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MEMORIA

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EFFECTS OF ALTERNATIVE PHOSPHORUS FERTILIZERS ON PHOSPHORUS MOBILIZATION BY DIFFERENT COVER CROPS IN TWO SOILS

by

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Esta es la memoria del trabajo fin de Carrera realizado por la alumna Carolina Rodríguez Garraus en la "University of Natural Resources and Applied Life Sciences (BOKU) " de Viena, Austria. En ella se presenta un pequeño resumen en castellano del trabajo en sí, por lo que sería conveniente leer el trabajo original para tener una visión global de los resultados.

INTRODUCCIÓN

Este trabajo de fin de Carrera forma parte del proyecto de investigación "IMPROVE-P (Improved Phosphorus Resource efficiency in Organic agriculture Via recycling and Enhanced biological mobilization)" llevado a cabo por el departamento de Agricultura Orgánica de la universidad de BOKU en Viena, y consiste en el análisis de los efectos de dos fertilizantes alternativos de fósforo (APFs): ceniza de lodo de aguas residuales tratadas y digestatos, en la movilización de este elemento mediante el uso de tres cultivos de cobertura: *Fagopyrum esculentum, Phacelia tanacetifolia y Trifolium pratense,* en dos suelos diferentes, uno con un alto contenido en carbonatos y otro sin ellos.

El motivo de este trabajo reside en la importancia del fósforo para el crecimiento de los cultivos, ya que forma parte de moléculas principales como ácidos nucleicos, fosfolípidos y ATP, por lo que las plantas no pueden crecer sin un suministro fiable de este nutriente. Sin embargo la concentración de fósforo disponible para las plantas es muy baja en la mayoría de los suelos, y por ello se usan fertilizantes fosfatados cuyo principal componente es la roca fosfatada, que es un recurso no renovable y limitado. Debido a ello y al aumento de la población y demanda de comida en el mundo, resulta necesario encontrar nuevos métodos de reciclar los recursos de fósforo, ya que con los actuales no se va a poder mantener el ritmo actual de consumo más de 100 años.

De acuerdo con numerosos autores, el reciclaje de fósforo a partir de materiales que normalmente se pierden en forma de residuos de lodos de depuradoras, de animales y vegetales, puede reducir el uso de fertilizantes químicos, y además el fósforo de estos materiales orgánicos se mineraliza por los organismos del suelo en formas disponibles para las plantas. Por otra parte se ha demostrado en numerosos estudios que la incorporación de cultivos de cobertura puede estimular la movilización de fósforo en el suelo.

Por todo ello, la cuestión principal de este experimento es comprobar si la combinación de cultivos de cobertura con APFs reciclados podría representar una opción sostenible para aumentar la eficiencia de fósforo, así como reemplazar la aplicación de roca fosfatada.

Para ello los objetivos de este estudio fueron:

- Comprobar en cuál de dos suelos diferentes, uno ácido y uno calcáreo, hubo más movilización de fósforo así como cuál de ellos era mejor para cultivar.

- Comprobar si *Trifolium pratense* (trébol rojo), al ser una leguminosa, movilizó más fósforo que los otros dos cultivos estudiados (*Fagopyrum esculentum*(trigo sarraceno*) y Phacelia tanacetifolia* (phacelia)).

- Comprobar si alguno de los APFs reciclados produjo más biomasa, mayor concentración de fósforo en los cultivos de cobertura así como una mayor cantidad de fosforo en la biomasa aérea que la roca fosfatada.

- Comprobar si no hubo interacción entre los suelos, cultivos de cobertura, APFs, suelos y cultivos de cobertura, suelos y APFs, cultivos de cobertura y APFs y suelo con cultivo de cobertura y con APFs.

En esta breve memoria explicativa en castellano se incluyen los siguientes apartados:

- Materiales y métodos
- Resultados
- Discusión
- Conclusión

MATERIALES Y MÉTODOS

MATERIALES

- **Suelos:** Los dos suelos se recogieron de campos de dos localidades austriacas tras haber cultivado en ellos trigo**.** El suelo libre de carbonatos y ligeramente ácido (Cambisol) fue tomado de Gföhl, mientras que el suelo con carbonatos y neutro (Chernozem) se recogió de Münchendorf, ambas localidades pertenecientes a Baja Austria. Las macetas de 324 cm^2 fueron rellenadas exactamente con 4 kg de suelo. En la siguiente tabla se pueden observar los parámetros químicos de ambos suelos.

Parameter/Soil	Carbonate-free soil (a)	Carbonate soil (c)
Texture	loam	loamy sand
pH $_{CaCl2}$	6,4	7,5
P_{CAL} [mg kg^{-1}] (August 2014)	3,06	19,32
P_{CAL} [mg kg^{-1}] (September 2014 after harvest)	26,5	16,2
Mineral N [mg kg^{-1}]	12,3	10,9
Total N [g kg^{-1}]	1,85	4,55
Total C [g kg^{-1}]	17,11	92,28
C inorganic [g kg^{-1}]	0,20	52,70
C organic [g kg^{-1}]	16,90	39,50
C/N ratio	9,14	8,68
$CaCO_3$ [g kg^{-1}]	1,48	439,57
Water-holding capacity %	45	63

- **Cultivos de cobertura:** Las semillas de los tres cultivos de cobertura diferentes (trigo sarraceno, phacelia y trébol rojo) se sembraron en macetas de acuerdo a las recomendaciones de una empresa de cultivo de semillas (Saatzucht Gleisdorf Ges.mbH) para kg ha^{-1} en cantidad triple. El riego fue principalmente por lluvia, y solo en el caso de que las macetas estuvieran secas se aplicó el sistema de riego (250 ml por maceta los Lunes, Miércoles y Sábados a la 1 a.m.)

- **Fertilización:** La fertilización estuvo a cargo de Lina Weissengruber (compañera del proyecto IMPROVE-P) antes de mi inicio en este experimento. Usó ceniza lodos de depuradora de magnesio tratado de un socio del proyecto de la ETH Zürich, roca fosfatada ["P26 Naturphosphat"] de Timac AGRO Düngemittelsproduktion und Handels-GmbH y digestato procedente de la planta de biogás "Biogas Bruck / Leitha GmbH & Co KG". Todas las macetas tenían el mismo contenido de nitrógeno y potasio, siendo el fósforo el único elemento limitante.

DESCRIPCIÓN DEL EXPERIMENTO

Se plantaron las semillas de cada uno de los tres cultivos en ambos suelos, con la aplicación de los dos APFs bajo condiciones climáticas normales, simulando estar al aire libre, durante más o menos 3 meses en Tulln (Baja Austria), sin olvidar la incorporación de un control positivo (fertilizante de roca fosfatada) y dos controles negativos (sin fertilizantes, sin cultivos de cobertura para cada suelo). Se dispusieron cuatro réplicas para cada tratamiento (32 combinaciones) y dos réplicas más para los controles negativos, por lo que 130 macetas fueron estudiadas en total.

Por otra parte se elaboró un código para el etiquetado de las macetas: el suelo libre de carbonato fue llamado suelo a, y el carbonatado, suelo c. Los cultivos de cobertura y los APFs se representaron con letras mayúsculas:

B para el trigo sarraceno, T para el trébol rojo, P para Phacelia y E para ningún cultivo de cobertura; y para los APFs: A para cenizas, D para digestato, R para la roca fosfatada y O la aplicación de ninguna enmienda. Las réplicas se representaron con números de 1 a 5. En la siguiente tabla se muestra un pequeño resumen de todos los tratamientos:

Carbonate free soil(a)				
Cover crop/APF	**No fertilizer (O)**	**Ash(A)**	**Digestate(D)**	**Rock Phosphate(R)**
No cover crop(E)	aEO1-5	aEA1-4	aED1-4	aER1-4
Buckwheat (B)	aBO1-4	aBA1-4	aBD1-4	aBR1-4
Red Clover(T)	aTO1-4	aTA1-4	aTD1-4	aTR1-4
Phacelia (P)	aPO1-4	aPA1-4	aPA1-4	aPR1-4

Carbonate soil(c)				
Cover crop/APF	**No fertilizer (O)**	**Ash(A)**	**Digestate(D)**	**Rock Phosphate(R)**
No cover crop(E)	cEO1-5	cEA1-4	cED1-4	cER1-4
Buckwheat (B)	cBO1-4	cBA1-4	cBD1-4	cBR1-4
Red Clover(T)	cTO1-4	cTA1-4	cTD1-4	cTR1-4
Phacelia (P)	cPO1-4	cPA1-4	cPA1-4	cPR1-4

MEDICIONES Y MÉTODOS

- **Muestras de suelo:** Tras el periodo de vegetación se recogieron muestras de suelo, de tal manera que se juntaron todas las réplicas del mismo tratamiento, tanto en tubos guardados en el frigorífico, como en bolsas de papel almacenadas en una habitación para secarse durante una semana. Una vez secas las muestras se tamizaron (< 2 mm) y se procedió a la extracción de las 32 muestras mediante el método calcio-acetato-lactato para determinar el fósforo disponible para las plantas (P_{CAL}). Posteriormente se determinó la concentración de fósforo mediante el método del molibdato de ÖRNOM.

  Para comparar ambos suelos y ver en cuál de ellos había más absorción de fósforo se calculó la diferencia entre el P_{CAL} de cada tratamiento y el P_{CAL} del control (ningún cultivo de cobertura). Si esta diferencia resulta menor que cero se puede asumir que existe absorción de P por parte del cultivo de cobertura. Además, para comparar la cantidad de P absorbida por el mismo cultivo con la aplicación de diferentes APFs, se calculó el porcentaje de absorción.

- **Muestras de plantas:** Tras el periodo de vegetación se recogieron todas las plantas de cada maceta y se colocaron en bolsas de plástico, contando previamente el número exacto de plantas de cada maceta. Posteriormente se secaron las muestras durante una semana en un horno a 60ºC, se pesaron y se separaron 2-3 gramos de cada una para la posterior digestión. El resto de las plantas fue colocado de nuevo en las macetas para simular una rotación de cultivos para los siguientes experimentos en los que yo no participé. Tras la digestión de cada muestra, así como 9 blancos y 9 referencias, se determinó la concentración de fósforo del material vegetal mediante el método "molybdate blue colorimetry" y posteriormente utilizando un UV/VIS espectrofotómetro.

  Para calcular la posible movilización de fósforo por parte de los cultivos de cobertura, se calculó primero la cantidad total de P en la biomasa de las plantas de todas las macetas y después se comprobó si este dato era mayor que la variación de P_{CAL} (ΔP_{CAL}) en el suelo. También se calculó el contenido total de carbono y nitrógeno, así como el ratio C/P.

Por otra parte es importante definir que, para explicar la posible mineralización de fósforo necesaria para el crecimiento de los cultivos, los resultados de concentración de fósforo y no los de cantidad total de fósforo en la biomasa aérea, fueron determinantes.

ANÁLISIS ESTADISTICO

Todos los datos fueron tratados con el programa SPSS para obtener un análisis de varianza (ANOVA) en el que los factores fijos fueron el suelo, APF y cultivo de cobertura. El nivel de significancia fue 0.05.

RESULTADOS

SUELOS

En las muestras de suelos se analizó el P_{CAL} y la posible movilización de fósforo en el suelo. Observando las figuras del documento original en el apartado 3.1.1.a se puede concluir que hay diferencias entre ambos suelos, siendo el suelo libre de carbonatos el que tuvo más P_{CAL} para la situación control (sin cultivo de cobertura y sin APF). Además tras la aplicación de fertilizantes en las macetas sin cultivos de cobertura se observó que los niveles de P_{CAL} aumentaron en el suelo con carbonatos, siendo la aplicación de las cenizas de lodos con la que se obtuvo el valor más alto, mientras que en el suelo sin carbonatos, únicamente la aplicación de digestatos aumento esta concentración. Por otro lado, el efecto de los cultivos de cobertura en la concentración de P_{CAL} también difiere dependiendo del tratamiento y el suelo.

A continuación se compararon todos los datos de P_{CAL} de todos los tratamientos de ambos suelos con el control para analizar la posible movilización del fósforo. Sin embargo estos resultados, a pesar de estar comentados en el documento original, mostraron valores inconsistentes, de tal manera que no se utilizaron en el presente trabajo, porque no se pudieron interpretar.

PLANTAS

En las muestras de plantas se analizó en primer lugar la biomasa total por maceta, el número de plantas presente en cada maceta, la biomasa de cada planta, la concentración de fósforo en las plantas, la cantidad total de fósforo presente en la biomasa de cada maceta, así como la diferencia entre la cantidad total de P y ΔP_{CAL}, el contenido total de carbono y nitrógeno y el ratio C/P.

En general se pudo ver cómo había una interacción significativa entre cultivos de cobertura y suelo, siendo el trigo sarraceno el que alcanzó la mayor biomasa en ambos suelos con la aplicación de todos los fertilizantes. Por otra parte, tanto el trigo sarraceno como phacelia tuvieron mayores valores de biomasa en el suelo libre de carbonatos que en el suelo carbonatado, mientras que el trébol rojo los tuvo en el suelo con carbonatos. Además se pudo observar cómo la aplicación de cenizas de lodos de depuradora fue la única que tuvo efecto positivo en el suelo ligeramente ácido (su valor superó al control: sin fertilizante) y sin embargo en el suelo neutro fue la aplicación de digestatos. Los resultados de biomasa por planta siguen el mismo patrón que los resultados de biomasa total.

En cuanto a los resultados de número de plantas por maceta se pudo observar cómo el trébol rojo obtuvo el mayor número de plantas en general, sin embargo fue el cultivo con menos biomasa por planta, por ello presentó la menor biomasa total. Al igual que en los resultados de biomasa por maceta, se pudo afirmar que existe una interacción entre suelo y cultivo de cobertura, así como con los APFs, manteniendo que la aplicación de lodos fue la mejor para el suelo libre de carbonatos y digestatos para el suelo con carbonatos.

Al analizar la concentración de fósforo en plantas, se pudo afirmar que, en general fue mayor en el suelo libre de carbonatos que en el suelo con carbonatos, siendo el trébol rojo el cultivo de cobertura con la mayor concentración de fósforo de los tres cultivos, con valores parecidos en ambos suelos, mientras que la concentración de fósforo en trigo sarraceno y phacelia fue bastante más alta en el suelo libre de carbonatos que en el suelo con carbonatos. Por otra parte se pudo observar cómo la concentración de P fue mayor con la aplicación de cenizas de lodos de depuradora y digestatos que con roca fosfatada y sin fertilizante.

En cuanto a la cantidad total de fósforo en la biomasa aérea de las plantas, en general fue más alta en el suelo libre de carbonatos para el trigo sarraceno y phacelia, mientras que el trébol rojo alcanzó la mayor cantidad de P en el suelo con carbonatos. Por otra parte con la aplicación de cenizas todos los cultivos de cobertura alcanzaron la mayor cantidad de fósforo en el suelo ligeramente ácido, mientras que en el suelo con carbonatos fue con la aplicación de digestatos. Al igual que en los apartados anteriores existe una interacción entre suelo y cultivo de cobertura así como con los APFs, afirmando que la cantidad de fósforo total con la aplicación de todos los fertilizantes es mayor en el suelo libre de carbonatos.

Con relación a la posible movilización de fósforo por las plantas, a pesar de que se ha comentado en el trabajo original, como se encontraron valores inconsistentes de P_{CAL}, no se interpretó la movilización de este elemento en el suelo.

En cuanto al contenido total de carbono y nitrógeno, se pudo observar como no hubo mucha diferencia en el contenido total de nitrógeno entre ambos suelos, aunque hubo más en el suelo libre de carbonatos, y además el trébol rojo fue el cultivo de cobertura con los niveles más altos de nitrógeno con la aplicación de todos los fertilizantes. Teniendo en cuenta el contenido total de carbono, las plantas que crecieron en el suelo con carbonatos tuvieron más carbono que aquellas cultivadas en el suelo libre de carbonatos, siendo el trigo sarraceno el que obtuvo los mayores valores en ambos suelos. Por otra parte, se analizó el radio C/N y se observó que el trigo sarraceno obtuvo el mayor ratio en ambos suelos, mientras que el trébol rojo obtuvo los menores ratios en ambos suelos.

Por último se analizó el ratio C/P y se pudo afirmar cómo ese ratio fue mayor en el suelo con carbonatos para todos los cultivos de cobertura con la aplicación de todos los fertilizantes. Además el trigo sarraceno tuvo el mayor ratio en ambos suelos, mientras que el trébol rojo obtuvo el menos ratio en el suelo con carbonatos. Por otra parte se pudo afirmar que hubo una interacción significativa entre suelos y cultivos de cobertura.

DISCUSIÓN

SUELOS

Los suelos tuvieron un gran impacto en la disponibilidad de fósforo que domina los resultados estadísticos, como se esperaba. La concentración P_{CAL} para la situación control (sin cultivo de cobertura, sin fertilizante), la biomasa total por maceta, concentración de fósforo en la biomasa así como la cantidad total de fósforo fue significativamente mayor en el suelo ligeramente acido, libre de carbonatos.

En general los valores de P_{CAL} se consideran muy bajos, especialmente en el suelo con carbonatos al principio del experimento, lo que junto a los valores inconsistentes encontrados en el suelo ligeramente ácido, hacen que no pueda estar segura en la fiabilidad de estos resultados, por tanto su interpretación no tiene sentido y no pude realizar una buena comparación de ambos suelos. La principal causa de estos valores inconsistentes pudo ser que se recogió únicamente una réplica por muestra, cuando es necesario al menos dos, así que para resolver este problema se deberían analizar más muestras de suelo con el mismo tratamiento.

Sin embargo, obviando estos resultados se pueden discutir otros conceptos. Numerosos estudios indican que la roca fosfatada está más disponible en suelos con pH bajos, así que los tratamientos con este fertilizante deberían incrementar el fósforo en la solución del suelo en los suelos ácidos. Sin embargo el efecto de este fertilizante en el suelo libre de carbnatos no difirió mucho del efecto en el suelo con carbonatos. Esto se puede explicar porque nuestro suelo no es estrictamente ácido, ya que tiene un pH de 6.4, por eso el efecto de la roca fosfatada no fue el esperado.

Por otra parte se pudo observar cómo las plantas crecieron mejor en el suelo libre de carbonatos, ya que a pesar de tener menos plantas por maceta, éstas alcanzaron una mayor biomasa, que es un mejor indicador. Esto se puede explicar teniendo en cuenta el pH de los suelos. En los suelos ligeramente alcalinos la cantidad de hierro es a menudo demasiado baja y el contenido de calcio es demasiado alto, lo que reduce la captación de potasio y magnesio y las plantas no absorben P debido a la fijación, lo que resulta en un menor crecimiento.

Además es importante destacar que las propiedades de los suelos (pH y contenido en carbonatos) resultaron determinantes en este experimento, como se puede observar en todos los resultados, ya que el contenido de CaCO3 y el pH neutro del suelo carbonato de explican la baja movilidad y la dificultad de las plantas en el acceso de P en este suelo debido a la fijación de P por la formación de fosfatos cálcicos insolubles.

CULTIVOS DE COBERTURA

En primer lugar se pudo observar cómo el trigo sarraceno fue el que alcanzó mayor biomasa en ambos suelos, mientras que el trébol rojo fue el que menos tuvo. Esto se puede explicar porque el periodo de vegetación fue demasiado corto para el trébol rojo, ya que no mostró flores antes de la cosecha. Sin embargo este cultivo fue el que tuvo los mayores niveles de concentración de fósforo en plantas, como se esperaba, ya que según numerosos estudios, las leguminosas son capaces de acceder más fácilmente al fósforo en el suelo.

Por otra parte, se encontraron interacciones significativas entre suelo y cultivo de cobertura, que confirman el efecto dominante de las propiedades del suelo en la biodisponibilidad de fósforo. Los carbonatos en el suelo alcalino inmovilizan el fósforo y por ello las plantas encuentran problemas en captarlo desde la solución del suelo. Por ello la concentración de fósforo para trigo sarraceno y phacelia fueron menores en el suelo con carbonatos que en el suelo libre de ellos. Sin embargo, para el trébol rojo, la concentración es parecida en ambos suelos porque puede acceder por igual al fósforo soluble en el suelo, al ser leguminosa y además este cultivo soporta pH más altos que el trigo sarraceno, por lo que puede crecer mejor en el suelo con carbonatos.

FERTILIZANTES ALTERNATIVOS DE FÓSFORO

Uno de mis objetivos en este trabajo era comprobar si alguno de los APFs tenía mejores resultados que la aplicación de roca fosfatada. Observando mis resultados se puede observar cómo con la aplicación de cenizas de lodo se produjo más biomasa y más cantidad total de fósforo en la biomasa que con la aplicación de roca fosfatada, y más concentración de fósforo con la aplicación tanto de cenizas como digestatos que con roca fosfatada, por lo que se puede afirmar que hubo un efecto positivo con a aplicación de ambos APFs, aunque no se pudo compararlos.

Por otra parte se puede decir que hubo una interacción significativa entre suelos y APFs, reflejando que la aplicación de cenizas de lodos es el mejor APF para el suelo libre de carbonatos, que se puede explicar mediante informes que defienden que el fósforo en cenizas de lodos de aguas residuales puede ser más accesible por las plantas, especialmente en suelos más ácidos. Sin embargo en el suelo carbonatado, es la aplicación de digestatos con la que se obtienen mejores resultados. Esto se puede explicar porque los aniones orgánicos provenientes de la descomposición de los materiales orgánicos pueden competir con el fosfato por los sitios de adsorción y el digestato es un material descompuesto, por lo que puede haber tenido esta ventaja en el suelo carbonato ya que los otros APFs suministran fósforo inorgánico.

CONCLUSIÓN

- Las propiedades del suelo tienen un efecto dominante en la movilización del fósforo. La biomasa y la concentración de fósforo es mayor en plantas que crecieron en el suelo libre de carbonatos. Sin embargo, debido a los valores inconsistentes de P_{CAL} no puedo afirmar que haya más movilización en este suelo.

- El trigo sarraceno y phacelia tuvieron más biomasa y concentración de fósforo en el suelo libre de carbonatos, mientras que el trébol rojo obtuvo más biomasa en el suelo con carbonatos y la mayor concentración de fósforo en ambos suelos, por lo que se pued concluir que es el cultivo mejor adaptado en ambos suelos. . Sin embargo, debido a los valores inconsistentes de P_{CAL} no puedo afirmar que haya más movilización en este cultivo.

- Ambos APfs superaron el efecto de la roca fosfatada, siendo la aplicación de cenizas de lodos de depuradora más adecuada en el suelo libre de carbonatos y la aplicación de digestatos en el suelo con carbonatos.

- Aunque no se esperaban interacciones, los suelos tuvieron diferentes resultados, así como los cultivos de cobertura y los fertilizantes, por lo que se puede afirmar que las características y propiedades de todos ellos son muy importantes y consistentes en este experimento.

- Se espera que la incorporación de trébol rojo en el suelo libre de carbonatos aumentará la absorción de P por el cultivo siguiente, debido a su baja relación C / P y alto contenido de P.

- Para mejorar este trabajo se deberían recoger más réplicas de las muestras de suelo para repetir los resultados de P_{CAL}, así como más estudios para investigar la biodisponibilidad de P.

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Carolina Rodríguez Garraus
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ABSTRACT

Phosphorus (P) is an essential nutrient for the plants, which is necessary for performing many metabolic processes. However, the concentration of this element in an available form to plants in the soil, results very low on many occasions, in such a way that it limits crop growth leading to very poor yields. As a result, in modern agriculture, the application of phosphate fertilisers whose main source is the rock phosphate, which is a limited and non-renewable resource, is needed. Therefore, different ways to recycle phosphorus from different waste materials to improve its mobility in soil and guarantee the availability of this element in the crops, are sought.

In the present study, the effect of applying different alternative phosphorus fertilizers (APFs), sludge sewage treated ash and digestate, as well as phosphate rock and the application of none fertilizer for different cover crops: *Fagopyrum esculentum, Phacelia tanacetifolia y Trifolium pratense,* as well as no cover crop in two different soils: one slightly acidic and the other carbonated and neutral, wanted to be determined in the P mobility potential. Because APFs can increase the efficiency of P cycle since they could be produced by recovering P wastes; and regarding the use of these cover crops, their importance lies in the accumulation of high P concentrations by some plant species which exploit sources of this element that are not available for most crops. Among the important plant nutrients, only P was limiting in the experiment.

For this, both soil samples, using the calcium-acetate-lactate method, as well as plant samples after plant digestion, were analysed. Phosphorus content results were obtained in samples of soil and plants as well as biomass per pot and per plant, number of plants, total amount of phosphorus on the above-ground biomass and carbon and nitrogen contents for these latter categories, as well as the ratio C/P. By analysing these results, the following conclusions were obtained. All effects of P mobilization were more pronounced in the carbonate-free soil due to the negative effect of the carbonates in the carbonate soil. However, inconsistent values were found in P_{CAL}(plant available phosphorus) analysis, therefore it could not be concluded that there is more P mobilization in this soil. Although *Trifolium pratense* showed the highest P concentration and a narrow C/P ratio , it could not be concluded that it mobilizes more P than the other cover crops. . Both APFs overcame the effect of rock phosphate, being ash most suitable in the carbonate-free soil, and digestate in the carbonate soil.

