	C	PiLow	PiHigh	PoLow	PoHigh
NO_3^-	↓	↔	↔	↔	↑
NH_4^+	↓	↔	↔	↔	↑

A reduction in the size and number of nodules defines a nitrogen deficiency, which manifests as chlorosis in old leaves.

	C	PiLow	PiHigh	PoLow	PoHigh
Totatl microbial counts	↑	↔	↔	↔	↔
Totatl fungal counts	↑	↔	↔	↔	↔
Totatl Nitrogenfixeers	↑	↔	↔	↔	↔

P deficiency in the control treatment (- P), with purple leaves due and increased anthocyanin production. to decreased chlorophyll biosynthesis.

The concentration of P in the nodules had two times more than the concentration in the shoot and four times more than in the root of *Vicia villosa*.

The control presented poor rooting due to the low concentration of P in the soil, which leads to a low production of ATP (Energy currency of the plant).

	C	PiLow	PiHigh	PoLow	PoHigh
P-Olsen	↓	↔	↑	↔	↔
P-NaOH	↓	↔	↑	↔	↔
P-Citrate	↓	↔	↑	↔	↔
Concentration P Shoot	↓	↔	↑	↔	↔
Agronomic PUE	↓	↑	↑	↔	↔

	C	PiLow	PiHigh	PoLow	PoHigh
Nodule Biomass	↓	↔	↑	↔	↔
Shoot Biomass	↓	↔	↑	↔	↔
Root Biomass	↓	↔	↔	↑	↔
Biological PUE	↔	↓	↓	↑	↑

Figure 6.1. 1 Graphical abstract of the conclusions of chapter 2

6.2. Chapter 3

Planting and cultivating legume cover (*Vicia villosa*) during the cold fallow season is recommended as an important agronomic practice to improve total biomass productivity and soil fertility in mono-rice (*Oryza sativa L.*).

The calculation of how much N fertilizer is actually possible to save in the presence of a good green manure cannot ignore a number of factors such as temperature trends, the type of soil and the variety of rice. The examination of the production results of 2019 shows how the paddy yield of the green manure plots reaches the maximum production with an integration of 80 kg ha^{-1} of mineral N while in its absence it is necessary to add 120 kg of N ha^{-1}. We could therefore estimate a saving of 40-50 kg of mineral N, which corresponds, taking into account the estimated apparent efficiency of 30 %, to a biomass N supply of 150 kg ha^{-1}.