Further studies that exceed the scope of this work will examine the fertilising effect of the cover crops on wheat after incorporation of above-ground biomass into the soil. However, the incorporation of *Trifolium pratense* into the carbonate-free soil is expected to increase P uptake by the following crop due to its low C/P ratio and high P content that fuel mineralisation.

KEYWORDS: Phosphorus, organic agriculture, alternative phosphorus fertilizers, cover crops, phosphorus mobilization.

ZUSAMMENFASSUNG

Phosphor (P) ist sowohl ein wesentlicher Nahrungsmittel für die Pflanzen als auch ein unersetzlicher Stoff, der für viele Vorgänge beim pflanzlichen Stoffwechsel nötig ist. Die Konzentration dieses Stoffes im Boden ist aber oft so niedrig, dass das Wachstum und die Leistungen der Pflanzen stark beeinträchtigen oder verringern. Aus diesem Grund ist notwendig innerhalb der modernen Agrarwirtschaft die Verwendung von Phosphatdünger, deren Ursprung der Phosphogips ist. Phosphorgips ist ein von Natur aus beschränkter und nicht wiederverwertender Bodenschatz. Deshalb sucht man unterschiedliche Materialien um das Phosphor aus abbaubaren Materialien wiederverwerten zu können, um ihre Mobilität verbessern zu können und somit die Verfügbarkeit dieses Stoffes zu garantieren.

In folgender Studie wurde die Wirkung der Anwendung verschiedener zur Phosphor alternativen Düngemittel auf Zwischenfruchtbau analysiert. Zuerst wurde auf einem Bodenanteil Klärschlamm als Düngemittel angewendet. Bei dem nächsten Bodenanteil Phosphorgips und der dritte Bodenanteil blieb ohne jegliche Düngemittel. Die Zwischenfrüchte dieser Studie waren: Fagopyrum esculentum (echter Buchweizen), Phacelia tanacetifolia (Reinfarn-Phazelie) und Trifolium pretense (Wiesen-Klee).

Zuletzt wurde auch einen Bodenanteil untersucht mit zwei verschiedenen Teilen ohne Zwischenfruchtanbau: der eine Teil mit einem sauren und Carbonatfreien Boden und der andere Bodenanteil hatte einen Ph-neutralischer und mit Carbonatreichem Boden.

Die Wichtigkeit der zur Phosphor alternativen Dünger besteht in der Tatsache, dass einige Pflanzenarten hohe Phosphorkonzentrationen sammeln, die in anderen Anbauarten nicht verfügbar sind.

In dieser Studie wurden sowohl Bodenmuster durch die Calcium- Acetat- Lactat Methode analysiert als auch Pflanzenmuster nach einer “Pflanzenverdauung”.

Man wurden Ergebnissen von Phosphorinhalt auf dem Boden und in den Pflanzen und Biomasse und Kohlenstoff und Stickstoff in den Pflanzen. Beim Analysieren dieser Ergebnissen hat man folgendes beobachtet: Alle Wirkungen bei der Mobilisierung des Phosphors waren gröβer auf Böden ohne Kohlenstoff wegen der niedrigen Phosphorfixierung. Troztdem hat man keine beständige Werte bei der Phosphor (cal) gefunden. Deshalb konnte man nicht schlieβen, dass dieser Boden gröβere Phosphor-Mobilisierung hatte. Es war auch nicht auszuschlieβen, dass bei Trifolium Pratense im Vergleich zu den anderen Pflanzenarten höhere Phosphor Konzentrationen aufweisen. Beide alternative Düngemittel und überstiegen die Wirkung des Phosphorgips. Klärschlamm war bei kohlenfreien Boden wirkungsvoller und bei Kohlenreichen Böden mit digestatos.

In andere Studien, die in dieser Arbeit nicht berücksichtigt wurden, wird der Düngemitteleffekt der Zwischenfrüchte analysiert bei Weizen Anbau nach Biomassenhinfügung auf dem Boden. Allerdings ist die Einbeziehung von Trifolium pratense, in dem Carbonat-freien Boden voraussichtlich P-Aufnahme durch die Folgekultur aufgrund seiner niedrigen C / P-Verhältnis und hoher P-Gehalt, die Kraftstoffmineralisierung zu erhöhen.

SCHLUSSELWÖRTER: Phosphor, Bio Landwirtschaft, Alternative Düngemittel zur Phosphor, Zwischenfrucht Anbau, Phosphor Mobilisierung.

RESUMEN

El fósforo (P) es un nutriente esencial para las plantas, el cual es necesario para la realización de numerosos procesos metabólicos. Sin embargo la concentración de este elemento en forma disponible para las plantas en el suelo, resulta en muchas ocasiones muy baja, de tal forma que limita el crecimiento de los cultivos dando lugar a rendimientos muy pobres. Debido a ello, en la agricultura moderna es necesaria la aplicación de fertilizantes fosforados cuya fuente principal es la roca fosfórica, que es un recurso limitado y no renovable. Por ello se buscan diferentes formas de reciclar el fósforo a partir de diferentes materiales de derecho para mejorar su movilidad en el suelo y así garantizar la disponibilidad de este elemento en los cultivos.

En el presente estudio, se determinó el efecto de la aplicación de diferentes fertilizantes alternativos de fósforo (APFs),ceniza de lodo de aguas residuales tratadas y digestatos, así como roca fosfatada y ningún tipo de fertilizante, para diferentes cultivos de cobertura: *Fagopyrum esculentum, Phacelia tanacetifolia y Trifolium pratense* , así como el caso de ningún cultivo en dos suelos diferentes, uno ligeramente ácido y libre de carbonatos y el otro con carbonatos y neutro, en el potencial de la movilidad del fósforo. Debido a que los APFs pueden incrementar la eficiencia del ciclo del fósforo al producirse recuperándolo de desechos, y en cuanto al uso de estos cultivos de cobertura, su importancia reside en que algunas especies acumulan altas concentraciones de P y explotan fuentes de este elemento que no están disponibles para la mayoría de los cultivos.

Para ello se analizaron tanto muestras de suelo mediante el método calcio-acetato-lactato, como muestras de plantas tras sufrir una digestión. Se obtuvieron resultados de contenido de fósforo en las muestras de suelo y plantas, así como biomasa por maceta y por planta, número de plantas por maceta contenidos de carbono y nitrógeno en la biomasa de las plantas y el ratio C/P. Al analizar estos resultados se obtuvieron las siguientes conclusiones. Todos los efectos de movilización de P fueron más pronunciados en el suelo sin carbonatos debido al efecto negativo de los carbonatos en el suelo carbonatado, sin embargo se encontraron valores inconsistentes en el análisis de P_{CAL}(fósforo disponible para las plantas), por lo que no se pudo concluir que este suelo tuviera mayor movilización de P, ni tampoco que a pesar de que *Trifolium pratense* mostrara la mayor concentración de P y un estrecho ratio C/P, este cultivo sea más efectivo en la movilización de fósforo en comparación con los otros dos. Ambos APfs superaron el efecto de la aplicación de roca fosfatada, siendo la ceniza de lodo de aguas residuales más adecuada en el suelo sin carbonatos y la aplicación de digestatos en el suelo carbonatado.

Otros estudios que exceden el alcance de este trabajo examinarán el efecto fertilizante de los cultivos de cobertura en trigo tras la incorporación de su biomasa en el suelo. Sin embargo, se espera que la incorporación de *Trifolium pratense* en el suelo libre de carbonatos incremente la absorción de fósforo por los siguientes cultivos debido a su estrecho ratio C/P y su alta concentración de fósforo que estimula la mineralización de este elemento.

PALABRAS CLAVE: Fósforo, agricultura orgánica, fertilizantes alternativos de fósforo, cultivos de cobertura, movilización del fósforo.

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INTRODUCTION

Phosphorus (P) is an important plant macronutrient, making up about 0.2% of a plant´s dry weight. It is a component of key molecules such as nucleic acids, phospholipids and ATP, and consequently, plants cannot grow without a reliable supply of this nutrient (Theodorou & Plaxton, 1993). It is crucially involved in most major metabolic processes, e.g. it takes on a prime role in energy transfer as adenosine triphosphate (ATP). As phospholipids, phosphorus is part of cell membranes and as part of nucleotides; phosphorus is a major component to build up DNA and RNA. Furthermore, plants are dependent on phosphorus to secure energy production in photosynthesis (Smil, 2000, Ruttenberg, 2009). Therefore, P is essential for life, yet is frequently the element that most limits biological productivity in ecosystems. (Turner B.L et al., 2003)

On the other hand, the concentration of plant available P is low in most soils, therefore the fertilization with P fertilizers is needed. Rock phosphate has been the main raw product for the production of P fertilizers since the last century until today, but the actual P resources in the world will not be able to maintain the present rate of consumption for more than 100 years (Berg and Shaum, 2005; Cordel et al., 2009; Steen, 1998; Stewart et al., 2005). Although the application of untreated rock phosphate is permitted in organic agriculture in the European Union, as it is a non-renewable and limited resource, it contradicts the idea of a closed nutrient cycle which is one of the central principles of the organic farming (Nelson et al. 2007).

The increasing world population, the consequent higher demand of food and the idea of a closed nutrient cycle makes it necessary to find ways to recycle P resources (Cabeza, 2010). According to Schröder et al. (2011) recycling P from materials that are usually wasted as sewage sludge, animal and plant residues could reduce the fertiliser import significantly, hence is a key requirement for sustainable P. These organic materials contain P that is mineralized by soil organisms to release plant-available forms (Arcand et al. 2010). However, P is often adsorbed by solid components that make it inaccessible for plants and result in suboptimal growth (Chien and Meneon 1995). It can be said that P is a key limiting factor in many terrestrial ecosystems because in most soil P is bound to soil minerals or organic matter (Arcand et al. 2010). According to Kamh et al. (1999) the incorporation of cover crops into cropping systems may contribute to a more efficient utilization of soil and fertilizer P, since cover crops may increase P cycling rates via plant uptake and P release during microbial decomposition. Moreover, cover crops and associated microbes may also change rhizosphere properties and stimulate soil P mobilization (Maltais-Landry, Scow, & Brennan, 2014). In addition, some plants, especially legumes, accumulate P in their biomass in above-average concentrations and increase soluble P via root exudates, which makes them applicable as cover crops or as green manure in organic agriculture (Nuruzzaman et al. 2005, Hassan et al. 2012).

Embedded in the broader research project IMPROVE-P (Improved Phosphorus Resource efficiency in Organic agriculture Via recycling and Enhanced biological mobilization) of the Division of Organic Farming (IFÖL) of the University of Natural Resources and Life of Vienna started on June 2013; my present TFC (master thesis) proposes to focus on the analysis of the effects of two alternative phosphorus fertilisers (APFs) in P mobilization by three cover crops in two different soils.

Part I: State of knowledge and objective

1.1 Phosphorus reserves and consumption

According to Cordell (2009) phosphorus is a non-renewable resource which is obtained mainly from rock phosphate mine. Recent approaches calculate that with the current rate of consumption, P will be exhausted in about 50-100 years (Steen, 1998). Therefore, it is really necessary to use P resources in an efficient way and also find other P resources by recycling it.

If the world situation is analysed (Figure 1), China and India are the largest consumers of phosphorus fertilisers, demanding 34% and 19% of global consumption, respectively, and their consumption shows increasing trends (20% and 80 % from 2002 to 2009, respectively) (FAOSTAT 2012). However, in Europe, consumption decreased by about 20 % in the same period. On a worldwide scale, population growth, changes towards meat-rich diets and growing demands for bioenergy crops, will push an increasing demand for phosphorus fertilisers in the future (Tirado and Allsopp, 2012).

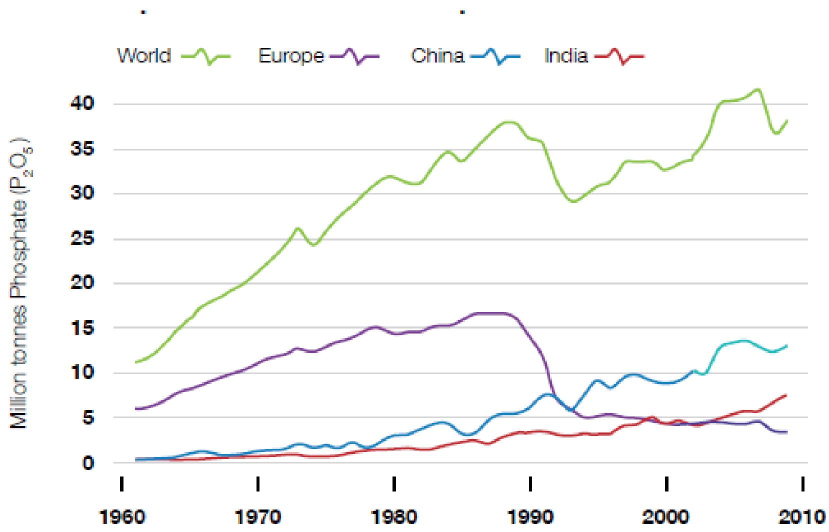

Figure 1: Phosphate fertiliser consumption in the world and in China, India and Europe from 1961 to 2009 (adapted from Tirado and Allsopp, 2012; data source: FAOSTAT 2012).
There had been a global increase in phosphorus consumption until 1995, when there was a reduction in the use of mineral phosphorus due to the economic crisis in ex-communist countries and environmental restrictions in Western European countries. After 1995 there was another increase in world phosphate consumption caused by the development of it use in Asia, especially in China.

Furthermore, the fertilizer industry recognises that the quality of reserves is declining and the cost of extraction, processing and shipping is increasing too (Cordell, 2009); so the introduction of alternatives and the recovery of the resource after being used is needed. According the European Fertilizer Manufactures Association (2000) farmers in Europa and North America are improving the use of phosphorus, avoiding over fertilization and including straw and animal manure into agricultural soils in order to recycle phosphorus.

1.2 Phosphorus recycling

In view of the ongoing need to find P resources, P recycling is needed. There are several sources, like the reuse of wastewaters and sewage sludge, animal bones, human faeces and urine, wood ash or compost that could make the agricultural P cycles more efficient.

Reuse of wastewaters and sewage sludge is not an attribute of our modern society (Cabeza, 2010). Regarding Kirchmann et al. (2005), ancient cultures implemented channel systems to evacuate wastewaters. For example in Rome, they had the “Cloaka maxima” to transport wastes outside the city, or in China, Corea and Japan, the wastes were incinerated and transported to the land. According to Kirchmann et al. (2005) the wastewaters began to be managed in sewage plants after the introduction of toilets in European cities, as the municipal wastes were discharged into water bodies which resulted in water eutrophication. However, the application of sewage sludge directly to the land has some problems, since it contains heavy metals (Balmer, 2004; Kirchmann et al., 2005). Because of that, new technologies are being introduced in order to reduce heavy metal contents.

1.2.1 Alternative phosphorus fertilisers

As the use of chemical fertilizers is not permitted in the organic agriculture, organic farmers frequently use many other strategies to contribute to P recycle and to ensure availability of P to crops. In this project, two APFs are studied and discussed:

- **Sewage sludge ash**

According to the EPA (United States Environmental Protection Agency) sewage sludge is the solid, semisolid or liquid untreated residue generated during the treatment of domestic sewage in a treatment facility. After being treated and processed, it becomes a bio-solid which can be recycled and applied as a fertilizer. However, Egle and Reichel (2012) point out that sewage sludge deposition, which is not permitted in Austria, it has to be burned or composted under strict regulations, since, according to Marani (2003) and Harrison (2006) it is often contaminated with organic pollutants and heavy metals.

The European Project SUSAN (Sustainable and Safe Re-use of Municipal Sewage Sludge for Nutrient Recovery) is aimed to develop a sustainable and safe strategy for nutrient recovery from sewage sludge using thermal treatment. A schematic of the strategy is presented in Fig. 1. With this, the organic pollutants are completely destroyed in a first step (mono-incineration), but the ashes still contain heavy metal compounds which have to be separated by a thermochemical treatment, adding chlorine additives. After this thermochemical treatment phosphates become more available (Adam et al. 2009).

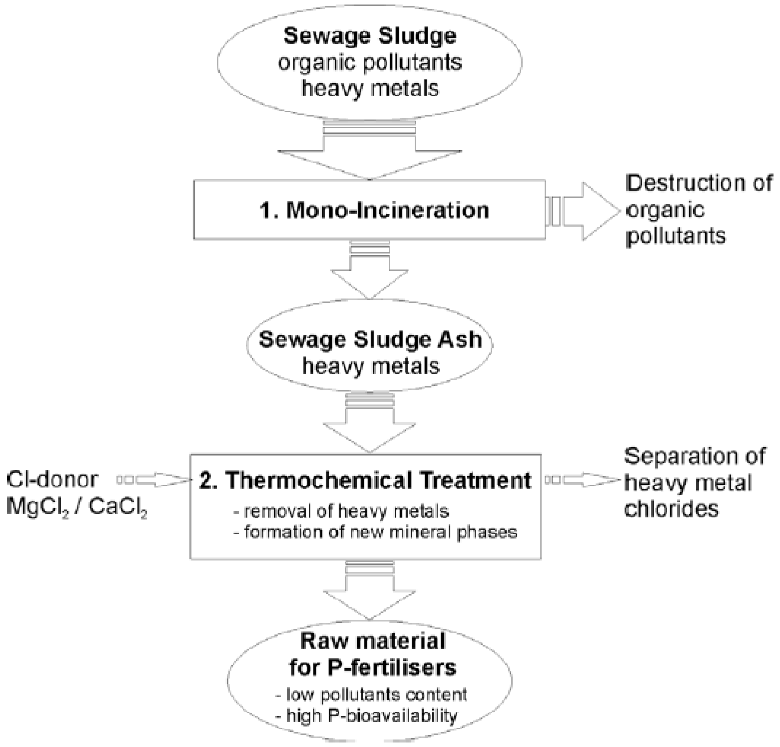

Figure 2: Schematic of the pathways of organic and inorganic pollutants during the thermal treatment (adapted from Adam et al. 2009)

According to Adam et al. (2009) sewage sludge ashes exhibit high phosphorus contents of approximately 20 % P_2O_5 and are therefore suitable raw-materials for P-fertilizer production. Besides, it is said that the P-bioavailability is significantly increased during thermochemical treatment. Egle et al. (2013) concluded that this treatment of incinerated sewage sludge is the most economic and produces less pollution compared with various P recovery technologies from wastewater.

- **Digestate:**

Digestate, also called biogas effluents, biogas residues or biogas slurry when animal manures are digested, is the residual product of anaerobic digestion, and it can be used as fertilizer (Müller and Möller, 2012). According to Marianna et al. (2012) it is the by-product of methane and heat production in a biogas plant, which can be a solid or a liquid material depending on the biogas technology as we can see in figure 3. It contains a high proportion of mineral nitrogen (N) available for plants and other macro- and microelements necessary for plant growth. Moreover, the organic fractions of digestate can contribute to soil organic matter, influencing the biological, chemical and physical soil characteristics and soil amendment; making it an effective fertilizer for crop plants.

Furthermore, Börjesson and Berglund (2007) assumed all phosphorus in the digestate to be in available forms, therefore digestate seems to be a useful material for supplement missing nutrients of soil, especially of the P and K. However, most available results from field experiments indicated no effects of anaerobic digestion on manure P availability (Adam et al, 2009).

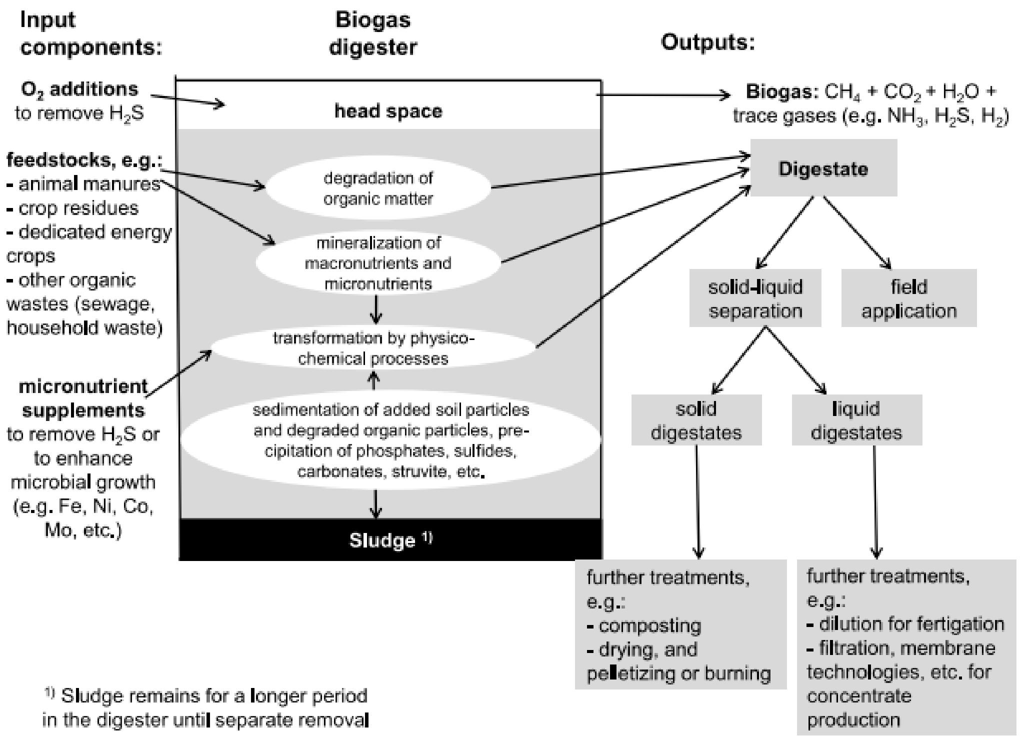

Figure 3: Overview of the matter flows and processes during anaerobic digestion and possible treatments of the resulting digestates (adapted from Adam et al. 2009)

1.3 Phosphorus in soil

All the reactions and interactions between soil compounds take place in the soil solution (Lindsay, 1979). In the case of phosphorus, it is taken up from the soil solution by plant roots as orthophosphate ions, principally $H_2PO_4^-$, which is predominant in soils with high pH (>7.2) and to a lesser extent HPO_4^{2-}, which is predominant in acid to neutral soils (pH 4-7.2) (Syers et al. 2008; Pierzynski et al., 2005). However, the soil compounds are very variable and control the intricate equilibrium in soil solution. For this reason a part of P, whose principal source in most soils derived from apatite, may precipitate in less soluble forms (precipitate-dissolution), others may be absorbed by the soil particle surface (adsorption-desorption), immobilized by biological fixation (immobilization-mineralization) or react in dependence on the soil pH and oxidation-reduction conditions (Pierzynski et al., 2005). This is represented in the figure below, where the P-cycle is explained.

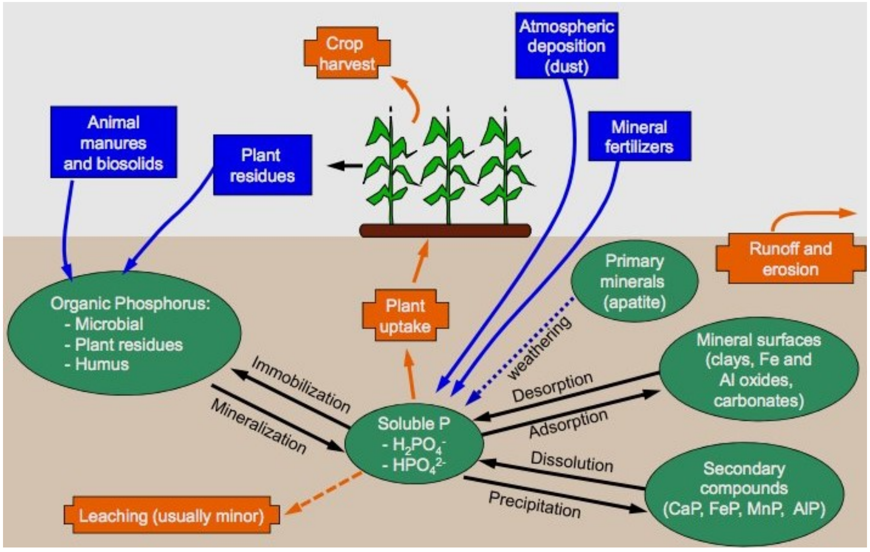

Figure 4: Phosphorus cycle. This figure represents the cycle of the phosphorus in the soil, green labels indicate how P- can appear in soil, blue ones, P-input to soil, and orange, P-outputs (losses or plant uptake). Phosphorus can be added to the soil by animal manures and bio-solids, plant residues, mineral fertilizers and atmospheric deposition, although the natural P sources in most soils are primary minerals derived from apatite.

The P availability depends on many factors of the soil, like the pH value of soil solution, which determinates the orthophosphate species (Figure 5), type and amount of clay minerals and oxide minerals such as Al and Fe (Stevenson and Cole, 1999).

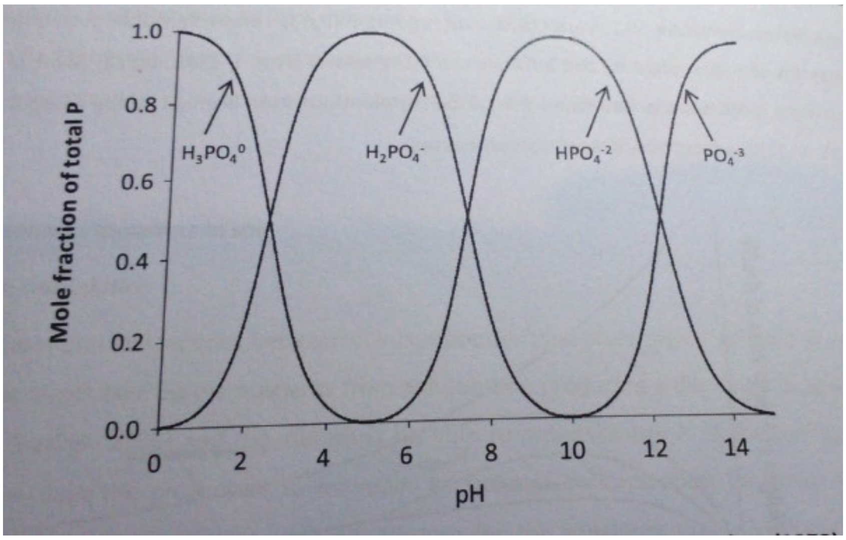

Figure 5: Influence of pH on the orthophosphate forms in soil solution. From Cabeza (2010)

In Figure 5, the different orthophosphate forms depending on the pH of the soil are shown. In an acid soil, the solubility of the monovalent ion ($H_2PO_4^-$) , which is the most readily absorbed by plants, increases. When the pH enhances, the release of iron and aluminium cations, which react with the phosphorus resulting in insoluble and unassimilable products, occurs. In alkaline soils there is a large amount of calcium (calcium carbonate) that reacts with the dominant bivalent ion (HPO_4^{2-}), resulting insoluble compounds.

About the concentration of phosphorus in soil solution, it is usually around 0.03 to 0.5 mg L^{-1} (1 µM to 16 µM) which is very low (Barber, 1995). However, in soils that have been fertilized, it is possible to find high concentration of P in soil solution (1 mg L^{-1} or 32 µM) and in some cases, where the soil has been heavily fertilized, the concentration of P can reach 7 to 8 mg L^{-1} (Brady and Weil, 2002; Pierzynski et al., 2005). According to Blume et al. (2010) about 0.1 % of the total soil P (between 0.001- 5 mg P L^{-1} depending on soil type and fertilization) can be taken up by plants, while more than 90 % of it is fixed in primary and silicate minerals, absorbed by calcium, iron and aluminium in secondary minerals or bound to organic matter.

1.4 Phosphorus in plants

Phosphorus enters the plant through root hairs, root tips and the outermost layers of root cells as orthophosphates. This uptake is also facilitated by mycorrhizal fungi, which grow in association with the roots of many crops (see figure 6). Once inside the plant, P may be stored in the root or transported to the upper portions of the plant. Through various chemical reactions, it is incorporated into organic compounds, including nucleid acids (DNA and RNA), phosphoproteins, phospholipids, enzymes, sugar phosphates and energy-rich phosphate compounds, as adenosine triphosphate (ATP). It is in these organic forms, as well as the inorganic phosphate ion that P is moved throughout the plant, where it is available for further reactions.

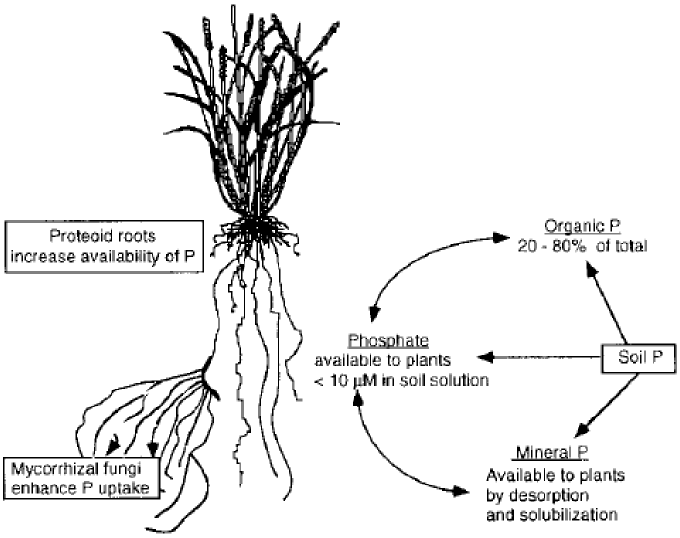

Figure 6: Plant acquisition of soil P. From Schachtman et al. (1998)

As it has been said, the uptake of P poses a problem for plants, since the concentration of this mineral in the soil solution is low and the plant requirements are high. Therefore, plants must have specialized transporters at the root/soil interface for extraction of P from solutions of micromolar concentrations, they may modify their root architecture, develop large amounts of fine root hairs or make mycorrhizal symbiosis to enhance the root surface. Plants also develop mechanisms for transporting P across membranes between intracellular compartments and for altering soil chemical properties in order to absorb P, like the excretion of protons during nitrogen fixation and ion uptake as well as release of organic acids. Enzymes like phosphatases and phytases can also be released by the plant roots to enhance P mineralisation (Schachtman et al., 1998; Shen et al. 2011).

Some plants, especially legumes, have these mechanisms for P uptake that heightens the amount of P, which can be extracted from the soil, and also the P use efficiency (Eichler-Löbermann et al. 2009). On the other hand, some plant species from other families also show P accumulation, like buckwheat, which, according Arcand et al. (2010), mobilised P from rock phosphate into its shoot tissues, or phacelia which increases the P availability for following crops as green manure (Eichler-Löbermann et al. 2009).

Because of it, these two last plants (buckwheat and phacelia) have been selected, as well as red clover (legume), for this experiment.

1.5 Objective

Considering the literature above, phosphorus is one of the most important elements for the growth of plants, however, as it is a limited and non-renewable resource; more and more methods to incorporate P into the soil, are rising. Nevertheless, in organic agriculture, the use of synthetic fertilizers is not permitted; because of that, different alternatives are being sought in order to improve the P mobility in the soil and assure the availability of this element for the crops.

One of these alternatives is studied in this project. The main research question is if a combination of P-accumulating plants (buckwheat, phacelia and red clover) with recycled APFs (Mg-treated sewage sludge ashes and digestates) could present a sustainable option of increasing P efficiency and also could replace the application of rock phosphate, which is a non-renewable resource and sometimes shows bad bioavailability.

Trying to find a sustainable alternative to the application of rock phosphate as fertilizer and also, discover which cover crop is the best one to mobilise P and make it accessible to plants are the main aims of this project.
For this, the objectives of this study were to:

a) Test in which of two different soils, one acidic and one calcareous, there was more P mobilization and also which one was better to cultivate in it. Due to the carbonate presented in the calcareous one, the availability of phosphorus in this soil should be less than in the other one. For this purpose, the P_{CAL} was analysed.

b) Test if red clover, as it is a legume, should mobilize more P from the soil than the other cover crops (buckwheat and phacelia) studied.

c) Test if one of the recycling APF would produce more biomass, higher P concentration in cover crops and amount of phosphorus in the above-ground biomass than the rock phosphate.

d) Test if there was no interaction between soils, cover crops, APFs, soil with cover crop, soil with APFs, cover crop with APFs or soil with cover crop with APFs. Therefore, I wanted to test it with some statistics using ANOVA and post-hoc Tuckey-HSD tests for every parameter.

Part II: Materials and methods

2.1 Materials

2.1.1 Soils

Two soils were collected from two Austrian locations for the present project. The carbonate-free soil (Cambisol) was taken from Gföhl, Lower Austria; while the carbonate soil (Chernozem) was collected from Münchendorf, Lower Austria (see figure 7). Both soils were from a field after growing wheat. 324 cm^2 plastic pots were filled with exactly 4 kg of soil. The chemical parameters charactised before the experimental set up are given in table 1.

Table 1. Chemical soil parameters (Lina Weisssengruber, personal communication)

Parameter/Soil	Carbonate-free soil (a)	Carbonate soil (c)
Texture	loam	loamy sand
pH $_{CaCl2}$	6,4	7,5
P_{CAL} [mg kg^{-1}] (August 2014)	3,06	19,32
P_{CAL} [mg kg^{-1}] (September 2014 after harvest)	26,5	16,2
Mineral N [mg kg^{-1}]	12,3	10,9
Total N [g kg^{-1}]	1,85	4,55
Total C [g kg^{-1}]	17,11	92,28
C inorganic [g kg^{-1}]	0,20	52,70
C organic [g kg^{-1}]	16,90	39,50
C/N ratio	9,14	8,68
$CaCO_3$ [g kg^{-1}]	1,48	439,57
Water-holding capacity %	45	63

Figure 7: Location of Gföhl and Münchendorf in a map of Austria

2.2.2 Cover crops

Seeds from three different cover crops (Buckwheat, Phacelia and Red Clover) were sown in pots according to recommendations of a seed breeding company (Saatzucht Gleisdorf Ges.mbH) for kg ha^{-1} in triple amount. In table 2, we can see the amount of seeds sown, in different units, as well as the date of seeding and the planting depth of each cover crop. The thousand grain weight for each cover crop is: 35 g for buckwheat, 1,62 g for phacelia and 1,7 g for red clover. Irrigation was by rain and irrigation-system (250 ml/per pot on Monday, Wednesday and Saturday at 1 a.m.), but only if it was dry.

Table 2. Seeding data (provided by Lina Weisssengruber)

	Buckwheat	Phacelia	Red Clover
Recommended seed density (kg ha^{-1})	60-80	8-12	10-22
Seeds (kg ha^{-1})	90	20	20
Seeds 3fold (kg ha^{-1})	270	60	60
Seeds per pot (g)	0,87	0,19	0,19
Seeding date	1/08/2014	1/08/2014	1/08/2014
Seeding depth (cm)	2-3	1-1,5	1-2

Image 1: Buckwheat (Source: Google images)

- **Buckwheat** (*Fagopyrum esculentum*): It is an herbaceous annual plant of the family Polygonaceae used in several occasions as a cover crop, which grows on a wide variety of soil types, including acidic soils (pH 4–6). It has a branching root system that reaches deeply into the moist soil that can acidify its rhizosphere and can absorb concentrations of P (Arcand et al., 2010) .According to Possinger (2013) it has been identified as a P-efficient crop that putatively increases soil-P availability for the next crop rotation.

Image 2: Phacelia (Source: Google images)

- **Phacelia** (*Phacelia tanacetifolia*): It is an herbaceous, non-leguminous annual plant of the family Boraginaceae, native to the arid southwest region of the United States and Mexico (Porcuna, 2011). It is comparable to buckwheat in many ways. Cultural differences are that buckwheat germinates more readily, especially at higher soil temperatures, and phacelia is more tolerant of cold and drought (Gilbert, 2003). It prefers well-drained soils within a pH range of 5.5-6.8. As buckwheat, this crop has been selected from the group of the most promising P accumulating plants (Arcand et al. 2010).

Image 3: Red Clover (Source: Google images)

- **Red clover** *(Trifolium pratense):* It is an herbaceous, short-lived perennial plant of the family Fabaceae, native to Europe, Western Asia and Northwest Africa. Red clover can be used as a cover crop that provides many benefits such as fixing nitrogen, protecting soil from erosion, competing with weeds, as well as supplying forage needs, and also, while many other legumes, it can grow quicker and produce more biomass(SARE, 2012).

It grows in all soil types, but prefers deep ones and with good level of bases, it also supports slightly acidic soils (pH 6-7.5).This crop was included in the experiment because, according to Gerke and Meyer (1995), legumes have been shown to have enhanced P mobilising mechanisms and red clover showed high P-levels.

2.2.3 Fertilization

The fertilization were given by Lina Weissengruber before my start in this experiment. She used magnesium-treated sewage sludge ash from a project partner of the ETH Zürich, rock phosphate [“Naturphosphat P26”] from Timac AGRO Düngemittelsproduktion und Handels-GmbH and digestate coming from the biogasplant “Biogas Bruck/Leitha GmbH & Co KG”. In the following table, detailed nutrient content of the fertilisers are shown.

Table 3. Nutrient contents of APFs (provided by suppliers)

APF/ Nutrient (mg/kg)	P	N	K	Mg	Fe	Zn	Cu	Mn
SSA DM	62000	0	15000	54300	153000	275,33	227	913,7
Digestate DM	20116,268	30000	33411	n.s	n.s	170	22	n.s
Rock Phospate DM	113310	0	0	n.s.	n.s	n.s.	n.s.	n.s

List of abbreviations: P, phosphorus; N, nitrogen; K, potassium; Mg, magnesium; Fe, iron; Zn, zinc; Cu, copper; Mn, manganese; n.s., not specified by supplier. SSA: ash DM: dry matter.

The targetlevel of P was 50 mg/kg (agreement in the consortium), the N and K was a result of the digestate needed for the P target level and the N, K-level in digestate. All pots got the same N and K level, being P the only limiting element. The additional mineral fertlization was with KCl and ammonium nitrate. . At the end, the pots had 50 mg/kg P of different sources, ore no P fertilizer, 83.04 mg K/kg and 74.57 mg N/kg . The exact applied amount of APFs in this experiment is shown in table 4.

Table 4. Amount of APFs applied (provided by suppliers)

	g of added APF per kg soil	g of addded APF per pot	Fertilizer total for all pots g
Ash	0,806	3,226	103,226
Digestate	26,812	107,251	3432,044
Rock Phosphate	0,441	1,765	56,482
No fertilizer	0	0	0

2.2 Experimental setup

Each of the three cover crops was grown on each of the two soils and with each of the two APFs under net house conditions for more or less 3 months in Tulln (Lower Austria), without forgetting the addition of one positive control (rock phosphate fertilizer) and two negative controls (no fertilizers, no cover crop for each soil). There were four replicas for each treatment (32 combinations) and two more replicates for the negative controls, so 130 pots were tested in total.

For the labelling, a code was developed: carbonate free soil was called soil a, and the carbonate one, soil c. The cover crops and the APFs were represented by capital letters: B for Buckwheat, T for Red clover, P for Phacelia and E for no cover crop; and for the APFs: A for ash, D for digestate, R for rock phosphate and O for no amendment. The replicas are depicted by numbers from 1 to 5.

Table 5 shows a little summary of all the treatments:

Table 5. Overview of all the treatments in the experimental setup.

Carbonate free soil(a)				
Cover crop/APF	**No fertilizer (O)**	**Ash(A)**	**Digestate(D)**	**Rock Phosphate(R)**
No cover crop(E)	aEO1-5	aEA1-4	aED1-4	aER1-4
Buckwheat (B)	aBO1-4	aBA1-4	aBD1-4	aBR1-4
Red Clover(T)	aTO1-4	aTA1-4	aTD1-4	aTR1-4
Phacelia (P)	aPO1-4	aPA1-4	aPA1-4	aPR1-4

Carbonate soil(c)				
Cover crop/APF	**No fertilizer (O)**	**Ash(A)**	**Digestate(D)**	**Rock Phosphate(R)**
No cover crop(E)	cEO1-5	cEA1-4	cED1-4	cER1-4
Buckwheat (B)	cBO1-4	cBA1-4	cBD1-4	cBR1-4
Red Clover(T)	cTO1-4	cTA1-4	cTD1-4	cTR1-4
Phacelia (P)	cPO1-4	cPA1-4	cPA1-4	cPR1-4

The code for the labelling follows the next order: first the kind of soil (a/c), then the crop (E/B/T/P), after it the APF(O/A/D/R) and finally the replica, which is indicated with numbers, from 1 to 4 or to 5 in 2 cases; as it is shown in the previous table.

In the following images, all the pots in the net house before the harvest, at the moment of the harvest and after it are shown. We can see how all the pots have the same size, and also the same amount of soil. In addition, the conditions of the experimental set up, like the environmental conditions, the tubes for the irrigation when it was necessary, are presented in order to get an idea of the experiment.

Image 4: Pots before the harvest in the net-house (Photo taken by my camera on 29/09/ 2014)

Image 5: Pots before the harvest in the net-house (Photo taken by my camera on 29/09/ 2014)

Image 6: Pots at the moment of the harvest in the net-house (Photo taken by my camera on 29/09/ 2014)

Image 7: Pots after the harvest in the net-house (Photo taken by my camera on 29/09/ 2014)

2.3 Measurements

2.3.1 Soil samples

a) Sample collection

After the vegetation period (3 months after the seeding), soil samples were collected; all the replicas from the same treatment were put together; partly stored in vials in the fridge (for mineral nitrogen for other experiments) and partly in paper bags stored in a room to be air-dried during a week.

b) Analysis of P using CAL- extraction of soil (ÖNORM L1087)

Before the extraction, the air-dried soil had to be sieved (< 2 mm). All 32 samples were extracted with the calcium-acetate-lactate method to determine the plant available P (P_{CAL}) . For this, 5 g (range between 4,95 – 5,05 g) were weighed into 500 mL PE-bottles and mixed with 100 mL of the CAL-work solution which was a dilution of the stock solution(pH 4.1) in a ratio of 1:5 described below:

Stock solution: 77g $C_6H_{10}CaO_6$*5 H_2O (calcium lactate) and 39,5 g $Ca(CH_3COO)_2$*H_2O (calcium acetate) solved in about 600 mL of hot Aqua bidest. , mixed then with 89,5 mL CH_3COOH (acetate) and top up with water to 1 L.

As half samples contained carbonate, the lids had to be closed after CO_2 escaped from the soil. After this, all the samples were shaken in the overhead mixer for two hours at 20 revolutions per minute at room temperature, settled for 10-15 minutes and then filtered through Whatman filter paper. All the samples were stored in the fridge until analysis.

The method used to determinate phosphorus concentration (P_{CAL}) was Molybdate Method 1 of the ÖRNOM. 100 µL of sample solution or standard (0.5, 1, 5, 10, 15, 20, 25 mg P/L) were mixed with 1,6 mL of a 1:10 diluted ammonium heptamolybdate solution [12,6 g $(NH_4)_6MO_7O_{24}$ * 4 H_2O in 400 mL H_2O plus 140 mL H_2SO_4 p: 1,84 g/mL, plus 0,5 g $K(SbO)C_4H_4O_5$ *0,5 H_2O (potassium antimony) in H_2O, all filled up to 1000 mL with H_2O] and 200 µL of L(+) Ascorbic acid solution [4,4 g $C_6H_8O_6$ in 1L H_2O]. Then, after 15 minutes reaction time, the absorption of the samples was measured at a wavelength of 660 nm on a UV/VIS spectrophotometer.

To calculate the P_{CAL} from the absorption of each sample, the following formula should be applied:

Absorption: m* P_{CAL} (mg P/l) +b; where:
m is the slope of the line calculated from the data of the standards. Its value is 0,0267.
b is the intercept calculated from the data of the standards. Its value is -0,00175.

Table 6. Values of absorption and P_{CAL} of the standards in CAL- extraction of soil

NAME	ABSORTION	P_{CAL} (mg P/l)
Standard 1	0,024	0,5
Standard 2	0,036	1
Standard 3	0,122	5
Standard 4	0,255	10
Standard 5	0,395	15
Standard 6	0,498	20
Standard 7	0,702	25

In order to compare both soils and see in which one the P uptake is higher the difference between the P_{CAL} of each treatment and P_{CAL} of the control one (no cover crop) is calculated. If this difference is below zero, we can assume that there is a P uptake by cover crop. Moreover, to compare the amount of P uptake by the same cover crop with different APF, absorption rate in % has been calculated as follow:

We can say that the P_{CAL} of the control is the 100% of P in the soil for each treatment, and the difference between P_{CAL} for each treatment and P_{CAL} of the control is the percentage of P uptake by the plant; i. e., it must be applied this simple rule of three:

P_{CAL} control → 100%
P_{CAL} of each treatment - P_{CAL} of the control → x

In order to determine the better soil to cultivate in, the results from biomass, number of plants per pot and biomass per pot, explained in the next section, were used. We can take on that the soil where the crop growth is higher will be more suitable for this purpose.

2.3.2 Plant samples

a) Harvest

After the vegetation period , all the plants from each pot were cut and placed in paper bags, counting the exact number of plants of each one. Then, they were dried during a week in an oven at 60 ºC. The temperature in the oven is a limiting factor; drying the sample at lower temperatures does not remove water from all tissues while drying at higher temperatures may decompose the sample thus reducing the dry weight and the nitrogen could evaporate. It is important to indicate that buckwheat showed flowers as well as phacelia, while red clover did not flower during the vegetation period.

b) Weight

All the dry samples were weighted with a balance and 2-3 grams were separated and crushed in small paper bags in order to use them for the digestion process. The rest were crushed too in small pieces and settled again into the pots to simulate crop rotation for following experiments.

c) **Plant digestion** (Zhao, F. et al., 1994)

About 0,200 g of oven-dried plant material (some variance, ± 0,010 g was tolerable), from the 2-3 grams which were separated previously, was weighed and transferred into tubes of known weight. 5 mL 65% HNO_3, about 1mL 30% H_2O_2 and 1 drop of 1-Octanol were added into each tube and, after placing the coolers on the tubes, they were incubated in the fume hood at room temperature overnight. The next morning the tubes were heated for approximately 4 hours at about 150 ºC in a heating block and then let to cool for 1 hour. After that, the tubes were filled with distilled water to approximately 50 mL, weighed again, mixed with a vortex-shaker, filtered through Whatman paper (Nr. 42, i.e. 2,5 µ) and stored in 50 mL vials. Coolers were rinsed and removed.
9 blanks (without plant material) and 9 references (Oriental Basma Tobacco Leaves (INCT-OBTL-5)), were added.

d) Analysis of P using molybdate blue colorimetry (Murphy J. and Riley J. P.,1962)

Phosphorus concentrations in the extracts were determined using molybdate blue colorimetry and then a UV/VIS spectrophotometer. The principle of this technique is the following: a sample containing the phosphate is mixed with an acid solution of Mo^{VI}, for example ammonium molybdate, to produce $PMo_{12}O_{40}^{3-}$, which has an α-Keggin structure. This anion is then reduced by, for example, ascorbic acid or $SnCl_2$, to form the blue coloured β-keggin ion, $PMo_{12}O_{40}^{7-}$.The amount of the blue coloured ion produced is proportional to the amount of phosphate present and the absorption can be measured using a colorimeter to determine the amount of phosphorus (Barrows et al, 1985)

1mL of each sample, including 2 blanks (1mL of water, no plant material form digestion) and 11 standards (10, 25, 50, 75, 100, 150, 200, 250, 300, 350 ,400 µg L^{-1}), was mixed with 0,2 mL of a staining reagent described below:

<u>Staining reagent for samples containing no H_2SO_4</u>

- 10 mL of a solution of 25,5 g 96% H_2SO_4 top up to 100 mL with distilled water.
- 3 mL of a solution of 10 g ammonium heptdamolybdate tetrahydrate top up to 250 mL with distilled water.
- 1 mL of a solution of 0,28 g potassium antimonyltartrate hydrate in water, top up to 100 mL.
- 6 mL of 0,88 g ascorbic acid in water, top up to 50 mL.

Then, the absorption of the samples was measured at a wavelength of 881 nm on a UV/VIS spectrophotometer.

To calculate the phosphorus concentration (g/kg) of each sample the following formulas should be applied:

P (g/kg): $\frac{\text{P in digestion(mg/L)} * \text{Volume of the tube after the digestion(mL)}}{\text{sample weight (g)} * 1000}$

P in digestion (mg/L): P(µg/L)*50*0,001

P(µ/L): $\frac{\text{Absorption} - b}{m}$, where

m is the slope of the line calculated from the data of the standards. Its value is 0,00054.
b is the intercept calculated from the data of the standards. Its value is 0,018.

Table 7. Values of absorption and P(µg/L) of the standards after the plant digestion

NAME	ABSORTION	P_{CAL} (mg P/l)
Standard 1	10	0,011
Standard 2	25	0,033
Standard 3	50	0,055
Standard 4	75	0,067
Standard 5	100	0,067
Standard 6	150	0,084
Standard 7	200	0,136
Standard 8	250	0,171
Standard 9	300	0,193
Standard 10	350	0,196
Standard 11	400	0,230

These data are used to find out if there is mobilization of phosphorus and which cover crop mobilizes more than the rest. This is going to be tested by calculating first the amount of P in plant biomass of all the pots and then, finding out if this amount of P is higher than the variation of P_{CAL} (ΔP_{CAL}) in the soil. To calculate the ΔP_{CAL} of each pot, P_{CAL} from the control ones are going to be used.

If there is more amount of P in plant biomass of red clover than in the other cover crops, it can be assumed that this cover crop mobilizes more P than the others in the above ground. However, to be sure that there is mobilization in the soil, it is necessary to compare the amount of P in plant biomass individually for each pot with the variation of P_{CAL} in the soil, because it will be the P uptake by the cover crop. If this value is higher than 0, it can be assumed that it must be P mobilization in the soil.