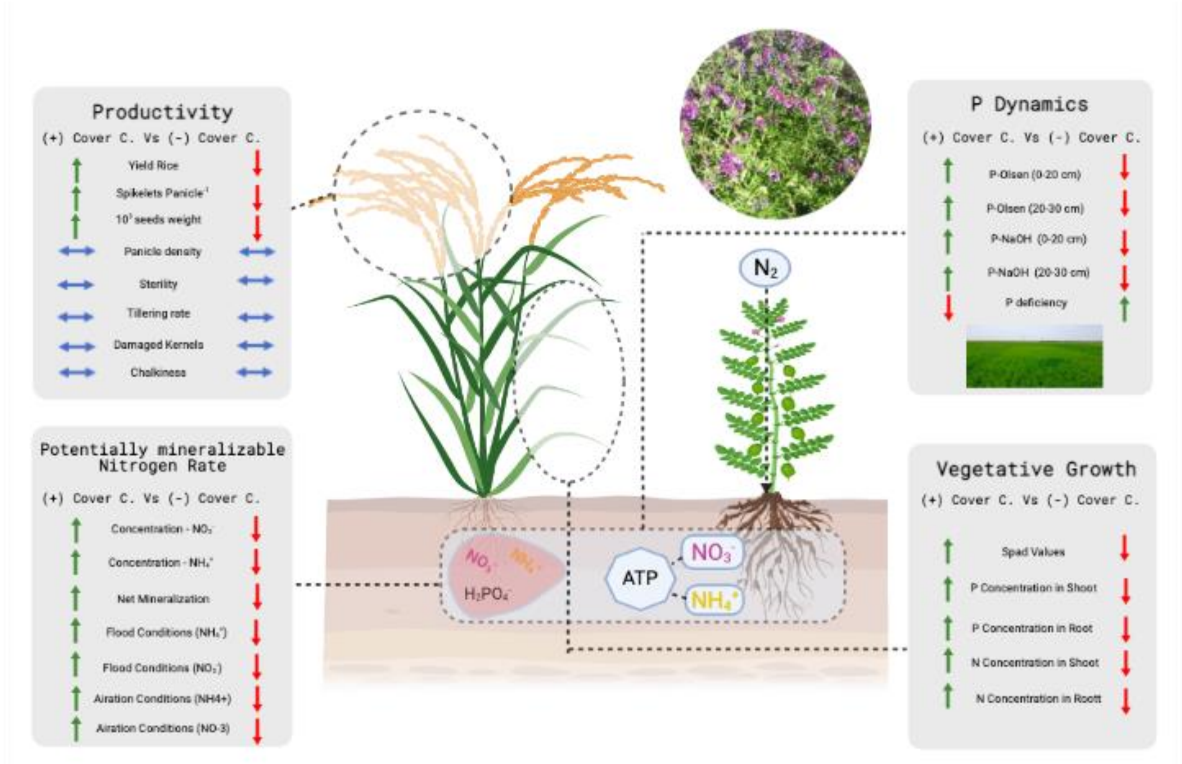


Figure 6.2. 1 Graphical abstract of the conclusions of chapter 3

Labile P deficiency is an important factor limiting the growth of rice in paddy soils. The hairy vetch, with higher N and P concentrations, as well as lower C:N and C:P ratios, significantly increased available soil P through high organic P input and transformation to labile P. This increased in available P in the soil may be closely related to a high soil enzyme activity induced by vetch addition, which had significant direct and indirect effects on grain production. The positive effect of vetch on increasing grain yield with low N nutrient level can be attributed to its good functions in increasing available P in the soil, promoting P uptake and enhancing the interactive effect of N and P. This distintive ability of the vetch is very important. Useful to reduce the application of chemical fertilizers in agroecosystems, maintain a sustainable supply of P for rice cultivation and reduce eutrophication.

6.3. Chapter 4

Struvite is an alternative P source, even though it has a slow-release effect, under constant water depth conditions (flooding condition). Struvite presents a slow release of P; thus, it is not recommended to be used alone in fertilization plans in short-cycle crops such as rice, but it should be combined with high soluble fertilizers. In addition, this combination could be more in favor under reduced conditions (flooding), in soils where there are high concentrations of low crystallinity Fe (**Figure 6.3. 1**).

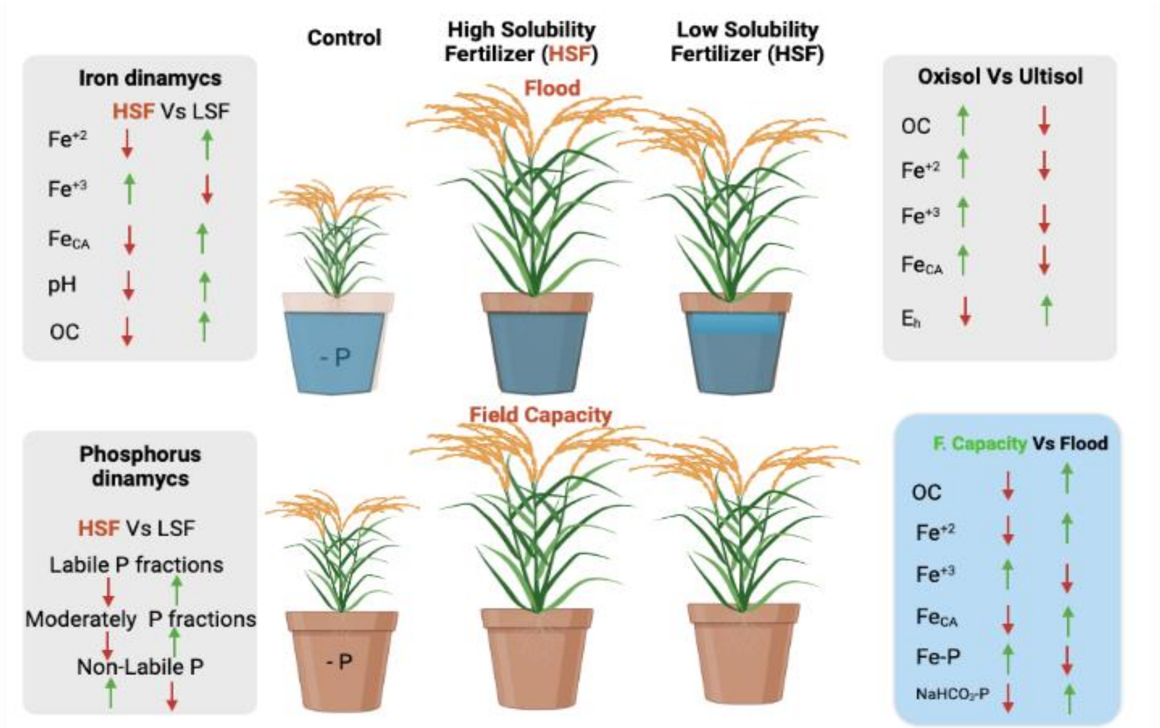


Figure 6.3. 1 Graphical abstract of the conclusions of chapter 4

Physical-chemical and microbiological characteristics, such as clay content, OM, CEC, Fe concentration, pH, P-fixing capacity, should be integrated to correctly determine P availability from these fertilizers. Weathered and acidic soils may present different P dynamics depending on soil moisture and irrigation regimes adopted or naturally occurring. Soils subject to reducing conditions may better allow the release of P (P-Labile), however it is important to establish a monitoring of P availability, which will also depend on the type of system to be implemented in rice crops.