Here, we can see how it will be calculated:

- Amount of P in plant biomass - $\Delta P_{CAL} > 0$ → must be mobilization
    - Amount of P in plant biomass (mgP/pot) = P concentration (mg P/g DW) * Dry weight (gDW/pot)
    - $\Delta P_{CAL} = P_{CAL}$ of each pot - P_{CAL} control

e) Plant total carbon and nitrogen

Elemental analyses of total nitrogen and carbon (and sulfur) is performed to provide carbonate and organic carbon and to get some idea of the composition of the organic matter.

The total nitrogen and carbon are determined using a CHNS analyser. For this 0.1-0.5 g homogenised sample of every replica of each treatment were mixed twice to obtain 48 mixed samples and 80 miligrams of the samples were weighed and analysed in the Vario Macro cube, Modus CN from Elementar Analysensysteme GmbH (Germany).

f) C/P ratio

The C/P ratio determines whether there is net mineralization of phosphorus or net immobilization (from webpage of University of Hawaii at Manoa). For this, C/P ratios were calculated from the amount of P measured for every pot and the C concentrations in mg per pot of the mixed plant replicas.

g) P mineralization (INTERPRETATION)

To explain the possible P mineralization necessary for the following crops, results from P concentration was used instead of total amount of P in the above-ground biomass, since according to Arcand et al. (2010), a narrow C/P ratio (less than 300) and a high P concentration are the most important factors to affirm that there will be P mineralization. Therefore, this will be taken into account in the conclusions.

2.4 Data analysis

Descriptive statistics, t-tests, univariate, multi-factorial analysis of variances (ANOVA) and post-hoc Tuckey_HSD tests in randomized block design for every parameter for the characterization of plants, were carried out using the statistical software PASW Statistics 18 (SPSS Inc.2009). Fixed factors were: soil, APF and cover crop; and the random factor was the block. In case the residues of ANOVA did not meet homogeneity of variance, data was logarithmically transformed in order to obtain more homogenous variances. The level of significance was 0,05. Microsoft Excel 2013 was used to generate all the graphics (mean and standard deviations) of plants and soils.

Part III: Results, discussion and conclusion

3.1 Results

The results will be presented as follow: first, the only factor studied for soil after the vegetation period, the P_{CAL} and a little analysis of possible P mobilization in soil. Then, all the factors studied for plants: biomass per pot and number of plants per pot and biomass per plant in order to explain the possible doubts on biomass; P concentration in plants; the amount of P in the above-ground biomass in each pot and the difference between amount of P and ΔP_{CAL} to find out if there is mobilization or not. Finally total carbon and nitrogen in plants and the ratio C/P.

3.1.1 Soils

a) P_{CAL}

In this section, the P_{CAL} concentration in soil after the vegetation period is analysed for all the treatments in both soils.

As we can see in Figure 8 and Figure 9, there are some differences between both soils. First, it is important to emphasize that there is more P_{CAL} concentration in the carbonate free soil (26.01 mgP kg^{-1}) than in the carbonate one (15.54 mgP kg^{-1}) for the control situation (no cover crop, no APF).

Secondly, if we take a look at the effect of the application of APFs on the pots with no cover crop, we can affirm that in the carbonate soil, the positive effect of this application is higher than in the carbonate-free soil, being the application of ash the highest one; since in soil c, the P_{CAL} concentration in soil is higher after the enforcement of all fertilizers, while in soil a, only the application of digestate raises this concentration.

On the other hand, the effect of the cover crops in P_{CAL} concentration also differs depending on the treatment and the soil. With buckwheat for example, this concentration is higher than the control one after the application of ash and digestate, while with rock phosphate, it is lower, implying a plant uptake, in soil a. In soil c, though, P_{CAL} concentration after all fertilizations is higher than the control one. For red clover, the results are completely different. In soil a, only with ash the P_{CAL} concentration is lower than the control; while in soil c, it occurs with digestate and rock phosphate. Finally, the effect in P_{CAL} concentration after planting phacelia in soil c is mostly the same after the application of all the APFs, whereas in soil a, only with rock phosphate, a decrease of this concentration is observed compared to the control.

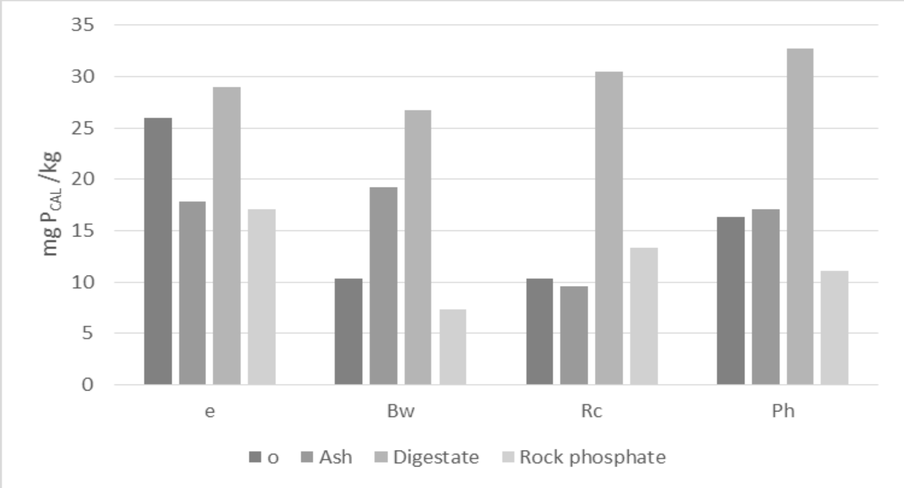

Figure 8. APF-Cover crop interaction for P_{CAL} in soil a.
Grey bars show mean value. No phosphorus addition (O), ash, digestate and rock phosphate on no cover crop (e), Buckwheat (Bw), Red clover (Rc) and Phacelia (Ph) on carbonate free-soil.

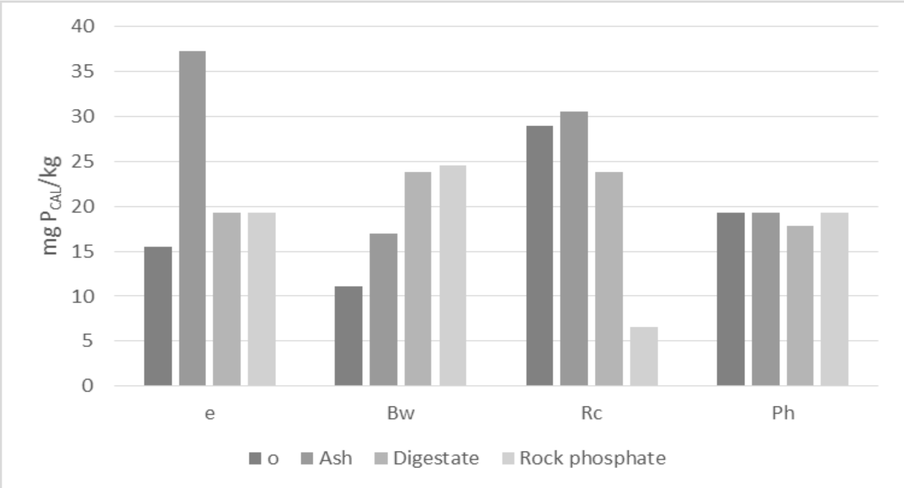

Figure 9 .APF-Cover crop interaction for P_{CAL} in soil c.
Grey bars show mean value. No phosphorus addition (O), ash, digestate and rock phosphate on no cover crop (e), Buckwheat (Bw), Red clover (Rc) and Phacelia (Ph) on carbonate soil.

b) Changes in P_{CAL} due to plant P uptake

Once described the above figures, P_{CAL} data (see Table 8 and 10) of all treatments of both soils are compared with the control one, in order to analyse a possible mobilization of the phosphorus in the soil.

In table 9, inconsistent values can be observed, the P_{CAL} concentration for control after the application of ash and rock phosphate is lower than with no fertilizer, which will be discussed later.

If we take a look at Table 9 and analyse the cover crops one by one, we can state how buckwheat absorbs P from the soil P_{CAL} pool with no fertilizer, the addition of digestate and rock phosphate, while red clover and phacelia absorbs P with the application of ash and rock phosphate and also with no fertilizer as well as buckwheat in the carbonate-free soil.

Table 8 . P_{CAL} of each treatment (mg P kg^{-1}) in soil a

APF/Cover crop	Bw	Rc	Ph	Control
O	10,3	10,3	16,3	26,0
Ash	19,3	9,5	17,0	17,8
Digestate	26,8	30,5	32,7	29,0
Rock phosphate	7,3	13,3	11,0	17,0

List of abbreviations: O, no fertilizer; Bw, Buckwheat; Rc, Red clover; Ph; phacelia; Control, no cover crop.

Table 9. P_{CAL} of each treatment – P_{CAL} control (mg P kg^{-1}) in soil a

APF/Cover crop	Bw	Rc	Ph
O	-15,7	-15,7	-9,7
Ash	1,5	-8,2	-0,7
Digestate	-2,2	1,5	3,7
Rock phosphate	-9,7	-3,7	-6,0

List of abbreviations: O, no fertilizer; Bw, Buckwheat; Rc, Red clover; Ph; phacelia; Control, no cover crop. Negative numbers indicate P uptake by cover crop.

However, in Table 11 inconsistent values are not observed. All the P_{CAL} concentrations after the fertilization with APFs are higher than the control one in soil c. If we analyse the cover crops, one by one, we can see how buckwheat absorbs P from the soil with the application of ash and without fertilizer, while red clover absorbs it with ash and rock phosphate, and phacelia, with ash and digestate.

Table 10 . P_{CAL} of each treatment (mg p/kg) in soil c

APF/Cover crop	Bw	Rc	Ph	Control
O	11,0	29,0	19,3	15,5
Ash	17,0	30,5	19,3	37,2
Digestate	23,8	23,8	17,8	19,3
Rock phosphate	24,5	6,6	19,3	19,3

List of abbreviations: O, no fertilizer; Bw, Buckwheat; Rc, Red clover; Ph; phacelia; Control, no cover crop.

Table 11. P_{CAL} of each treatment – P_{CAL} control (mg p/kg) in soil c

APF/Cover crop	Bw	Rc	Ph
O	-4,5	13,5	3,7
Ash	-20,2	-6,7	-18,0
Digestate	4,5	4,5	-1,5
Rock phosphate	5,2	-12,7	0,0

List of abbreviations: O, no fertilizer; Bw, Buckwheat; Rc, Red clover; Ph; phacelia; Control, no cover crop. Negative numbers indicate P uptake by cover crop.

In order to test and compare the amount of P uptake by the same cover crop with different APF, the absorption rate of P_{CAL} in % was calculated (see Tables 12 and 13).

Table 12. P_{CAL} decrease due to P uptake by cover crops related to P_{CAL} in the control with no cover crop in soil a(%)

APF/Cover crop	Bw	Rc	Ph
O	60,4	60,4	37,4
Ash	-	46,3	4,2
Digestate	7,7	-	-
Rock phosphate	57,1	22,0	35,2

List of abbreviations: O, no fertilizer; Bw, Buckwheat; Rc, Red clover; Ph; phacelia; Control, no cover crop

We can see in soil a how buckwheat absorbs more phosphorus (60.4 %) without fertilizer than with the application of rock phosphate (57.1 %) and digestate (7.7 %). For red clover, is also without fertilizer with wich takes more P from the soil (60.4 %), followed by ash (46.3 %) and then rock phosphate (22%). Like buckwheat and red clover, phacelia absorbs more P without fertilizer (37.4 %) than with rock phosphate (35.2 %) and ash (4.2%).

Table 13. P_{CAL} decrease due to P uptake by cover crops related to P_{CAL} in the control with no cover crop in soil c (%)

APF/Cover crop	Bw	Rc	Ph
O	28,9	-	-
Ash	54,3	18,1	48,2
Digestate	-	-	7,8
Rock phosphate	-	66,0	-

List of abbreviations: O, no fertilizer; Bw, Buckwheat; Rc, Red clover; Ph; phacelia; Control, no cover crop

With these data for soil c, it can be assumed that buckwheat absorbs more phosphorus (54.3%) with the application of ash than without fertilizer (28.9 %). For red clover, is the application of rock phosphate with which the cover crop takes more P from the soil (66 %), followed by ash (18.1 %). Like buckwheat, phacelia absorbs more P with ash (48.2%) than with digestate (7.8%).

3.1.2 Plants

a) Aboveground plant biomass

All following numbers are given in mg dry weight per pot. The mean of all treatments are tested first, then the interactions and finally, the main effects. Significances (p) and F (Fisher) value are given in Table 14.

Table 14. Factor effects of ANOVA for plant weight.

Factor	p	F
Soil	0,041	4,336
APF	0,000	7,272
Cover crop	0,000	303,988
Replica	0,078	2,369
Soil*Cover crop	0,000	13,978
Soil*APF	0,000	13,904
Cover crop*APF	0,002	4,041
Soil*Cover crop*APF	0,553	0,828

Fixed factors: Soil, alternative phosphorus fertilizer (APF), Cover crop. Random factor: Replica. Significant results: p< 0,05

In general, buckwheat reached the highest biomass in both soils with all the fertilizers applied. Moreover, the application of ash was with which all cover crops had the highest biomass in the carbonate free soil, while in the carbonate soil, the application of digestate was the better (Figure 10).

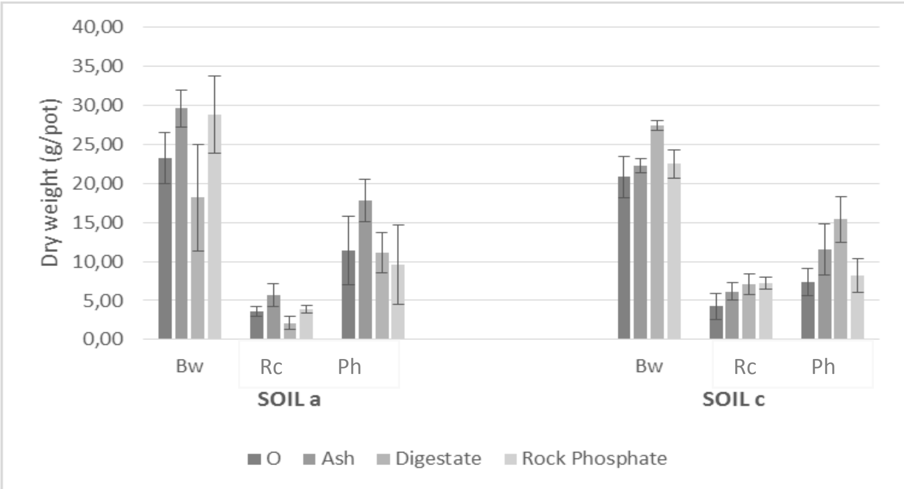

Figure 10. Mean of all treatments for plant weight.
Grey bars show mean value, error bars indicate two standard deviation of the mean (positive and negative). Buckwheat (Bw), Red clover (Rc), Phacelia (Ph) with no phosphorus addition(O), ash, digestate and rock phosphate on carbonate-free (a) and carbonate (c) soils

There is a significant interaction (p: 0.041) between soils and cover crops, however this interaction has no strong meaning since in both soils buckwheat has the highest dry weight followed by phacelia and red clover. It can be said that the difference in dry weight between the three cover crops is higher in the carbonate-free soil (a) than in the carbonate soil (c). However, the main interaction we can see in Figure 11, is that buckwheat and phacelia have higher values (24.982 ±6.349 g/pot ; 12.497 ± 4.746 g/pot respectively) in soil a than in soil c (23.276 ± 2.989 g/pot; 10.641 ± 4.024 g/pot respectively), and conversely red clover shows different behaviour, as its value in soil c (6.189 ± 1.685 g/pot) is higher than in soil a (3.845 ± 1.544 g/pot).

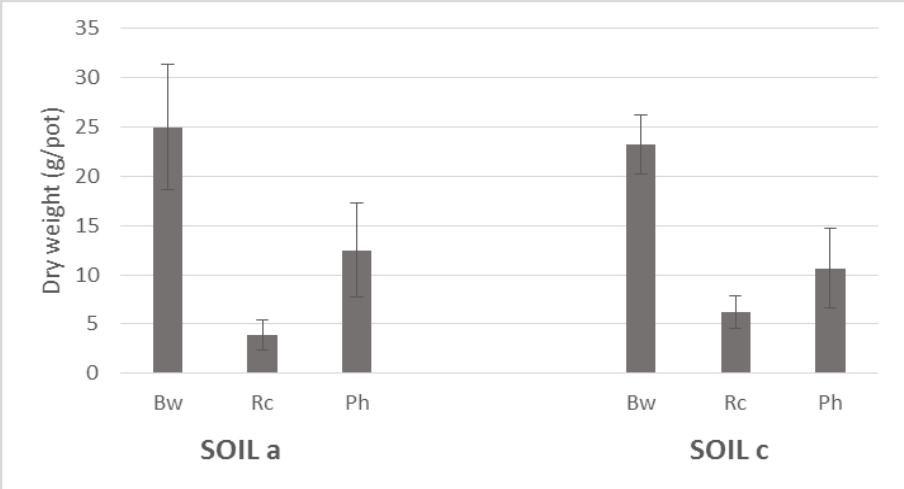

Figure 11. Soil-cover crop interaction (p: 0,041) for plant weight.
Grey bars show mean value, error bars indicate two standard deviation of the mean (positive and negative). Buckwheat (Bw), Red clover (Rc), Phacelia (Ph) on carbonate-free (a) and carbonate (c) soils.

Regarding the interaction between soil and APF (see Figure 12), although there is a significant interaction (p: 0.000), there is not too much difference in the dry weight of all cover crops with the application of rock phosphate between both soils . It can be observed, as the main interaction, that ash is the only APF that has a positive effect in soil a (its value is higher than the control), nevertheless the APF which has a positive effect in soil c is the digestate.

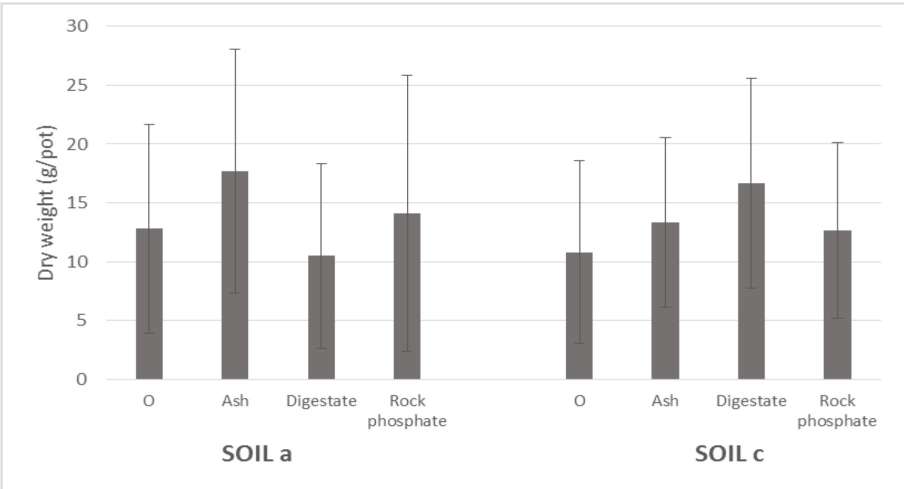

Figure 12. Soil-APF interaction (p: 0,000) for plant weight.
Grey bars show mean value, error bars indicate two standard deviations of the mean (positive and negative). No phosphorus addition(O), ash, digestate, rock phosphate on carbonate-free (a) and carbonate (c) soils.

With respect to the next interaction, cover crop with APF, although we can affirm that there are significant differences between the dry weight for the different treatments (p: 0.002), a very strong interaction is not seen. It can only be said that the best fertilizer for all the cover crops is ash, since with it, the highest values are seen (see Figure 13).

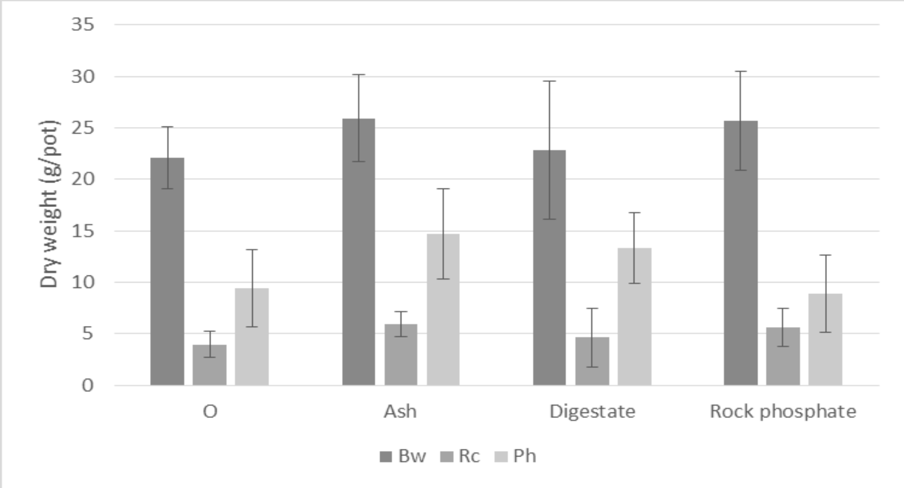

Figure 13. Cover crop-APF interaction (p: 0,002) for plant weight.
Grey bars show mean value, error bars indicate two standard deviations of the mean (positive and negative). Buckwheat (Bw), Red clover (Rc), Phacelia (Ph) on no phosphorus addition(O), ash, digestate and rock phosphate.

Finally, the main effects are tested (Figure 14). We can affirm that the mean of dry weight for all cover crops and all fertilizers in soil a is significantly different from that in soil c (p: 0.041), however their values are similar (13.775 ± 9.884 g/pot in soil a and 13.369 ± 7.901 g/pot in soil c). Regarding the fertilizers, it can also be stated that they are significant different (p: 0.000). The dry weight in pots with ash is significant different and higher (15.527 ± 9.039 g/pot) than with rock phosphate (13.388 ± 9.657 g/pot) and without fertilizer (11.801 ± 8.233 g/pot), but is no significant different with digestate (13.571 ± 8.794 g/pot).

The dry weight per pot in all cover crops is significantly different (p: 0.000) and also we can affirm that the biomass per pot in buckwheat is the uppermost value (24.129 ± 4.958 g/pot) followed by phacelia (11.569 ± 4.430 g/pot) and red clover (5.017 ± 1.986 g/pot).

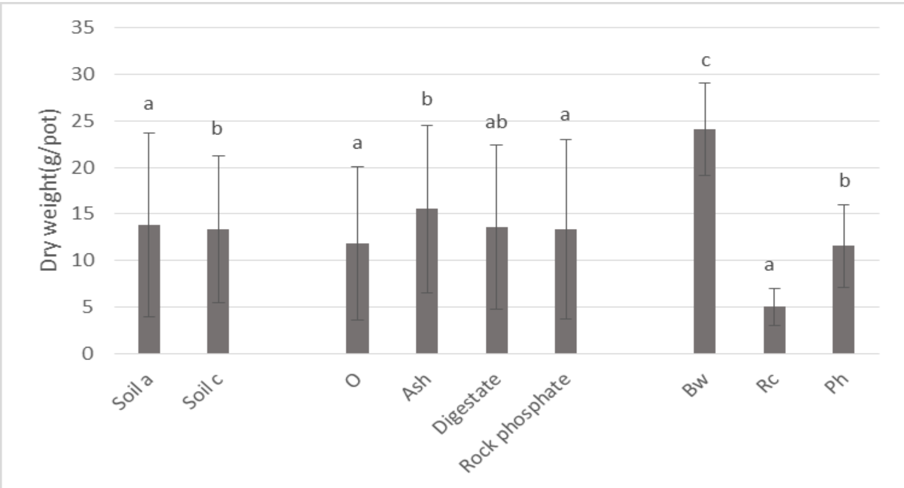

Figure 14. Mean plant weight for all factors.
Grey bars show mean value, error bars indicate two standard deviation of the mean (positive and negative). Carbonate-free (a) and carbonate (c) soils, no phosphorus addition(O), ash, digestate, rock phosphate, buckwheat (Bw), Red clover (Rc) and Phacelia (Ph). Bars with the same letter for the same factor are not significantly different (p>0,05)

b) Number of plants per pot

All following numbers are given in number of plants per pot. The mean of all treatments are tested first, then the interactions and finally, the main effects. Significances (p) and F(Fisher) value are given in table 15.

Table 15. Factor effects of ANOVA for number of plants per pot.

Factor	p	F
Soil	0,000	34,964
APF	0,023	3,396
Cover crop	0,000	125,725
Replica	0,001	6,084
Soil*Cover crop	0,000	21,019
Soil*APF	0,002	5,659
Cover crop*APF	0,005	3,433
Soil*Cover crop*APF	0,002	3,912

Fixed factors: Soil, alternative phosphorus fertilizer (APF), Cover crop. Random factor: Replica. Significant results: p< 0,05

In figure 15, we can see how red clover and phacelia had more number of plants than buckwheat in both soils with all the fertilizers. In soil a, the number of plants per pot of buckwheat was similar after the application of all treatments, as well as in soil c, while for red clover, the application of no fertilizer was with which this cover crop had the highest number of plants, and for phacelia, the highest number of plants was with the application of digestate in the carbonate free soil. In soil c, both phacelia and red clover reached the highest number of plants per pot with the application of digestates.

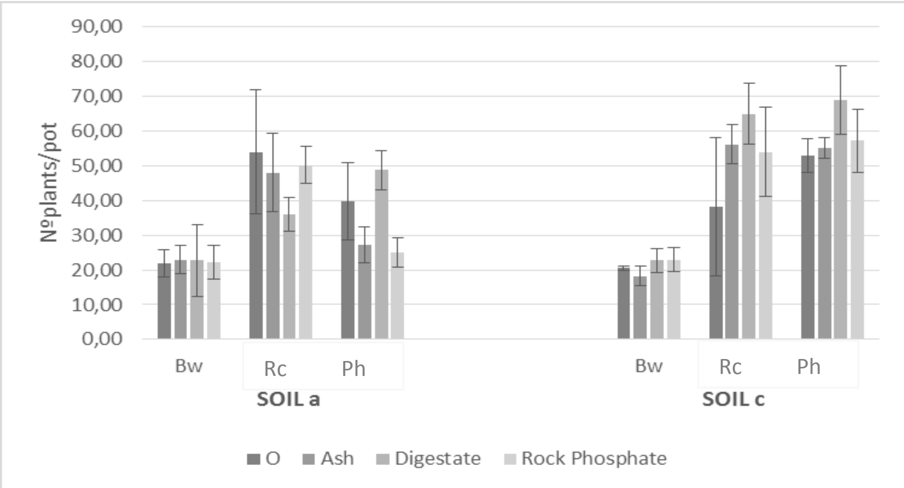

Figure 15.Mean of all treatments for plant number.
Grey bars show mean value, error bars indicate two standard deviation of the mean (positive and negative). Buckwheat (Bw), Red clover (Rc), Phacelia (Ph) with no phosphorus addition(O), ash, digestate and rock phosphate on carbonate-free (a) and carbonate (c) soils.

There is a significant interaction (p: 0,000) between soils and cover crops, so it can be said that there is a different performance of cover crops in both soils. We can see in Figure 16 that the difference between the number of plants per pot is higher in the carbonate-free soil (a) than in the carbonate soil (c), as with dry weight. On the other hand, it can be affirmed that the number of plants of buckwheat in both soils is similar (22.50 plants/pot in soil a and 21.12 plants/pot in soil c), while this number differs in the other cover crops being higher in soil c for red clover and phacelia. Moreover, in soil c, phacelia is the cover crop with the highest number of plants per pot, while in soil a, red clover is the uppermost.

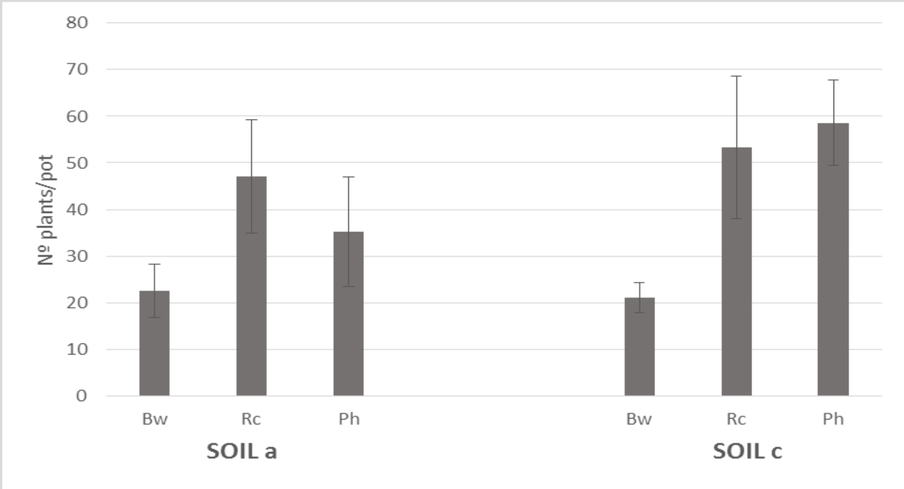

Figure 16. Soil-cover crop interaction (p: 0,000) for plant number.
Grey bars show mean value, error bars indicate two standard deviation of the mean (positive and negative). Buckwheat (Bw), Red clover (Rc), Phacelia (Ph) on carbonate-free (a) and carbonate (c) soils.

Regarding the interaction between soil and APF (see Figure 17) the number of plants with the application of rock phosphate is higher in carbonate soil than in the carbonate-free one. It can be observed, as the main interaction, that none of the APFs has a positive effect in soil a (its value is higher than the control), nevertheless the APF which has a positive effect in soil c is the digestate.

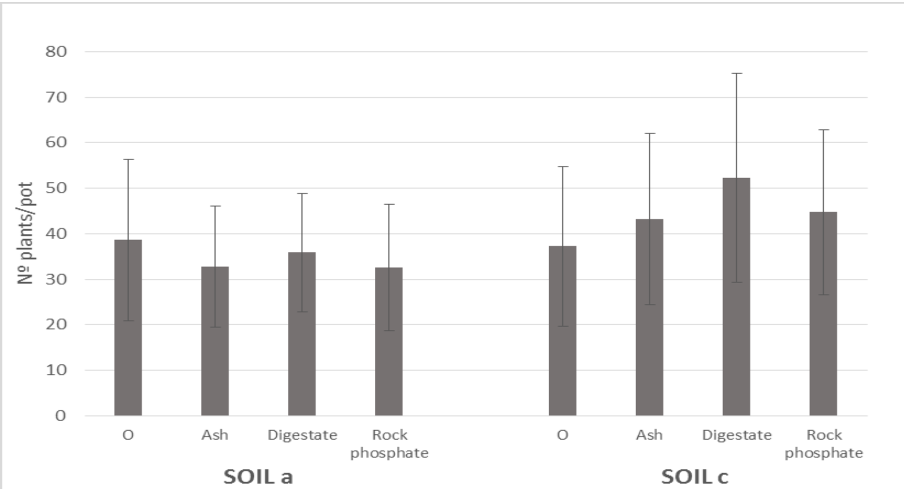

Figure 17. Soil-APF interaction (p: 0,002) for plant number.
Grey bars show mean value, error bars indicate two standard deviation of the mean (positive and negative). No phosphorus addition(O), ash, digestate, rock phosphate on carbonate-free (a) and carbonate (c) soils.

With respect to the next interaction, cover crop with APF, although we can affirm that there are significant differences between the number of plants per pot for the different treatments (p: 0,005), a very strong interaction is not seen. It can only be said that phacelia replies in a different way depending on the APF , performing higher with digestate than ash, while the other cover crops have not too many differences between the different APFs (see Figure 18).

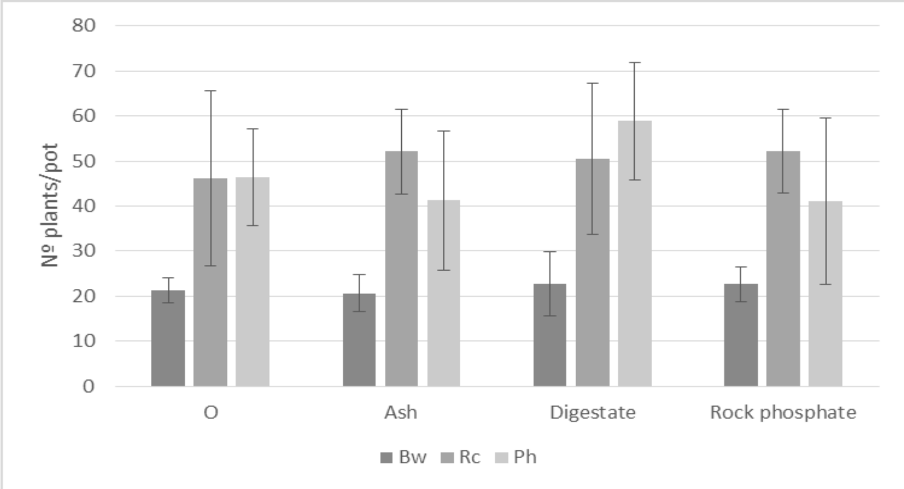

Figure 18. Cover crop-APF interaction (p: 0,005) for plant number.
Grey bars show mean value, error bars indicate two standard deviation of the mean (positive and negative).
Buckwheat (Bw), Red clover (Rc), Phacelia (Ph) on no phosphorus addition(O), ash, digestate and rock phosphate.

In the following Figure (19), the soil-covercrop-APF interaction is represented. It can be observed firstly that buckwheat performs similar with all the APF and in both soils, while red clover and phacelia have more effects with soil and APF. Secondly, we can affirm that with all fertilisers there is a higher number of plants in soil c than in soil a. However, if we focus on the number of plants without fertilizer, red clover has more number of plants per pot in soil a, while phacelia has more in soil c. Otherwise, the best APF in the carbonate-free soil is digestate for all cover crops, although it has no positive effect (number of plants with no fertilizer is higher); whereas for the carbonate soil, the best APF for red clover is ash, and for phacelia is digestate.

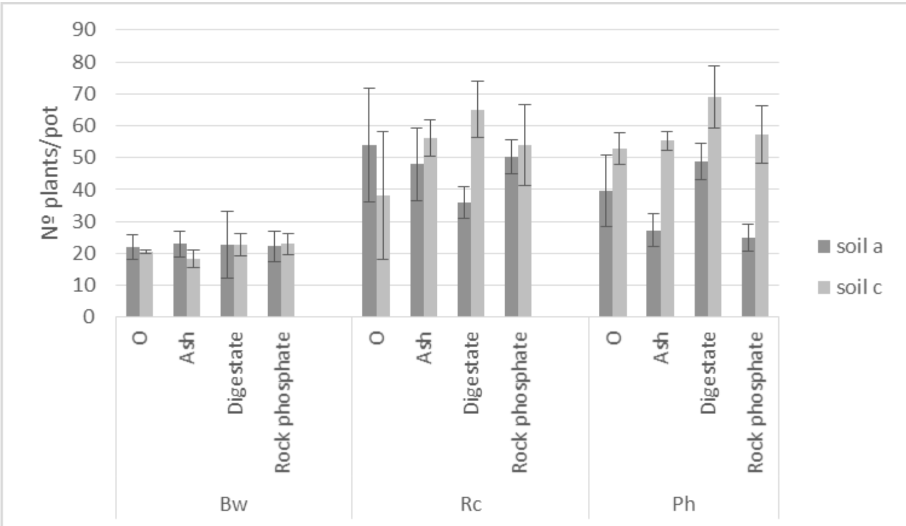

Figure 19. Soil-Cover crop-APF interaction (p: 0,002) for plant number.
Grey bars show mean value, error bars indicate two standard deviation of the mean (positive and negative).
Buckwheat (Bw), Red clover (Rc), Phacelia (Ph) on no phosphorus addition(O), ash, digestate and rock phosphate on carbonate-free (a) and carbonate (c) soils.

Finally, the main effects are tested in Figure 20. It can be affirmed that the mean of number of plants per pot for all cover crops and all fertilizers in soil a is significantly different and less than in soil c (p: 0,000) (34.92 ± 14.33 plants/pot in soil a and 44.38 ± 19.63 plants/pot in soil c). Regarding the fertilizers, it can also be stated that they are significantly different (p: 0,023). However, we can only affirm that the number of plants per pot with digestate is significantly different and higher than the application of ash and without fertiliser (44.04 ± 20.08; 38 ± 16.82; 37.91 ±17,24 plants/pot respectively)

The number of plants per pot in all cover crops are significantly different (p:0,000), buckwheat features the lowest number, followed by phacelia and red clover (21.81 ± 4.63; 46.91 ± 15.80; 50.21 ± 13.98 plants/pot respectively).

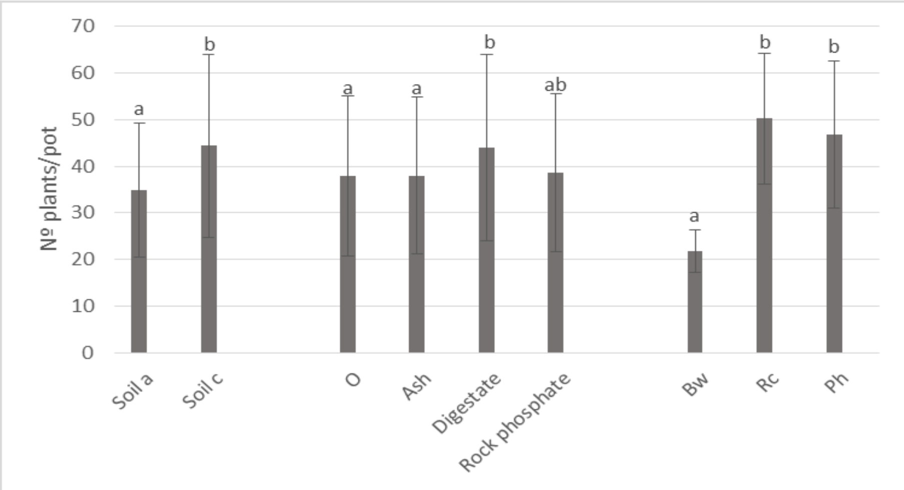

Figure 20. Mean plant number for all factors.
Grey bars show mean value, error bars indicate two standard deviation of the mean (positive and negative). Carbonate-free (a) and carbonate (c) soils, no phosphorus addition(O), ash, digestate, rock phosphate, buckwheat (Bw), Red clover (Rc) and Phacelia (Ph). Bars with the same letter for the same factor are not significantly different (p>0,05)

c) Biomass per plant

All following numbers are given in g dry weight per plant. The mean of all treatments are tested first, then the interactions and finally, the main effects. Significances (p) and F(Fisher) value are given in table 16.

Table 16. Factor effects of ANOVA for biomass per plant.

Factor	p	F
Soil	0,118	2,507
APF	0,000	8,513
Cover crop	0,000	609,254
Replica	0,002	5,647
Soil*Cover crop	0,000	30,839
Soil*APF	0,000	7,939
Cover crop*APF	0,194	1,493
Soil*Cover crop*APF	0,219	1,421

Fixed factors: Soil, alternative phosphorus fertilizer (APF), Cover crop. Random factor: Replica. Significant results: p< 0,05

As well as in the results of biomass per pot, in Figure 21 we can see how buckwheat had the highest biomass per plant in both soils. Moreover, red clover showed more biomass per plant in the carbonate free soil than in the carbonate soil after the application of all treatments as well as phacelia. On the other hand the application of ash in the carbonate free soil is with which all the cover crops had the highest biomass per plant, while in the calcareous soil it deppends on the cover crop. For buckwheat is the application of ash with which it had the highest biomass, for red clover, the application of rock phosphate and for phacelia, the application of digestate.

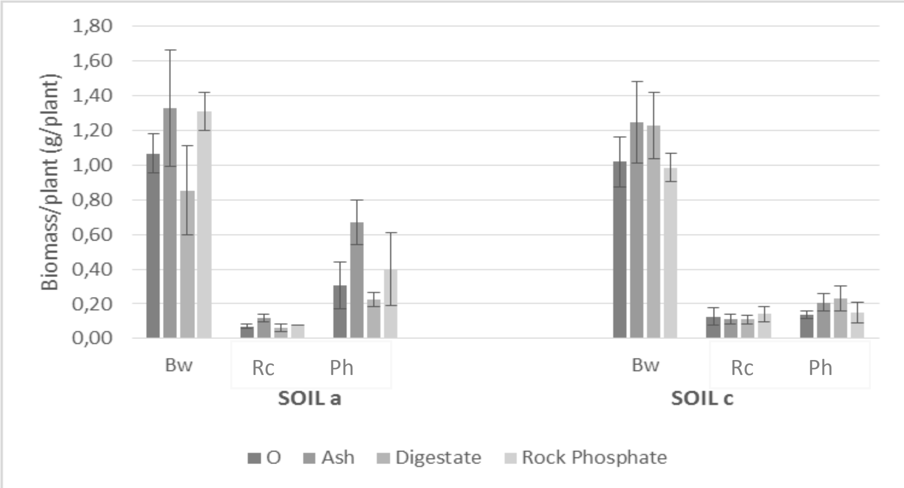

Figure 21. Mean of all treatments for biomass per plant.
Grey bars show mean value, error bars indicate two standard deviation of the mean (positive and negative). Buckwheat (Bw), Red clover (Rc), Phacelia (Ph) with no phosphorus addition(O), ash, digestate and rock phosphate on carbonate-free (a) and carbonate (c) soils.

First of all, a significant (p: 0,000) soil-cover crop interaction is analysed in Figure 22. It can be affirmed that the cover crop which has more biomass per plant is buckwheat in both soils, followed with a big difference by phacelia and red clover. As happens in the two previous cases (dry weight and number of plants per pot), there is a higher difference between the biomass per plant of the cover crops in soil a than in soil c, where the biomass per plant of red clover and phacelia is similar.

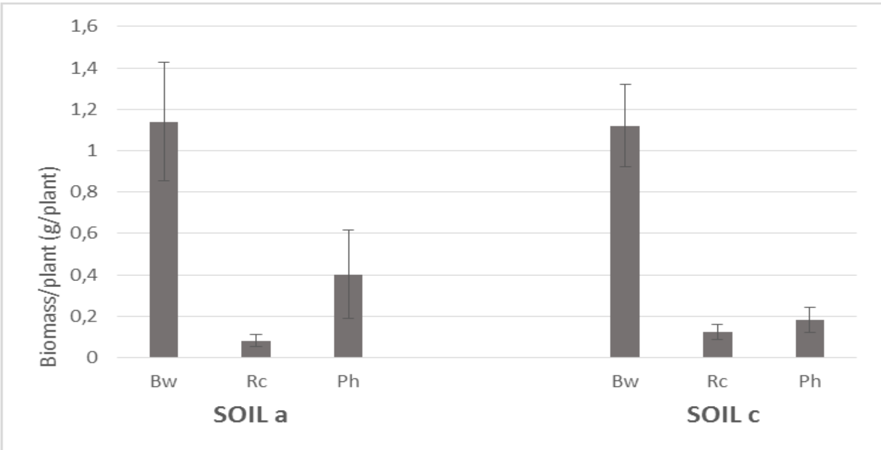

Figure 22. Soil-cover crop interaction (p: 0,000) for biomass per plant.
Grey bars show mean value, error bars indicate two standard deviation of the mean (positive and negative). Buckwheat (Bw), Red clover (Rc), Phacelia (Ph) on carbonate-free (a) and carbonate (c) soils.

As happens with dry weight (Figure 12), although there is a significant interaction (p: 0,000) between soil and APF for the factor biomass per plant (Figure 23)there is not much difference between the application of rock phosphate in both soils. In general, the behaviour of this factor is similar to the dry weight, so the main interaction is the same. Ash is the only APF that has a positive effect in soil a (its value is higher than the control), nevertheless the APFs which have a positive effect in soil c is the digestate and ash.

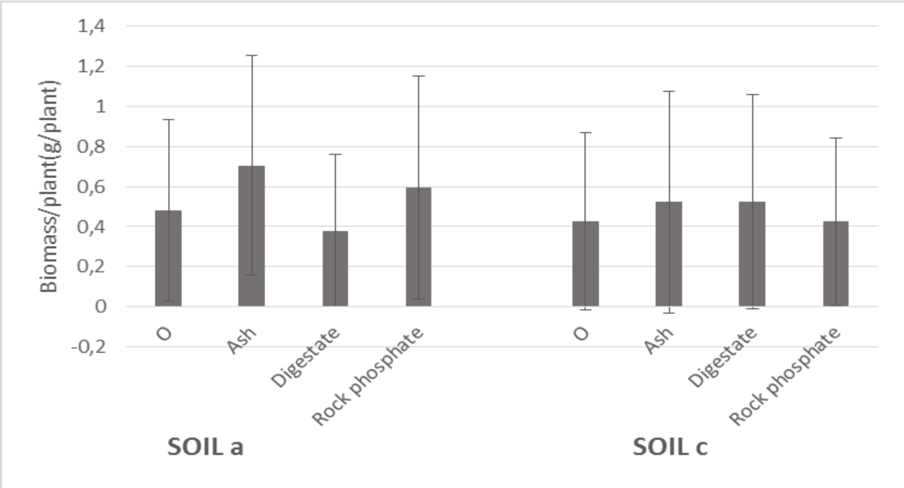

Figure 23. Soil-APF interaction (p: 0,000) for biomass per plant.
Grey bars show mean value, error bars indicate two standard deviation of the mean (positive and negative).
No phosphorus addition(O), ash, digestate, rock phosphate on carbonate-free (a) and carbonate (c) soils.

Finally, the main effects are tested in Figure 24. In this case we cannot affirm that there is a significant difference between both soils, since the p-value is higher than 0,005 (0,119). Regarding the APFs, it can be stated that they are significant different (p: 0,000). However, we can only affirm that the biomass per plant with the application of ash is significant different and higher than the other ones (0.614 ± 0.547 g/plant for ash; 0.454 ± 0.440 g/plant for no fertilizer; 0.452 ± 0.460 g/plant for digestate and 0.510 ± 0.490 g/plant for rock phosphate).

The biomass per plant in all cover crops is significantly different (p: 0,000), and also can affirm that the biomass in red clover (0.760 ± 0.418 g/pot) is the uppermost value followed by buckwheat (0.611 ± 0.573g/pot) and phacelia (0.152 ± 0.06 g/pot), which differs with the results from biomass per pot (Figure 13).

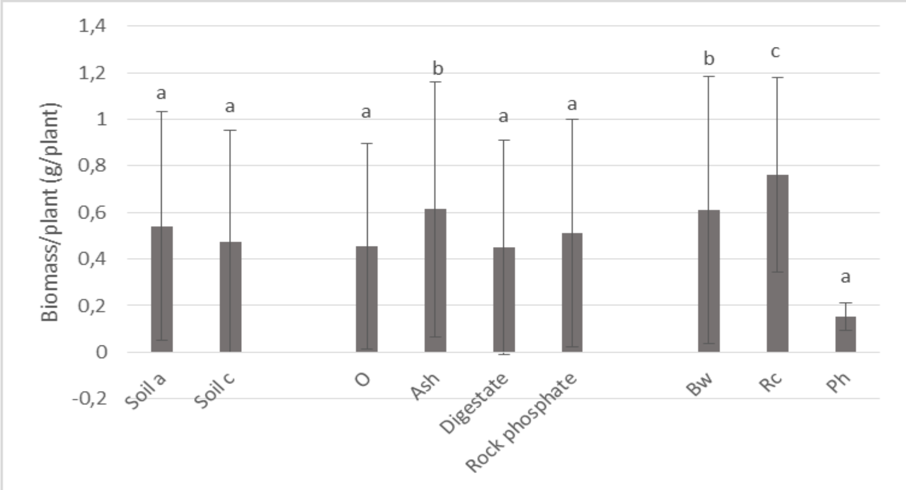

Figure 24. Biomass per plant for all factors.

Grey bars show mean value, error bars indicate two standard deviation of the mean (positive and negative). Carbonate-free (a) and carbonate (c) soils, no phosphorus addition(O), ash, digestate, rock phosphate, buckwheat (Bw), Red clover (Rc) and Phacelia (Ph). Bars with the same letter for the same factor are not significantly different (p>0,05)

d) Concentration of phosphorus in plants

All following numbers are given in g of phosphorus per kg of plant. The mean of all treatments are tested first, then the interactions and finally, the main effects. Significances (p) and F(Fisher) value are given in table 17.

Table 17. Factor effects of ANOVA for biomass per plant.

Factor	p	F
Soil	0,000	51,891
APF	0,000	8,748
Cover crop	0,000	72,218
Replica	0,152	1,820
Soil*Cover crop	0,000	13,855
Soil*APF	0,143	1,871
Cover crop*APF	0,174	1,556
Soil*Cover crop*APF	0,074	2,023

Fixed factors: Soil, alternative phosphorus fertilizer (APF), Cover crop. Random factor: Replica. Significant results: p< 0,05

In Figure 25, the phosphorus concentration for all treatments is shown. In general, the P concentration of the plants grown in soil a is higher in this soil than in soil c. Red clover had the highest P concentration in the carbonate soil with all the fertilizers, while in the carbonate free soil, it had the highest concentration only with no fertilizer, since phacelia was the cover crop with more P concentration after the application of ash, digestate and rock phosphate.