6.4. General conclusions, environmental significance and future perspectives

Chapters 1 and 2 carried out in Italy establish important conclusions related to aspects of the dynamics of P in legumes as well as their availability for next cropping system.

Cover crops improved biological and physiological processes as a response to P addition in organic or inorganic form, influencing the N biological fixation efficiency. This in turn resulted in a better availability of nutrients in soil, increasing the foliar and root biomass (including nodules) of *Vicia villosa.* The concentration of P in the different parts of the legume plant was affected by the form of P, with better values with Pi. The use of legumes as a cover crop has great benefits, not only allowing for the reduction of N fertilizers, but also advantages in the rice productivity and quality (CHAPTER 3).

CHAPTER 4, focused on P dynamics in tropical acid soils, highlighted the important role of organic matter in soils with a high P fixation capacity as well as the type of short-term Fe minerals. In these soils the use of slow-release phosphate fertilizers seems a good solution in combination with the use of highly soluble fertilizers, obtaining a good response for future crops and leaving an appropriate reserve of labile P for successive crops.

These results could allow the reduction of N fertilizer use such as urea, a highly polluting fertilizer and the reduction of conventional phosphate fertilizers, that can help to solve the issue of exhaustion of P reserves. The recovery of fertilizers from waste materials such as struvite could define a new era of fertilization with less polluting materials and guarantee P availability in the medium and long term. Thus, future researches should be addressed to: i) investigate the dynamics of organic P in the legume-rice cycles; ii) Evaluate the agronomic efficiency of new alternative fertilizers in acidic soil conditions and their possible use in combination with conventional phosphate fertilizers; iii) Determine how the use of alternative fertilizers can impact on C cycling and climate change mitigation.

6.5. Conclusions (Spanish version)

1. Los efectos de la disponibilidad de P en el crecimiento de *Vicia villosa* y la capacidad de BNF son biológicamente interesantes y agronómicamente relevantes, particularmente cuando se considera la importancia de este cultivo de cobertura de leguminosas para mejorar los aportes de N orgánico y reducir el uso de N mineral en los agroecosistemas.

2. El aumento de la disponibilidad de P del suelo mediante la adición de Pi mejoró el crecimiento de la planta (tres veces), la formación de nódulos (16 veces), absorción P (6 veces) y eficiencia en la BNF (7 veces).

3. Con la adición de Po, la veza pudo acceder a fuentes de P orgánico que podrían mejorar parcialmente el efecto de limitación de P en la fijación de N_2, aunque en menor medida con respecto a Pi y sin aumentar el crecimiento de la planta o la absorción de N a través de BNF

4. El cálculo de cuánto fertilizante nitrogenado es realmente posible ahorrar en presencia de un buen abono verde, no solo depende de la leguminosa, sino también de una serie de factores como las tendencias de temperatura, el tipo de suelo y el requerimiento nutricional de la variedad de arroz.

5. Se estima que en este estudio se podría tener un ahorro de 40-50 kg de nitrógeno mineral, lo que corresponde, teniendo en cuenta la eficiencia aparente estimada de un 30 %, con un aporte de N en biomasa de 150 kg ha^{-1}.

6. La capacidad distintiva de la veza para proporcionar N a mediano plazo es muy útil para reducir la aplicación de fertilizantes nitrogenados en arrozales, para mantener un suministro sostenible de N y P para el cultivo de arroz y reducir problemas como la eutrofización.

7. El efecto positivo de la veza en el aumento del rendimiento de grano con un aporte bajo de fertilización nitrogenada, puede atribuirse a sus buenas funciones para aumentar el P disponible en el suelo, promover la absorción de P y mejorar el efecto en la interacción de N-P.

8. La estruvita como fuente de P, a pesar de su lenta liberación, presentaba altas concentraciones de P lábil y moderadamente lábil al final del cultivo, esto debido al aporte de carbono orgánico en el fertilizante, materia prima para la activación microbiana y enzimática.

9. Las condiciones de inundación propiciaron mayor disponibilidad de P a corto y mediano plazo, debido a procesos como la desorción de P fijado por elementos como el Fe, micronutriente que se encuentra en altas concentraciones y puede llegar a ser tóxico en suelos tropicales.

10. El suelo Oxisol presentó mayores concentraciones de Fe^{+2} y P lábil y moderadamente lábil, además de una mayor activación microbiana y enzimática, esto debido a mayores contenidos de materia orgánica, mayor adsorción de P, con mayores contenidos de arcilla (Fracción más activa del suelo) y contenido mineralógico de Al y Fe, lo que conduce a la importancia de una caracterización fisicoquímica y microbiológica, en el manejo de la fertilización fosfatada.

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