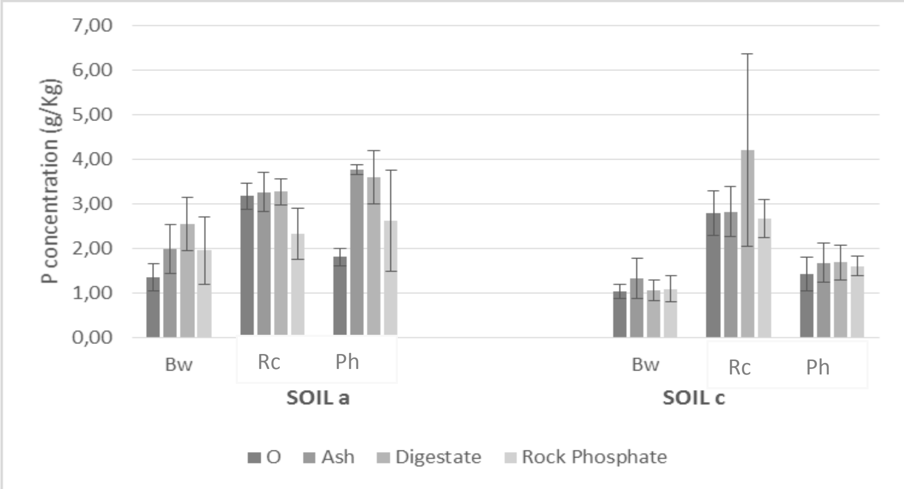

Figure 25. Mean of all treatments for P concentration in plants.
Grey bars show mean value, error bars indicate two standard deviation of the mean (positive and negative). Buckwheat (Bw), Red clover (Rc), Phacelia (Ph) with no phosphorus addition(O), ash, digestate and rock phosphate on carbonate-free (a) and carbonate (c) soils.

Firstly, it is interesting to enhance that red clover is the cover crop with the highest concentration of phosphorus in both soils. It must also be said that there is a significant interaction between cover crops and soils (p: 0,000) from which we can conclude that there is no difference between the P concentration in red clover for both soils, while for the other two cover crops, there is a big difference. The concentration of this element is higher in soil a for buckwheat as well for phacelia than in soil c (see Figure 26).

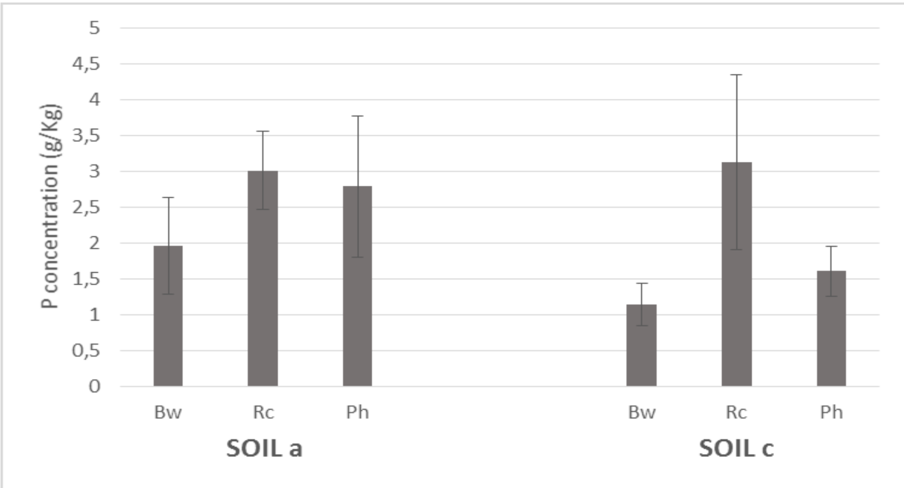

Figure 26. Soil-cover crop interaction (p: 0,000) for P concentration in plants.
Grey bars show mean value, error bars indicate two standard deviation of the mean (positive and negative). Buckwheat (Bw), Red clover (Rc), Phacelia (Ph) on carbonate-free (a) and carbonate (c) soils.

Regarding the main effects in Figure 27, we can state that there is a significant difference between the P concentration in both soils (p: 0,000), being higher in soil a (2.593 ± 0.887 g/Kg) than in soil c (1.961 ± 1.134 g/Kg). On the other hand, there is also a significant difference between the APFs, since its p value is 0,000. It can be affirmed that the concentration of P with the application of ash and digestate is significantly different and higher than the P concentration with the application of rock phosphate and without fertilizer.

Finally, the P concentration in all cover crops is significantly different (p: 0,000), red clover features the highest concentration, followed by phacelia and buckwheat (3.075 ± 0.938 g/Kg ; 2.201 ± 0.999 g/Kg; 1.555 ± 0.664 g/Kg respectively).

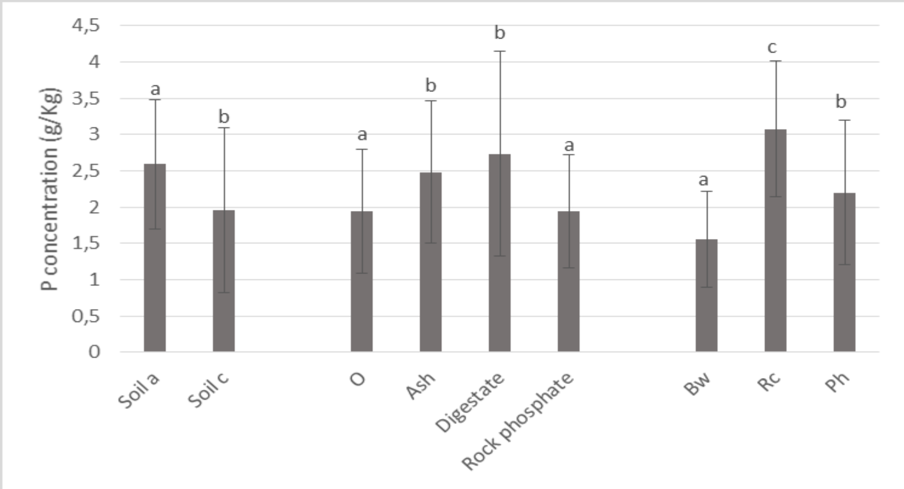

Figure 27. P concentration in plants for all factors.
Grey bars show mean value, error bars indicate two standard deviation of the mean (positive and negative) Carbonate-free (a) and carbonate (c) soils, no phosphorus addition(O), ash, digestate, rock phosphate, buckwheat (Bw), Red clover (Rc) and Phacelia (Ph). Bars with the same letter for the same factor are not significantly different (p>0,05)

e) Total amount of P in the above-ground biomass of plants

Multiplying the concentration of phosphorus (mg/g) by the weight dry (g/pot) of each pot, the total amount of P in above-ground plant biomass is obtained and with this data (see Table v in the appendix), statistics were done. The interactions are tested firstly, and then, the main effects. Significances (p) and F(Fisher) value are given in table 18.

Table 18. Factor effects of ANOVA for amount of phosphorus

Factor	p	F
Soil	0,005	8,234
APF	0,000	41,877
Cover crop	0,000	10,839
Replica	0,463	0,867
Soil*Cover crop	0,000	21,843
Soil*APF	0,002	5,285
Cover crop*APF	0,054	2,189
Soil*Cover crop*APF	0,074	2,026

Fixed factors: Soil, alternative phosphorus fertilizer (APF), Cover crop. Random factor: Replica. Significant results: p< 0,05

In general the amount of phosphorus in the above ground biomass of plants grown in the carbonate free soil was higher than in the carbonate soil for buckwheat and phacelia, while red clover reached higher amount of phosphorus in the carbonate soil than in the carbonate free soil (see Figure 28). On the other hand, with the application of ash, all cover crops had the highest amount of P in soil a, while in soil c, is the application of digestate.

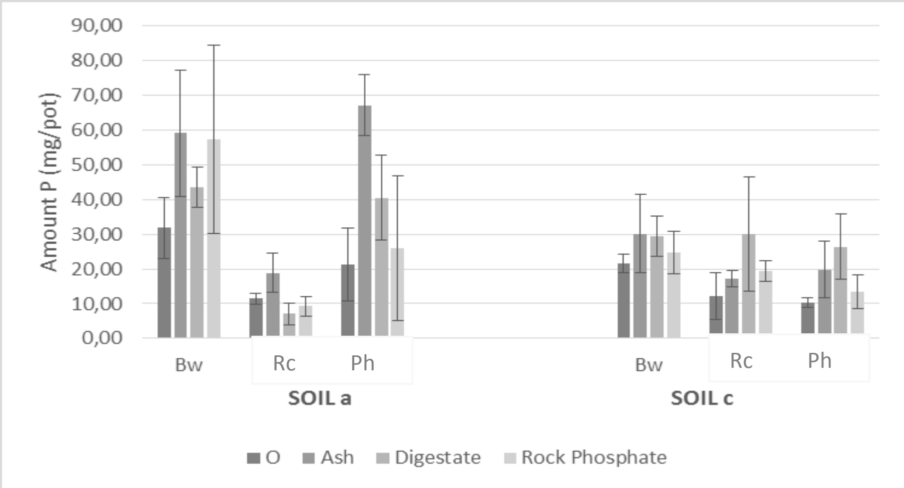

Figure 28.Mean of all treatments for amount of P in plants.
Grey bars show mean value, error bars indicate two standard deviation of the mean (positive and negative). Buckwheat (Bw), Red clover (Rc), Phacelia (Ph) with no phosphorus addition(O), ash, digestate and rock phosphate on carbonate-free (a) and carbonate (c) soils.

First of all, a soil-cover crop interaction (p:0,000) is analysed in Figure 29. We can see how the amount of P is different for each cover crop in both soils. In the carbonate free one the amount of P is higher for buckwheat and for phacelia, while in the carbonate one this value is higher for red clover.

Buckwheat showed the highest values in both soils, being higher in soil a (47.97 ± 19.16 mg P/pot) than in soil c (26.44 ± 7.38 mg P/pot). However, phacelia follows buckwheat in soil a with 37.12 ± 22.07 mg P/pot, but in soil c is the cover crop with the least amount of P (17.46 ± 8.79 mg P/pot. And, finally red clover has the least amount of P in soil a (11.64 ± 5.59 mgP/pot) and in soil c, it follows buckwheat with a value of 19.72 ± 10.58 mgP/pot.

There are more differences in the amount of phosphorus in the acidic soil between the cover crops than in the carbonate one.

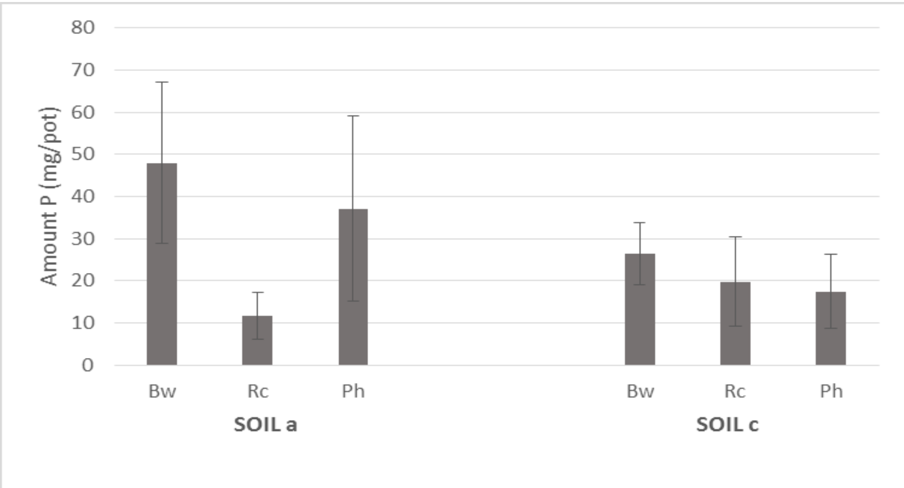

Figure 29. Soil-cover crop interaction (p: 0,000) for amount of P in plants.
Grey bars show mean value, error bars indicate two standard deviation of the mean (positive and negative). Buckwheat (Bw), Red clover (Rc), Phacelia (Ph) on carbonate-free (a) and carbonate (c) soils.

Secondly, in Figure 30, we can see an interaction between soil and APF (p: 0,002). The main thing is that the amount of phosphorus is higher after the application of all the fertilizers in both soils, being higher for all the treatments in soil a than in soil c. In the carbonate free one the APF with the highest value is ash (48.39 ± 24.768 mgP/pot) followed by digestate and rock phosphate (30.36 ± 18.73 and 28.68 ± 28.00 mgP/pot respectively), while in the carbonate soil the APF with the highest value is the digestate (28.65 ± 9.40 mgP/pot) followed by ash (22.37 ± 9.4 mgP/pot) and rock phosphate (19.19 ± 6.49), which is the APF with the lowest values in both soils.

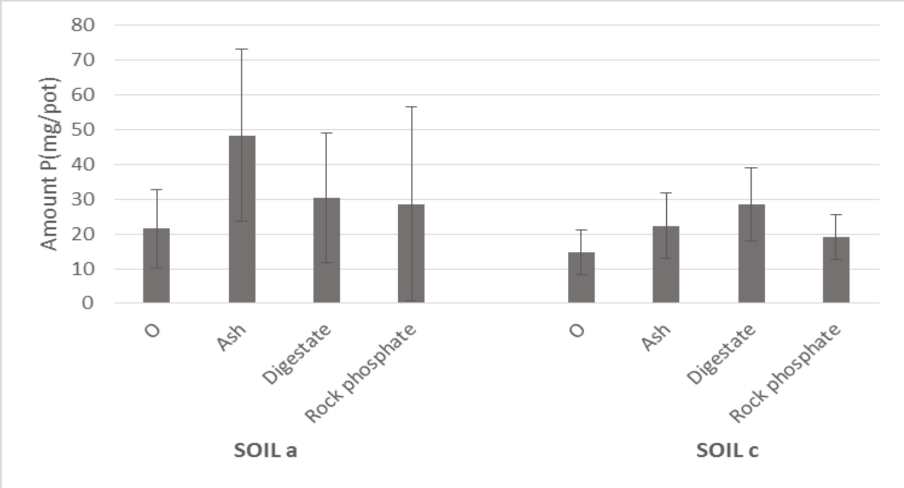

Figure 30. Soil-APF interaction (p: 0,002) for amount P in plants.
Grey bars show mean value, error bars indicate two standard deviation of the mean (positive and negative).
No phosphorus addition(O), ash, digestate, rock phosphate on carbonate-free (a) and carbonate (c) soils.

Regarding the main effects in Figure 31, we can state that there is a significant difference between the amount of P in both soils (p: 0,000), being higher in soil a (32,931 ± 23.00 mgP/pot) than in soil c (21,211 ± 9.625 mgP/pot). On the other hand, there is also a significantly difference between the APFs, since its p value is 0,000. It can be affirmed that the amount of P with the application of ash is significantly different and higher than the one without fertilizer and rock phosphate. Also, the amount of P is significantly different and higher with the application of digestate than in the one without fertilizer.

Finally, the P amount in all cover crops is significantly different (p: 0,000), buckwheat features the highest content, followed by phacelia and red clover (37.21 ± 17.99; 27.29 ± 19.81; 15.68 ± 9.28 mgP/pot respectively).

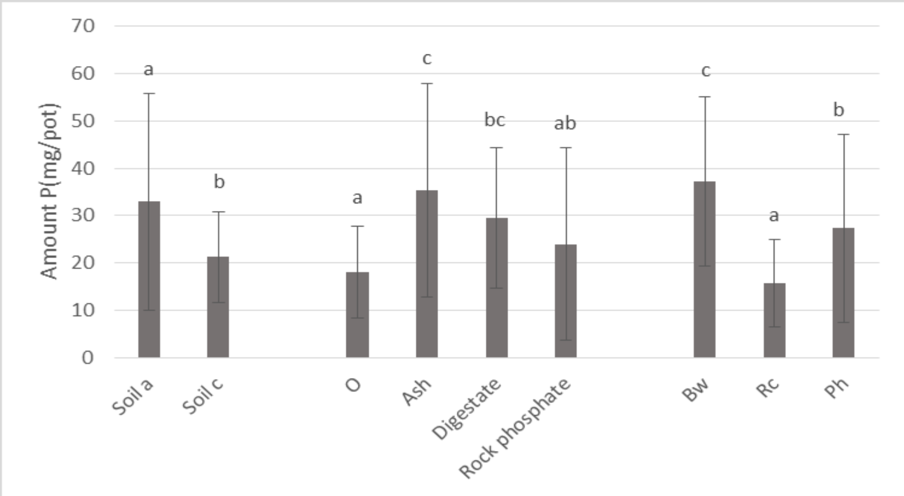

Figure 31. Amount P in plants for all factors.

Grey bars show mean value, error bars indicate two standard deviation of the mean (positive and negative). Carbonate-free (a) and carbonate (c) soils, no phosphorus addition(O), ash, digestate, rock phosphate, buckwheat (Bw), Red clover (Rc) and Phacelia (Ph). Bars with the same letter for the same factor are not significantly different ($p>0,05$)

f) Phosphorus mobilization by plants

In this subsection, the mobilization of phosphorus by the different cover crops with all the treatments is tested. For this, the total amount of P in plant biomass in mg per pot (previous section) was used and the decrease in P_{CAL} contents due to plant uptake from the P_{CAL} pool (ΔP_{CAL}) was calculated for each pot (4 kg of soil) as the P_{CAL} content of each treatment minus the P_{CAL} content of the control (no cover crop) .To affirm that there is P mobilization, the difference between amount P and ΔP_{CAL} must be more than 0, since this means that there is more P uptake in cover crop decrease in P_{CAL} content, therefore P uptake took place from other sources than the P_{CAL} pool, i. e. there is P mobilization.

In table 19, we can see a summary of the means of the difference between amount P and ΔP_{CAL} of all replicas for each treatment in soil a.

Table 19. Amount P in plant biomass - ΔP_{CAL} (mg /pot) for each treatment in soil a

APF/Cover crop	Bw	Rc	Ph
O	-31,0	-51,4	-17,6
Ash	53,2	-14,1	64,2
Digestate	34,6	1,1	25,5
Rock phosphate	18,4	-5,8	2,1

List of abbreviations: O, no fertilizer; Bw, Buckwheat; Rc, Red clover; Ph; phacelia; Control, no cover crop. Positive numbers indicate P mobilization. ΔP_{CAL} : decrease in P_{CAL} contents due to plant uptake from the P_{CAL} pool (the P_{CAL} content of each treatment minus the P_{CAL} content of the control (no cover crop))

If we take a look at these results, we can affirm that both buckwheat and phacelia mobilized phosphorus from non-P_{CAL} pools with the application of all fertilizers, while with red clover, only the addition of digestate produced this mobilization, in the carbonate-free soil.

On the other hand, in Table 20, we can see a summary of the means of the difference between amount P and ΔP_{CAL} of all replicas for each treatment in soil c.

Table 20. Amount P in plant biomass - ΔP_{CAL} (mg /pot) for each treatment in soil c

APF/Cover crop	Bw	Rc	Ph
O	3,5	-41,6	-4,8
Ash	-50,7	-9,8	-52,0
Digestate	11,5	12,1	20,4
Rock phosphate	3,8	-31,5	13,5

List of abbreviations: O, no fertilizer; Bw, Buckwheat; Rc, Red clover; Ph; phacelia; Control, no cover crop. Positive numbers indicate P mobilization. ΔP_{CAL} : decrease in P_{CAL} contents due to plant uptake from the P_{CAL} pool (the P_{CAL} content of each treatment minus the P_{CAL} content of the control (no cover crop))8

Taking into account these results, it can be stated that buckwheat mobilize the phosphorus with the application of digestate and rock phosphate as well as with no fertilization, while with red clover, only the addition of digestate produces this mobilization. In the case of phacelia, it mobilizes the phosphorus with digestate and rock phosphate.

g) Total nitrogen and carbon

The mean CN concentrations of plants for each treatment in each soil are shown in Tables 21,22,23 and 24 below.

If we take a look to Tables 21 and 22, we can see that there is not much difference in the total content of nitrogen between both soils, although there is more in soil a (21.65 g N/Kg) than in soil c (20.85 gN/Kg). Red clover is the cover crop with the highest levels of it for all the fertilizers followed by phacelia and buckwheat, which is the cover crop with the lowest values in both soils.

In the carbonate-free soil, the application of rock phosphate is the one with which the highest results are obtained for buckwheat and phacelia, while the higher content of nitrogen for red clover is without fertilizer. By contrast, in the carbonate soil there are more differences between the treatments than in soil a. The content of N is higher with the application of ash for buckwheat, with ash for red clover and with rock phosphate for phacelia.

Table 21. Total nitrogen (g/kg) for each treatment in soil a

APF/Cover crop	Bw	Rc	Ph
O	10,3	37,3	18,3
Ash	10,6	31,9	16,5
Digestate	11,9	35,0	21,6
Rock phosphate	12,8	34,9	18,6
MEAN	21,65		

List of abbreviations: O, no fertilizer; Bw, Buckwheat; Rc, Red clover; Ph; phacelia; Control, no cover crop.

Table 22. Total nitrogen (g/kg) for each treatment in soil c

APF/Cover crop	Bw	Rc	Ph
O	12,3	34,7	17,3
Ash	12,8	33,9	16,9
Digestate	11,6	35,0	15,1
Rock phosphate	11,0	31,4	18,1
MEAN	20,85		

List of abbreviations: O, no fertilizer; Bw, Buckwheat; Rc, Red clover; Ph; phacelia; Control, no cover crop.

Analysing the total content of carbon, in general, plants grown in the carbonate soil have more carbon (the mean of all treatments is 406.62 gC/Kg) than the plants grown in the carbonate-free soil (the mean of all treatments is 392.97 gC/Kg) (see Tables 23 and 24), being buckwheat the cover crop with the highest values in both soils.

In soil a, the application of digestate for buckwheat, no fertilizer for red clover and ash and rock phosphate for phacelia are the ones with the highest contents of carbon, while in soil c is no fertilizer for buckwheat and phacelia and rock phosphate for red clover.

Table 23. Total carbon (g/kg) for each treatment in soil a

APF/Cover crop	Bw	Rc	Ph
O	411,1	403,2	373,6
Ash	415,6	364,0	384,0
Digestate	418,7	390,9	377,8
Rock phosphate	410,6	382,0	384,0
MEAN	392,97		

List of abbreviations: O, no fertilizer; Bw, Buckwheat; Rc, Red clover; Ph; phacelia; Control, no cover crop.

Table 24. Total carbon (g/kg) for each treatment in soil c

APF/Cover crop	Bw	Rc	Ph
O	425,3	412,2	392,8
Ash	415,0	411,1	385,1
Digestate	417,3	404,8	390,6
Rock phosphate	423,6	413,4	388,0
MEAN	406,61		

List of abbreviations: O, no fertilizer; Bw, Buckwheat; Rc, Red clover; Ph; phacelia; Control, no cover crop.

Finally, the C/N ratio was tested. In Tables 25 and 26, we can see that buckwheat has the highest values, followed by phacelia and then red clover, which has the lowest C/N values, in both soils.

In soil a, buckwheat has the highest C/N ratio with the application of no fertilizer, red clover and phacelia with ash. However in soil c, buckwheat and red clover have the highest value with rock phosphate, while phacelia has the highest one with digestate.

Table 25. C/N ratio for each treatment in soil a

APF/Cover crop	Bw	Rc	Ph
O	40,1	10,8	20,5
Ash	39,0	11,4	23,2
Digestate	35,1	11,2	17,5
Rock phosphate	32,1	11,0	20,6
MEAN	22.7		

List of abbreviations: O, no fertilizer; Bw, Buckwheat; Rc, Red clover; Ph; phacelia; Control, no cover crop

Table 26. C/N ratio for each treatment in soil c

APF/Cover crop	Bw	Rc	Ph
O	34,6	11,9	22,8
Ash	32,3	12,1	22,8
Digestate	36,0	11,6	25,9
Rock phosphate	38,4	13,2	21,5
MEAN	23.6		

List of abbreviations: O, no fertilizer; Bw, Buckwheat; Rc, Red clover; Ph; phacelia; Control, no cover crop

h) C/P ratio

In this last subsection, the C/P ratio was analysed in the same way as the other factors. The mean of all treatments are tested first, then the interactions and finally, the main effects .Significances (p) and F(Fisher) value are given in table 27.

Table 27. Factor effects of ANOVA C/P ratio

Factor	p	F
Soil	0,000	62,346
APF	0,000	82,933
Cover crop	0,000	9,383
Replica	0,463	1,82
Soil*Cover crop	0,000	11,738
Soil*APF	0,160	1,777
Cover crop*APF	0,277	1,284
Soil*Cover crop*APF	0,081	1,976

Fixed factors: Soil, alternative phosphorus fertilizer (APF), Cover crop. Random factor: Replica. Significant results: $p < 0,05$

In Figure 32, the C/P ratio for all treatments is shown. In general, this ratio is higher in the carbonate soil for the three cover crops with the application of all the fertilizers. Buckwheat had the highest C/P ratio in both soils, while red clover had the lowest C/P ratio in the carbonate soil.

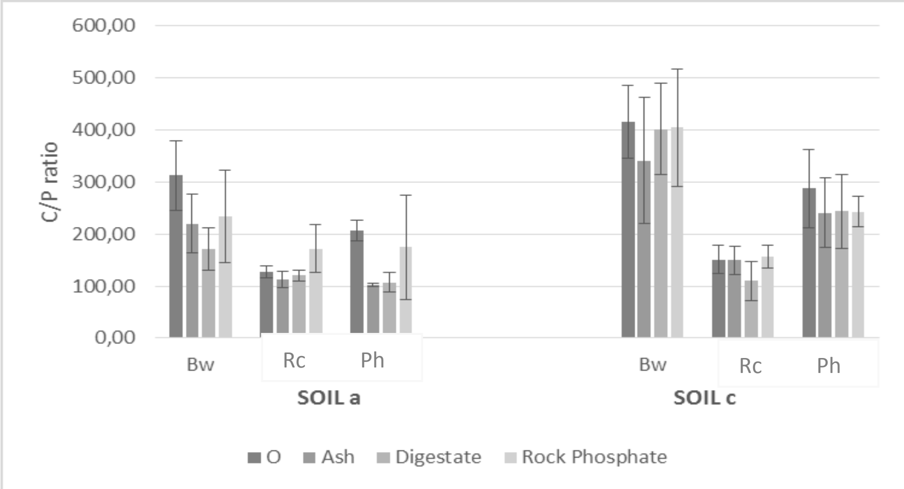

Figure 32. Mean of all treatments for C/P ratio in plants.

Grey bars show mean value, error bars indicate standard deviation of the mean. Buckwheat (Bw), Red clover (Rc), Phacelia (Ph) with no phosphorus addition(O), ash, digestate and rock phosphate on carbonate-free (a) and carbonate (c) soils.

There is a significant interaction (p: 0,000) between soils and cover crops, so it can be said that there is a different performance of cover crops in both soils. We can see in Figure 33 that there are more differences in C/P between the three cover crops in soil c than in soil a, and also the values are higher in the carbonate soil than in the carbonate-free one. On the other hand, in both soils buckwheat is the cover crop with the highest C/P ratio (234.28 ± 78.83 in soil a; 391.31 ± 94.76 in soil c), followed by phacelia (136.72 ± 61.75 in soil a; 253.73 ± 59.97 in soil c) and finally, red clover (133.01 ±32.99 in soil a; 141.74 ±32.09)

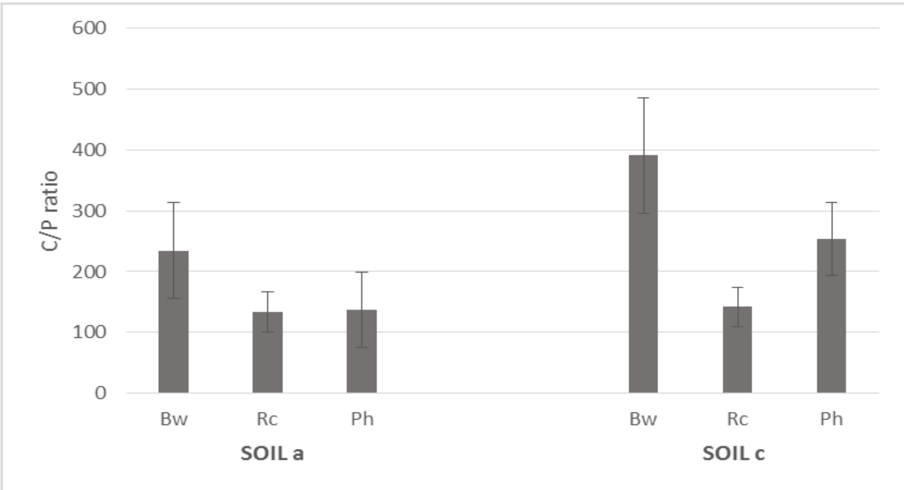

Figure 33. Soil-cover crop interaction (p: 0,000) for C/P ratio in plants.

Grey bars show mean value, error bars indicate standard deviation of the mean. Buckwheat (Bw), Red clover (Rc), Phacelia (Ph) on carbonate-free (a) and carbonate (c) soils.

Regarding the main effects in Figure 34, it can be stated that there is a significant difference between the C/P ratio in both soils (p: 0,000), being higher in soil c (262.26 ± 122.40) than in soil a (171.58 ± 75.12). On the other hand, there is also a significantly difference between the APFs, since its p value is 0,000. It can be affirmed that the C/P ratio with the application of ash and digestate is significantly different and lower than without fertilizer and rock phosphate.

Finally, the C/P ratio in all cover crops is significantly different (p: 0,000), buckwheat features the highest ratio, followed by phacelia and red clover (257.96 ±110.26; 211.52 ±105.36 ; 175.91 ± 104.93 respectively).

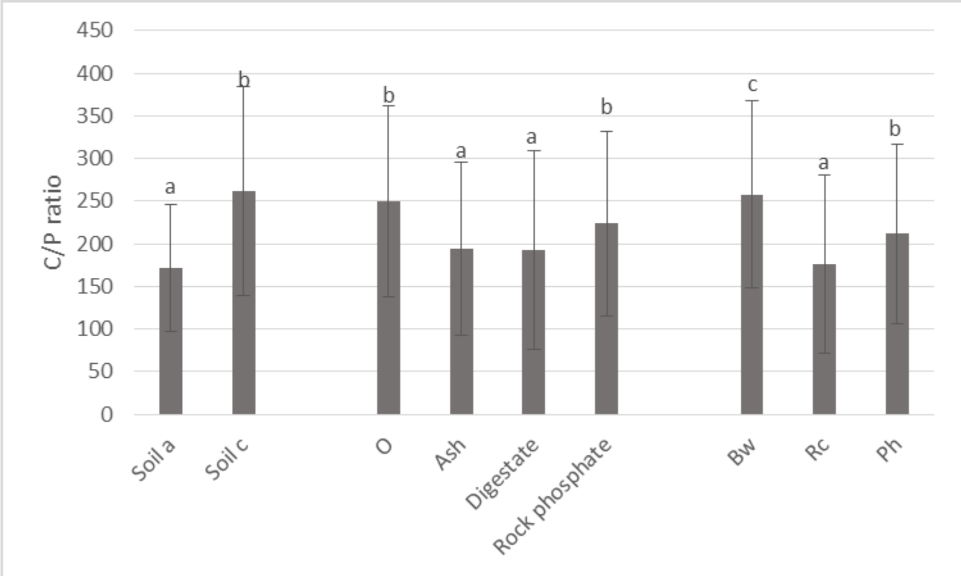

Figure 34. C/P ratio in plants for all factors.

Grey bars show mean value, error bars indicate standard deviation of the mean. Carbonate-free (a) and carbonate (c) soils, no phosphorus addition(O), ash, digestate, rock phosphate, buckwheat (Bw), Red clover (Rc) and Phacelia (Ph). Bars with the same letter for the same factor are not significantly different (p>0,05)

3.2 Discussion

The main aim of this experiment was to try to find solutions to the problematic with P outlined in the introduction; since according to Cabeza et al. (2010) phosphorus has no substitute for plants, and without its presence, the growth of any organism is not possible. These results will be discussed as follows, taking into account my objectives: firstly the discussion about both soils, then about cover crops, including its interactions or no interaction with the soils, and finally the APF, including too the possible interaction with soils.

3.2.1 Soils

The soils had a significant impact on the phosphorus availability that dominate the statistical results, as expected. The P_{CAL} concentration for the control situation (no cover crop, no APF), aboveground plant biomass per pot, P concentration in the biomass as well as total amount of P were significantly higher in the slightly acidic, carbonate-free soil (Figures 8,9, 14,27,31 in section 3.1).

In general the P_{CAL} values of the soils are considered to be very low, especially in the carbonate soil (Figures 8 and 9 in chapter 3.1.1) taking into account the classification of AGES (2006) (see table 28). However, there were much lower P_{CAL} levels (3.06 mP Kg^{-1}) in the carbonate free soil at the beginning of the experiment (see table 1) which results were too low comparing with other experiments (Cabeza et al., 2013; 2010) that already indicated values of 20-25 mg P kg^{-1} as a low supply level (Cabeza et al.; 2013). Moreover, although it was expected a similar P_{CAL} in both soils regarding to Cabeza (2010) who affirmed that CAL method can extract P in alkaline soils due to its low pH (4.1) and in acid soils, due to acetate and lactate anions, can compete with phosphates adsorbed to Fe and Al (Cabeza, 2010), different values were found (see Figures 8 and 9). Furthermore, some inconsistent values were found in the P_{CAL} concentration in the acidic soil (see Table 8) which have no sense, since its levels after the application of fertilizer for the control samples, i. e. no cover crop, were lower than with no fertilizer. For all these reasons, I cannot be sure that the results are reliable, so the interpretation of P_{CAL} for this soil is a meaningless and therefore it cannot make a good comparison between both soils. The main cause of these inconsistent values may be that there was only one replicate per sample. In order to solve it, more replicas of the same treatment should be analysed.

Table 28. Classification of the phosphorus contents (source: AGES, 2006)

Nutrition	mg P_{CAL}/1000g
Very low	under 26
Low	26-46
Sufficient	47-111
High	112-174
Very high	over 174

Rock phosphate is a sparingly water soluble P fertilizer which is governed by soil chemical and biological properties as well as other factors like the rock characteristic and the crop species (Cabeza et al., 2013; Chien et al., 2003). Several works indicated that rock phosphate is more available at low soil pH values (Casanova et al., 2002; Casanova, 1995; Chien and Menon, 1995 ; Havlin et al., 2005; Kanabo and Gilkes, 1987) , while dissolution of rock phosphate can be significantly slowed in soils of high pH and high calcium concentration (Bolan et al., 1997) So the treatments with rock phosphate should be able to increase the P in soil solution in acid soils (Cabeza, 2010). However, the effect of this fertilizer in the carbonate-free soil, was not very different than the effect in the carbonate soil, although higher in the slightly acidic one (see

Figures 8, 9 in chapter 3.1.1 and Figures 12, 16, 23, 30 in chapter 3.1.2). This is because, the carbonate-free soil is not acid, since it has a pH of 6.4 (see Table 1), making it slightly acid and because of that the effect of rock phosphate was not as expected.

On the other hand, although in the results of biomass (section 3.1.2.a) it can be stated that there was a significant difference between the soils (Table 14), their values were very similar. This may be due to the high standard deviation, which indicates that many values move away from the mean. In order to solve it, more replicas of the same treatment should be analysed. However, if we take a look at the number of plants per pot and biomass per pot data (see Figures 20 and 24 in chapter 3.1.2), these values indicate that plants grow better in the carbonate free soil, since although there were less plants per pot, these had more biomass/plant resulting in more biomass per pot, which is the best indicator. It can be explained for many reasons. Taking into account the pH of the soils, the carbonate one is slightly alkaline (see Figure 35). In these soils, the amount of Fe is often too low and the content of calcium is too high, which reduce the uptake of potassium and magnesium and plants do not absorb P due to the fixation, resulting in lower growth.

Soil Reaction	pH	Plant Growth
	>8.3	Too alkaline for most plants
	7.5	Iron availability becomes a problem on alkaline soils.
Alkaline soil	**7.2**	6.8 to 7.2 – "near neutral"
Neutral soil	**7.0**	6.0 to 7.5 – acceptable for most plants
Acid soil	**6.8**	
	6.0	
	5.5	Reduced soil microbial activity
	<4.6	Too acid for most plants

Figure 35. Soil pH and plant growth from Whiting et al. (20014)

Obviating the results of P_{CAL} as explained above, it can be assumed that the soil effect on plant biomass, P concentration, amount of P in plant biomass, plant C/P ratio and P mobilization from non-P_{CAL} pools, is consistent. The plants in the carbonate free soil showed a higher P concentration (Figures 25 and 27) resulting in a bit more biomass (Figures 10 and 14), therefore, a higher amount of P in plants was found (Figure 31) and also a narrower C/P ratio (Figure 34) than the plants in the carbonate soil which had less P concentration (Figures 25 and 27) resulting in less biomass (Figures 10 and 14), therefore less amount of P in plants was found (Figure 31). Moreover, the indirect relation between P concentration and C/P ratio in this soil was also fulfilled. All this indicates that the traits of the soil (pH and $CaCO_3$ content, above all) are determinant, since P adsorption and precipitation in the soil are related to pH value (Havlin et al., 2005). The $CaCO_3$ content and the neutral pH of the carbonate soil explain the low mobility and the difficulty of the plants in accessing P in the soil with carbonates due to the fixation of P attributed to the formation of insoluble calcic phosphates (Richardson et al. 2009 b; González and Tristán, 1959).

Finally, P sorption and mineralisation dynamics in the soil are not yet fully understood. It remains to be seen if P becomes bioavailable in the carbonate soil, for example due to organic anions that form with decomposition of plant biomass and compete for sorbing sites with phosphate (Nzigheba 1998, Oburger et al. 2011) or due to root activities of cover crops during their vegetation period (Nuruzzaman et al. 2005).

3.2.2 Cover crops

Following my objectives, these results should serve in order to find out if red clover, as a legume, mobilizes more phosphorus than the other cover crops.

As explained before P_{CAL} results were not reliable, therefore their interpretation has no sense. Taking into account the results of plant biomass, number of plants per pot and biomass per pot (section 3.1.2 b, c and d), it can be stated that the biomass of buckwheat is the highest one while red clover had considerably lower biomass than the other ones. It can be explained because red clover does not grow so big and also the vegetation period was too short for red clover which did not show flowers before the harvest, while buckwheat flowered.

However, in spite of its low biomass, red clover had the highest levels of P concentration in plants (Figures 25 and 27) as I expected, since regarding Pypers et al. (2006) there are several indications that legumes are capable of accessing sparingly soluble phosphorus in the soil through root-induced processes. These rhizosphere processes enable legumes to take up P, which is inaccessible to other species. Furthermore, according to Hassan et al. (2012 a) it is expected that legume residues contain more P and have lower C/P ratios than cereals, as shown in Figures 27 and 34. Buckwheat, on the other hand, had the highest P content in plan biomass because although its P concentration is the lowest one, it is the cover crop with the highest dry weight in the experiment.

Otherwise significant interactions between soil and cover crop for other factors were found (see chapter 3.1.2) which, when looked at separately, confirm the dominant effect of the soil properties on P bioavailability. In the carbonate-free soil, buckwheat had a higher biomass (Figure 11) and also has more amount P (Figure 29) than red clover and phacelia. According to Amann and Amberger (1989) buckwheat has special adaptations to lower pH. Passinger et al. (2013) suggested that direct rhizosphere chemical alteration (exudation of organic acid anions) may enhance P uptake by buckwheat. Results also showed a significantly interaction between soil and cover crops for P concentration in plants (Table 17 and Figure 26) that reflects how carbonates in the alkaline soil immobilize P and plants find problems in taking it from the soil solution; since P concentration of buckwheat and phacelia was lower in the carbonate soil than in the carbonate free one. However, for red clover, it is similar in both soils because it can equally access soluble phosphorus in soil as explained before.

In general, buckwheat and phacelia had better results in the carbonate-free soil (higher biomass, P concentration and amount P) than in the carbonate soil, contrary to red clover, which had better results in the carbonate soil. This can be explained because of the different pH range of plants growing (see section 2.2.2). Red clover supports higher pH than buckwheat, therefore it grows better in the carbonate soil.

As P_{CAL} results were inconsistent, it is not possible to interpret the P mobilization by cover crops, therefore it can not be stated that red clover, although had the higher P concentration, is the cover crop with higher P mobilization.

3.2.3 Alternative phosphorus fertilisers (APFs)

Plants take up P from soil solution and this represents the immediately available P pool which is buffered by echangeable P from the bulk soil (Mengel and Kirkby, 1987). In the present pot experiment P_{CAL} concentration in soil was affected by the different recycled P applied to the soil as well as other factors like the biomass, P concentration in plants, amount of P, total carbon and nitrogen and C/P ratio. As in the above sections, this discussion should analyse my objective; which APF has better results than rock phosphate in this experiment. Some works indicated that P recycling products might have similar effect to fertilizers with rock phosphate. Johnston and Richards (2003) worked with struvite from sewage sludge and found that that product was as effective as monocalcium phosphate in soils with neutral to slighty acid. Plaza et al. (2007) indicated that struvite from sewage sludge reached similar efficiency in comparison to triple superphosphate in an acid soil. Adam et al. (2009) carried out a pot experiment with a thermal P recycled product finding that P uptake by maize plants was similar to soluble commercial fertilizers.

Taking into account these studies and looking the results, it can be observed that more biomass was produced in general with the application of ash than with rock phosphate (Figure 14) and more amount of phosphorus in the above-ground biomass with the application of this APF (Figure 31) than with rock phosphate. In general, more P concentration in plants was produced by digestate and ash than by rock phosphate (Figure 27). Therefore it can be affirmed that a positive effect was found with the application of APFS, being not able to compare between them, since they were not significantly different.

It can be stated that there were significant interactions between soils and APFs factors for biomass per pot, number of plants per pot, biomass per plant and amount of P in the above-ground biomass (section 3.1.2) reflecting that ash is the best APF for the carbonate-free soil and digestate, for the carbonate one; because the highest values of biomass per pot (see Figure 12) and amount of P (see Figure 30) were found with the application of ash in the acidic soil. . There are reports that P in Mg-treated sewage sludge ashes can be accessed by plants (Nanzer, 2012), especially on more acidic soil. However, for the carbonate soil, this values were the highest with the application of digestate. This can be explained because organic anions present from decomposition of organic materials can compete with phosphate for adsorption sites (Nzigheba, 1998), and digestate is a decomposed material, so it may have had this advantage on the carbonate soil since the other APFs supplied inorganic P. Also this may be explained because organic matter increases P availability in four ways. First, it forms complexes with organic phosphate which increases phosphate uptake by plants; second, organic anions can also displace sorbed phosphate; third, humus coats aluminium and iron oxides, which reduces P sorption and finally, organic matter is also a source of phosphorus through mineralization reactions (University of Hawaii at Manoa). All these values are higher than rock phosphate and the control situation, which the application of no fertilizer, therefore, the application of these APFs have better results. Nevertheless, no interaction between concentration of phosphorus in plants and soils was found.

We found that digestates present a good alternative to rock phosphate in the carbonate-free soil and sewage sludge ashes in the carbonate soil, so I can conclude that both APFs improved P nutrition of the plants because plant biomass and plant P uptake is increased. However I can affirm which one mobilized the phosphorus due to the inconsistent values found in P_{CAL} results, as explained in the above sections.

3.3 Conclusion

Soil properties have a dominant effect on P mobilisation, in general plants reached higher biomass and P concentration when grown on the carbonate-free soil, where more P_{CAL} was measured, indicating growth-limiting P. However, some problems were found with P_{CAL} data, therefore, although carbonate-free soil seem to be better to cultivate in, I cannot be sure to affirm that there is more P mobilization in it.

Buckwheat and phacelia were found to have more dry weight and P concentration in the slightly acidic soil, while red clover showed more biomass in the carbonated soil and similar results of P concentration in both soils, which makes it the best adapted cover crop in both soils, because besides it had the highest P concentration of all. However, I cannot affirm that red clover mobilizes more P from the soil, since P_{CAL} values were inconsistent, therefore phosphorus mobilization cannot be interpreted.

In general, the application of APFs had positive results, since more biomass, amount of P in the above-ground plant biomass and P concentration was found with digestate and ash than with rock phosphate. Both APFs overcame the effect of rock phosphate, being ash most suitable in the carbonate-free soil, and digestate in the carbonate soil.

On the other hand, no interaction was expected, however we can affirm that soils had different results, as well as cover crops and fertilizers, and the characteristics of all of them were very important and consistent in the results.

Incorporation of red clover into the carbonate-free soil is expected to increase P uptake by the following crop due to its low C/P ratio and high P content that fuel mineralisation. Biomass with a higher C/P and the adsorption of P in the carbonated soil will probably result in a P immobilization with negative effects on the next crop.

Due to the inconsistent values found in P_{CAL} analysis, more replicates should have taken in order to take more conclusions in this experiment, since the interpretation of P uptake by plants and also the possible P mobilization could not be analysed. According to Torres-Dorante et al. (2006) and Cabeza (2010) P in soil solution explains more satisfactorily the P availability in soil and also the P uptake by plants than P_{CAL} for so for following studies, this factor could be tested. Otherwise, further studies are needed to investigate the bioavailability of P in different materials and to various plant species because they differ in their responses depending on species and soil type. Long term effects of APFs and cover crops may be seen in neutral to alkaline soils but little information on this topic calls for more research.

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APPENDIX

Table i: Values of number of plants per pot, dry weight, and biomass per plant for each pot of the experiment

Name	Nº plants/pot	Dry weight (bag+ sample) (g)	Dry weight (dry sample)(g)	Biomass/ plant (g_plant)
aEO1	-	-	-	-
aEA1	-	-	-	-
aED1	-	-	-	-
aER1	-	-	-	-
aBO1	23	36,56	27,15	1,181
aBA1	18	41,63	32,224	1,790
aBD1	26	36,54	27,134	1,044
aBR1	25	40,95	31,544	1,262
aTO1	31	12,14	2,734	0,088
aTA1	35	13,23	3,824	0,109
aTD1	39	12,64	3,234	0,083
aTR1	44	12,81	3,404	0,077
aPO1	39	26,93	17,524	0,449
aPA1	31	30,62	21,214	0,684
aPD1	43	17,43	8,024	0,187
aPR1	27	22,32	12,914	0,478
cEO1	-	-	-	-
cEA1	-	-	-	-
cED1	-	-	-	-
cER1	-	-	-	-
cBO1	20	32,26	22,854	1,143
cBA1	17	32,45	23,044	1,356
cBD1	21	37,26	27,854	1,326
cBR1	22	32,89	23,484	1,067
cTO1	21	11,98	2,574	0,123
cTA1	48	16,29	6,884	0,143
cTD1	56	16,68	7,274	0,130
cTR1	43	17,76	8,354	0,194
cPO1	53	17,36	7,954	0,150
cPA1	51	17,65	8,244	0,162
cPD1	60	29,15	19,744	0,329
cPR1	57	19,42	10,014	0,176
aEO2	-	-	-	-
aEA2	-	-	-	-
aED2	-	-	-	-
aER2	-	-	-	-
aBO2	27	34,17	24,764	0,917
aBA2	23	36,61	27,204	1,183
aBD2	36	29,05	19,644	0,546

Name	Nº plants/pot	Dry weight (bag+ sample) (g)	Dry weight (dry sample)(g)	Biomass/ plant (g_plant)
aBR2	21	39,77	30,364	1,446
aTO2	59	13,42	4,014	0,068
aTA2	42	15,65	6,244	0,149
aTD2	30	11,49	2,084	0,069
aTR2	48	13,05	3,644	0,076
aPO2	25	19,32	9,914	0,397
aPA2	21	25,32	15,914	0,758
aPD2	45	21,55	12,144	0,270
aPR2	29	12,01	2,604	0,090
cEO2	-	-	-	-
cEA2	-	-	-	-
cED2	-	-	-	-
cER2	-	-	-	-
cBO2	20	30,78	21,374	1,069
cBA2	15	32,32	22,914	1,528
cBD2	24	37,53	28,124	1,172
cBR2	21	31,04	21,634	1,030
cTO2	21	13,37	3,964	0,189
cTA2	57	15,36	5,954	0,104
cTD2	62	15,02	5,614	0,091
cTR2	43	16,29	6,884	0,160
cPO2	57	18,66	9,254	0,162
cPA2	56	23,94	14,534	0,260
cPD2	63	24,25	14,844	0,236
cPR2	47	19,59	10,184	0,217
aEO3	-	-	-	-
aEA3	-	-	-	-
aED3	-	-	-	-
aER3	-	-	-	-
aBO3	20	30,53	21,124	1,056
aBA3	23	40,36	30,954	1,346
aBD3	13	23,52	14,114	1,086
aBR3	27	41,44	32,034	1,186
aTO3	52	12,98	3,574	0,069
aTA3	58	16,65	7,244	0,125
aTD3	41	10,74	1,334	0,033
aTR3	53	13,62	4,214	0,080
aPO3	52	20,54	11,134	0,214
aPA3	25	28,15	18,744	0,750
aPD3	55	23,39	13,984	0,254
aPR3	25	23,37	13,964	0,559
cEO3	-	-	-	-
cEA3	-	-	-	-

Name	Nº plants/pot	Dry weight (bag+ sample) (g)	Dry weight (dry sample)(g)	Biomass/ plant (g_plant)
cED3	-	-	-	-
cER3	-	-	-	-
cBO3	21	31,52	22,114	1,053
cBA3	21	31,48	22,074	1,051
cBD3	19	36,58	27,174	1,430
cBR3	21	29,87	20,464	0,974
cTO3	54	16,06	6,654	0,123
cTA3	61	16,55	7,144	0,117
cTD3	65	18,27	8,864	0,136
cTR3	67	16,29	6,884	0,103
cPO3	56	16,65	7,244	0,129
cPA3	58	23,49	14,084	0,243
cPD3	82	22,88	13,474	0,164
cPR3	56	15,93	6,524	0,117
aEO4	-	-	-	-
aEA4	-	-	-	-
aED4	-	-	-	-
aER4	-	-	-	-
aBO4	18	29,54	20,134	1,119
aBA4	28	37,48	28,074	1,003
aBD4	16	21,22	11,814	0,738
aBR4	16	30,84	21,434	1,340
aTO4	74	13,63	4,224	0,057
aTA4	57	14,87	5,464	0,096
aTD4	34	11,28	1,874	0,055
aTR4	56	13,81	4,404	0,079
aPO4	43	16,55	7,144	0,166
aPA4	32	24,87	15,464	0,483
aPD4	52	19,74	10,334	0,199
aPR4	19	18,34	8,934	0,470
cEO4	-	-	-	-
cEA4	-	-	-	-
cED4	-	-	-	-
cER4	-	-	-	-
cBO4	21	26,41	17,004	0,810
cBA4	20	30,54	21,134	1,057
cBD4	27	36,08	26,674	0,988
cBR4	28	33,91	24,504	0,875
cTO4	57	13,18	3,774	0,066
cTA4	59	14,14	4,734	0,080
cTD4	77	16,04	6,634	0,086
cTR4	63	16,25	6,844	0,109
cPO4	46	14,44	5,034	0,109

Name	Nº plants/pot	Dry weight (bag+ sample) (g)	Dry weight (dry sample)(g)	Biomass/ plant (g_plant)
cPA4	56	18,74	9,334	0,167
cPD4	71	23,12	13,714	0,193
cPR4	69	15,49	6,084	0,088
aEO5	-	-	-	-
cEO5	-	-	-	-

The name is the code of the pot explained in table 5 in section 2.2 (experimental setup), which represents the treatment applied in each pot.

Table ii: Data and results from plant digestion and P concentration using molybdate blue colorimetry

Name	sample weight(g)	tube weight (g)	tube weight (g) after digestion	volume (mL)	Absortion (881 nm)	P (µg/l)	P (µg/l) (less Blanks)	P in digest (mg /L)	P (mg/kg)	P (g/kg)
aBO1	0,195	97,533	141,412	43,879	0,075	103,888	103,867	5,193	1168,6084	1,169
aBA1	0,199	116,704	169,286	52,582	0,084	120,407	120,386	6,019	1590,4888	1,590
aBD1	0,206	95,546	140,731	45,185	0,111	169,965	169,944	8,497	1863,8174	1,864
aBR1	0,192	116,517	171,221	54,704	0,132	208,511	208,489	10,424	2970,1038	2,970
aTO1	0,193	116,235	167,392	51,157	0,167	272,752	272,731	13,637	3614,5358	3,615
aTA1	0,207	115,065	169,478	54,413	0,133	210,346	210,325	10,516	2764,3484	2,764
aTD1	0,201	117,063	159,957	42,894	0,202	336,994	336,973	16,849	3595,5525	3,596
aTR1	0,193	97,264	149,138	51,874	0,126	197,498	197,476	9,874	2653,8578	2,654
aPO1	0,201	95,546	148,575	53,029	0,105	158,953	158,931	7,947	2096,5092	2,097
aPA1	0,195	116,396	168,853	52,457	0,166	270,917	270,896	13,545	3643,6859	3,644
aPD1	0,203	116,408	170,432	54,024	0,136	215,852	215,831	10,792	2871,9375	2,872
aPR1	0,199	116,224	165,994	49,77	0,002	-30,102	-30,123	-1,506		
cBO1	0,196	113,601	167,827	54,226	0,058	72,685	72,664	3,633	1005,1679	1,005
cBA1	0,209	96,671	154,986	58,315	0,088	127,749	127,728	6,386	1781,9291	1,782
cBD1	0,202	116,944	157,820	40,876	0,064	83,698	83,677	4,184	846,62419	0,847
cBR1	0,206	96,674	135,924	39,25	0,062	80,027	80,006	4,000	762,18892	0,762
cTO1	0,198	111,362	158,024	46,662	0,132	208,511	208,489	10,424	2456,6989	2,457
cTA1	0,198	115,165	167,789	52,624	0,128	201,169	201,147	10,057	2673,0251	2,673
cTD1	0,202	95,989	148,68	52,691	0,33	571,936	571,915	28,596	7459,0986	7,459
cTR1	0,198	116,95	165,577	48,627	0,129	203,004	202,983	10,149	2492,5372	2,493
cPO1	0,202	116,931	159,053	42,122	0,075	103,888	103,867	5,193	1082,9402	1,083
cPA1	0,202	113,786	168,750	54,964	0,066	87,369	87,347	4,367	1188,3583	1,188
cPD1	0,200	113,599	167,608	54,009	0,098	146,104	146,083	7,304	1972,448	1,972
cPR1	0,205	97,395	145,914	48,519	0,088	127,749	127,728	6,386	1511,5218	1,512
aBO2	0,191	116,049	170,319	54,27	0,086	124,078	124,057	6,203	1762,4556	1,762
aBA2	0,200	97,395	138,086	40,691	0,101	151,611	151,589	7,579	1542,0806	1,542
aBD2	0,201	114,089	168,36	54,271	0,113	173,636	173,615	8,681	2343,8476	2,344
aBR2	0,205	113,121	160,654	47,533	0,115	177,307	177,286	8,864	2055,3513	2,055
aTO2	0,201	96,951	140,931	43,98	0,173	283,765	283,744	14,187	3104,2447	3,104
aTA2	0,203	96,82	148,72	51,9	0,162	263,575	263,554	13,178	3369,0739	3,369
aTD2	0,198	115,188	166,678	51,49	0,148	237,878	237,857	11,893	3092,7418	3,093
aTR2	0,205	116,134	166,398	50,264	0,091	133,256	133,235	6,662	1633,3905	1,633
aPO2	0,204	116,414	166,925	50,511	0,093	136,927	136,906	6,845	1694,9103	1,695
aPA2	0,202	117,064	172,285	55,221	0,167	272,752	272,731	13,637	3727,8435	3,728
aPD2	0,191	116,672	166,466	49,794	0,165	269,081	269,060	13,453	3507,2209	3,507
aPR2	0,190	116,266	171,545	55,279	0,068	91,040	91,018	4,551	1324,055	1,324
cBO2	0,206	116,822	169,264	52,442	0,067	89,204	89,183	4,459	1135,178	1,135
cBA2	0,192	116,492	158,44	41,948	0,102	153,446	153,425	7,671	1676,0066	1,676
cBD2	0,203	116,377	163,378	47,001	0,061	78,191	78,170	3,909	904,94384	0,905
cBR2	0,199	96,852	147,573	50,721	0,061	78,191	78,170	3,909	996,19716	0,996

Name	sample weight(g)	tube weight (g)	tube weight (g) after digestion	volume (mL)	Absortion (881 nm)	P (µg/l)	P (µg/l) (less Blanks)	P in digest (mg /L)	P (mg/kg)	P (g/kg)
cTO2	0,202	116,112	166,491	50,379	0,118	182,814	182,793	9,140	2279,4322	2,279
cTA2	0,206	96,508	149,833	53,325	0,116	179,143	179,122	8,956	2318,3637	2,318
cTD2	0,204	114,768	162,557	47,789	0,17	278,259	278,238	13,912	3258,9944	3,259
cTR2	0,201	116,163	166,137	49,974	0,131	206,675	206,654	10,333	2568,9845	2,569
cPO2	0,200	113,459	166,421	52,962	0,066	87,369	87,347	4,367	1156,5245	1,157
cPA2	0,204	116,018	158,663	42,645	0,094	138,762	138,741	6,937	1450,1493	1,450
cPD2	0,207	98,231	152,756	54,525	0,065	85,533	85,512	4,276	1126,2179	1,126
cPR2	0,203	116,55	166,027	49,477	0,105	158,953	158,931	7,947	1936,8087	1,937
aBO3	0,194	95,744	144,529	48,785	0,065	85,533	85,512	4,276	1075,1813	1,075
aBA3	0,198	116,656	167,146	50,49	0,135	214,017	213,996	10,700	2728,4457	2,728
aBD3	0,198	116,474	170,527	54,053	0,129	203,004	202,983	10,149	2770,6647	2,771
aBR3	0,200	111,637	158,493	46,856	0,073	100,217	100,196	5,010	1173,6944	1,174
aTO3	0,203	98,529	152,954	54,425	0,138	219,523	219,502	10,975	2942,4647	2,942
aTA3	0,200	112,253	165,882	53,629	0,147	236,043	236,022	11,801	3164,3996	3,164
aTD3	0,200	116,367	158,774	42,407	0,195	324,146	324,125	16,206	3436,2886	3,436
aTR3	0,193	116,906	169,157	52,251	0,104	157,117	157,096	7,855	2126,5318	2,127
aPO3	0,201	115,063	158,867	43,804	0,102	153,446	153,425	7,671	1671,7964	1,672
aPA3	0,196	115,791	170,209	54,418	0,169	276,423	276,402	13,820	3837,0538	3,837
aPD3	0,199	117,349	167,889	50,54	0,177	291,107	291,086	14,554	3696,3532	3,696
aPR3	0,198	112,397	163,644	51,247	0,152	245,220	245,199	12,260	3173,1591	3,173
cBO3	0,200	116,227	168,487	52,26	0,053	63,507	63,486	3,174	829,44755	0,829
cBA3	0,199	116,798	160,453	43,655	0,07	94,711	94,689	4,734	1038,6097	1,039
cBD3	0,199	115,948	172,259	56,311	0,069	92,875	92,854	4,643	1313,7432	1,314
cBR3	0,206	96,488	146,855	50,367	0,081	114,901	114,880	5,744	1404,4046	1,404
cTO3	0,193	98,297	152,163	53,866	0,146	234,207	234,186	11,709	3268,0481	3,268
cTA3	0,200	111,636	164,821	53,185	0,129	203,004	202,983	10,149	2698,9107	2,699
cTD3	0,199	116,407	157,741	41,334	0,179	294,778	294,757	14,738	3061,1768	3,061
cTR3	0,200	114,113	161,385	47,272	0,171	280,094	280,073	14,004	3309,9038	3,310
cPO3	0,201	116,639	173,129	56,49	0,082	116,736	116,715	5,836	1640,1099	1,640
cPA3	0,201	115,306	166,496	51,19	0,108	164,459	164,438	8,222	2093,9223	2,094
cPD3	0,202	115,83	165,739	49,909	0,093	136,927	136,906	6,845	1691,2913	1,691
cPR3	0,210	96,490	138,763	42,273	0,102	153,446	153,425	7,671	1544,221	1,544
aBO4	0,197	116,799	167,645	50,846	0,08	113,065	113,044	5,652	1458,8445	1,459
aBA4	0,205	116,521	167,82	51,299	0,11	168,130	168,109	8,405	2103,3678	2,103
aBD4	0,208	115,305	158,255	42,95	0,19	314,968	314,947	15,747	3251,6789	3,252
aBR4	0,201	114,501	166,027	51,526	0,089	129,585	129,564	6,478	1660,6698	1,661
aTO4	0,196	115,962	169,732	53,77	0,14	223,194	223,173	11,159	3061,2297	3,061
aTA4	0,193	115,186	169,126	53,94	0,167	272,752	272,731	13,637	3811,1707	3,811
aTD4	0,200	116,021	168,663	52,642	0,142	226,865	226,844	11,342	2985,382	2,985
aTR4	0,199	97,534	147,309	49,775	0,146	234,207	234,186	11,709	2928,7966	2,929
aPO4	0,198	114,815	168,222	53,407	0,092	135,091	135,070	6,754	1821,6375	1,822
aPA4	0,199	96,446	149,329	52,883	0,178	292,943	292,921	14,646	3892,102	3,892

Name	sample weight(g)	tube weight (g)	tube weight (g) after digestion	volume (mL)	Absortion (881 nm)	P (µg/l)	P (µg/l) (less Blanks)	P in digest (mg /L)	P (mg/kg)	P (g/kg)
aPD4	0,201	96,448	139,551	43,103	0,238	403,072	403,050	20,153	4321,5623	4,322
aPR4	0,202	116,364	169,077	52,713	0,16	259,904	259,883	12,994	3390,8915	3,391
cBO4	0,204	114,504	170,351	55,847	0,066	87,369	87,347	4,367	1195,6116	1,196
cBA4	0,198	112,397	156,725	44,328	0,06	76,356	76,335	3,817	854,48488	0,854
cBD4	0,206	116,88	161,283	44,403	0,081	114,901	114,880	5,744	1238,1078	1,238
cBR4	0,200	116,381	167,398	51,017	0,072	98,382	98,360	4,918	1254,5129	1,255
cTO4	0,199	116,193	163,836	47,643	0,163	265,410	265,389	13,269	3176,8692	3,177
cTA4	0,198	110,522	158,944	48,422	0,18	296,614	296,592	14,830	3626,6663	3,627
cTD4	0,204	113,564	170,255	56,691	0,141	225,030	225,009	11,250	3126,4619	3,126
cTR4	0,198	98,526	148,247	49,721	0,121	188,320	188,299	9,415	2364,2461	2,364
cPO4	0,198	113,783	167,412	53,629	0,094	138,762	138,741	6,937	1878,9243	1,879
cPA4	0,198	116,934	168,080	51,146	0,104	157,117	157,096	7,855	2028,9955	2,029
cPD4	0,199	111,363	168,921	57,558	0,093	136,927	136,906	6,845	1979,9012	1,980
cPR4	0,203	98,232	136,228	37,996	0,104	157,117	157,096	7,855	1470,2	1,470
blank 1		113,124	165,577	52,453	0,026					0,000
blank 2		97,598	145,203	47,605	0,039					0,000
blank 3		114,501	168,135	53,634	0,025					0,000
blank 1.1		110,523	148,34	37,817	0,000					0,000
blank 2.1		96,951	161,711	64,76	0,007					0,000
blank 2.3		116,496	166,935	50,439	0,031					0,000
blank 1.2		114,815	169,677	54,862	0,031					0,000
blank 2.2		110,526	163,906	53,38	0,027					0,000
blank 2.3		114,152	174,756	60,604	0,005					0,000
reference 1	0,199	116,417	162,541	46,124	0,086	124,078	124,057	6,203	1437,6912	1,438
reference 2	0,205	113,561	159,416	45,855	0,11	168,130	168,109	8,405	1880,1523	1,880
reference 3	0,200	114,768	171,548	56,78	0,103	155,282	155,260	7,763	2203,9203	2,204
reference 1.1	0,214	116,876	168,589	51,713	0,097	144,269	144,247	7,212	1742,8662	1,743
reference 2.1	0,204	114,113	166,792	52,679	0,073	100,217	100,196	5,010	1293,6809	1,294
reference 3.1	0,195	97,598	150,936	53,338	0,068	91,040	91,018	4,551	1244,8057	1,245
reference 1.2	0,196	116,493	173,571	57,078	0,086	124,078	124,057	6,203	1806,3604	1,806
reference 2.2	0,211	116,88	174,966	58,086	0,104	157,117	157,096	7,855	2162,3381	2,162
reference 3.2	0,207	97,64	151,546	53,906	0,096	142,433	142,412	7,121	1854,3137	1,854

The name is the code of the pot explained in table 5 in section 2.2 (experimental setup), which represents the treatment applied in each pot. 9 blanks (no plant material) and 9 references (Oriental Basma Tobacco Leaves) are added as it is explained in section 2.3.1.c.

Table iii: Values of amount P, ΔP$_{CAL}$ and their difference in order to find out a possible P mobilization by plants for each pot.

Name	Amount P (mg/pot)	ΔPCAL	Amount P - ΔPCAL (mg/pot)
aBO1	31,732	62,867	-31,13
aBO2	43,645	62,867	-19,22
aBO3	22,712	62,867	-40,16
aBO4	29,372	62,867	-33,49
aBA1	51,252	5,987	45,26
aBA2	41,951	5,987	35,96
aBA3	84,456	5,987	78,47
aBA4	59,050	5,987	53,06
aBD1	50,573	8,981	41,59
aBD2	46,043	8,981	37,06
aBD3	39,105	8,981	30,12
aBD4	38,415	8,981	29,43
aBR1	93,689	38,918	54,77
aBR2	62,409	38,918	23,49
aBR3	37,598	38,918	-1,32
aBR4	35,595	38,918	-3,32
aTO1	9,882	62,867	-52,99
aTO2	12,460	62,867	-50,41
aTO3	10,516	62,867	-52,35
aTO4	12,931	62,867	-49,94
aTA1	10,571	32,930	-22,36
aTA2	21,036	32,930	-11,89
aTA3	22,923	32,930	-10,01
aTA4	20,824	32,930	-12,11
aTD1	11,628	5,987	5,64
aTD2	6,445	5,987	0,46
aTD3	4,584	5,987	-1,40
aTD4	5,595	5,987	-0,39
aTR1	9,034	14,968	-5,93
aTR2	5,952	14,968	-9,02
aTR3	8,961	14,968	-6,01
aTR4	12,898	14,968	-2,07
aPO1	36,739	38,918	-2,18
aPO2	16,803	38,918	-22,11
aPO3	18,614	38,918	-20,30
aPO4	13,014	38,918	-25,90
aPA1	77,297	2,994	74,30
aPA2	59,325	2,994	56,33
aPA3	71,922	2,994	68,93
aPA4	60,187	2,994	57,19
aPD1	23,044	14,968	8,08

Name	Amount P (mg/pot)	ΔPCAL	Amount P - ΔPCAL (mg/pot)
aPD2	42,592	14,968	27,62
aPD3	51,690	14,968	36,72
aPD4	44,659	14,968	29,69
aPR1			
aPR2	3,448	23,949	-20,50
aPR3	44,310	23,949	20,36
aPR4	30,294	23,949	6,34
cBO1	22,972	17,962	5,01
cBO2	24,263	17,962	6,30
cBO3	18,342	17,962	0,38
cBO4	20,330	17,962	2,37
cBA1	41,063	80,829	-39,77
cBA2	38,404	80,829	-42,43
cBA3	22,926	80,829	-57,90
cBA4	18,059	80,829	-62,77
cBD1	23,582	17,962	5,62
cBD2	25,451	17,962	7,49
cBD3	35,700	17,962	17,74
cBD4	33,025	17,962	15,06
cBR1	17,899	20,956	-3,06
cBR2	21,552	20,956	0,60
cBR3	28,740	20,956	7,78
cBR4	30,741	20,956	9,78
cTO1	6,324	53,886	-47,56
cTO2	9,036	53,886	-44,85
cTO3	21,746	53,886	-32,14
cTO4	11,990	53,886	-41,90
cTA1	18,401	26,943	-8,54
cTA2	13,804	26,943	-13,14
cTA3	19,281	26,943	-7,66
cTA4	17,169	26,943	-9,77
cTD1	54,257	17,962	36,30
cTD2	18,296	17,962	0,33
cTD3	27,134	17,962	9,17
cTD4	20,741	17,962	2,78
cTR1	20,823	50,893	-30,07
cTR2	17,685	50,893	-33,21
cTR3	22,785	50,893	-28,11
cTR4	16,181	50,893	-34,71
cPO1	8,614	14,968	-6,35
cPO2	10,702	14,968	-4,27
cPO3	11,881	14,968	-3,09
cPO4	9,459	14,968	-5,51

Name	Amount P (mg/pot)	ΔPCAL	Amount P - ΔPCAL (mg/pot)
cPA1	9,797	71,848	-62,05
cPA2	21,076	71,848	-50,77
cPA3	29,491	71,848	-42,36
cPA4	18,939	71,848	-52,91
cPD1	38,944	5,987	32,96
cPD2	16,718	5,987	10,73
cPD3	22,788	5,987	16,80
cPD4	27,152	5,987	21,17
cPR1	15,136	0,000	15,14
cPR2	19,724	0,000	19,72
cPR3	10,074	0,000	10,07
cPR4	8,945	0,000	8,94

The name is the code of the pot explained in table 5 in section 2.2 (experimental setup), which represents the treatment applied in each pot. Values > 0 represents P mobilization.

Table iv: Values of P_{CAL} for soil samples

sample name	Concentration Pcal (mg/kg)
aEO	26,014
aEA	17,781
aED	29,008
aER	17,033
aBO	10,297
aBA	19,278
aBD	26,762
aBR	7,303
aTO	10,297
aTA	9,549
aTD	30,504
aTR	13,291
aPO	16,284
aPA	17,033
aPD	32,750
aPR	11,045
cEO	15,536
cEA	37,240
cED	19,278
cER	19,278
cBO	11,045
cBA	17,033
cBD	23,769
cBR	24,517
cTO	29,008
cTA	30,504
cTD	23,769
cTR	6,555
cPO	19,278
cPA	19,278
cPD	17,781
cPR	19,278
soil a	26,529
soil c	16,223
Reference soil	126,530

The sample name represents the code for all the replicas with the same treatment.

Table v. Amount of phosphorus (mg/pot) for each pot

Name	Amount P (mg/pot)	Name	Amount P (mg/pot)	Name	Amount P (mg/pot)	Name	Amount P (mg/pot)
aBO1	31,732	aTD1	11,628	cBO1	22,972	cTD1	54,257
aBO2	43,645	aTD2	6,445	cBO2	24,263	cTD2	18,296
aBO3	22,712	aTD3	4,584	cBO3	18,342	cTD3	27,134
aBO4	29,372	aTD4	5,595	cBO4	20,330	cTD4	20,741
aBA1	51,252	aTR1	9,034	cBA1	41,063	cTR1	20,823
aBA2	41,951	aTR2	5,952	cBA2	38,404	cTR2	17,685
aBA3	84,456	aTR3	8,961	cBA3	22,926	cTR3	22,785
aBA4	59,050	aTR4	12,898	cBA4	18,059	cTR4	16,181
aBD1	50,573	aPO1	36,739	cBD1	23,582	cPO1	8,614
aBD2	46,043	aPO2	16,803	cBD2	25,451	cPO2	10,702
aBD3	39,105	aPO3	18,614	cBD3	35,700	cPO3	11,881
aBD4	38,415	aPO4	13,014	cBD4	33,025	cPO4	9,459
aBR1	93,689	aPA1	77,297	cBR1	17,899	cPA1	9,797
aBR2	62,409	aPA2	59,325	cBR2	21,552	cPA2	21,076
aBR3	37,598	aPA3	71,922	cBR3	28,740	cPA3	29,491
aBR4	35,595	aPA4	60,187	cBR4	30,741	cPA4	18,939
aTO1	9,882	aPD1	23,044	cTO1	6,324	cPD1	38,944
aTO2	12,460	aPD2	42,592	cTO2	9,036	cPD2	16,718
aTO3	10,516	aPD3	51,690	cTO3	21,746	cPD3	22,788
aTO4	12,931	aPD4	44,659	cTO4	11,990	cPD4	27,152
aTA1	10,571	aPR1		cTA1	18,401	cPR1	15,136
aTA2	21,036	aPR2	3,448	cTA2	13,804	cPR2	19,724
aTA3	22,923	aPR3	44,310	cTA3	19,281	cPR3	10,074
aTA4	20,824	aPR4	30,294	cTA4	17,169	cPR4	8,945

The name represents the code of the pot explained in table 5 in section 2.2 (experimental setup)

Table vi: Total Nitrogen and Carbon in % for each sample

Sample name	%N	%C
aBO a	1,087	41,378
aBO b	0,970	40,847
aBA a	1,056	41,753
aBA b	1,074	41,372
aBD a	1,191	41,758
aBD b	1,193	41,981
aBR a	1,333	41,106
aBR b	1,228	41,024
aTO a	3,697	40,360
aTO b	3,757	40,276
aTA a	3,031	34,429
aTA b	3,342	38,362
aDT a	3,521	38,960
aDT b	3,480	39,224
aTR a	3,518	38,421
aTR b	3,457	37,987
aPO a	1,702	37,184
aPO b	1,954	37,541
aPA a	1,632	38,581
aPA b	1,674	38,220
aPD a	2,106	37,764
aPD b	2,223	37,794
aPR a	1,857	38,653
aPR b	1,872	38,153
cBO a	1,251	42,659
cBO b	1,209	42,397
cBA a	1,266	41,424
cBA b	1,301	41,583
cBD a	1,102	41,989
cBD b	1,221	41,462
cBR a	1,115	42,261
cBR b	1,094	42,469
cTO a	3,501	41,064
cTO b	3,430	41,370
cTA a	3,343	41,005
cTA b	3,440	41,216
cTD a	3,494	40,576
cTD b	3,513	40,386
cTR a	3,191	41,496
cTR b	3,086	41,182
cPO a	1,777	39,187
cPO b	1,679	39,373
cPA a	1,650	38,500

Sample name	%N	%C
cPA b	1,732	38,522
cPD a	1,485	39,252
cPD b	1,535	38,872
cPR a	1,819	38,809
cPR b	1,799	38,799

The sample name represents the code for all the replicas with the same treatment. a and b at the end represent the two repetitions of the experiment.

Table vii: Total Nitrogen and Carbon in g/kg and C/N ratio for each treatment

Sample name	g N/Kg	g C/Kg	C/N
aBO	10,288	411,124	40,076
aBA	10,650	415,628	39,030
aBD	11,921	418,698	35,123
aBR	12,806	410,647	32,119
aTO	37,273	403,179	10,818
aTA	31,864	363,953	11,419
aTD	35,004	390,918	11,168
aTR	34,879	382,040	10,954
aPO	18,279	373,625	20,531
aPA	16,532	384,003	23,233
aPD	21,645	377,787	17,466
aPR	18,643	384,031	20,600
cBO	12,301	425,284	34,582
cBA	12,834	415,035	32,343
cBD	11,615	417,256	36,031
cBR	11,046	423,649	38,357
cTO	34,656	412,168	11,895
cTA	33,915	411,102	12,124
cTD	35,037	404,810	11,554
cTR	31,382	413,391	13,176
cPO	17,280	392,800	22,751
cPA	16,907	385,107	22,791
cPD	15,098	390,619	25,880
cPR	18,089	388,040	21,452

The sample name represents the code for all the replicas with the same treatment

Table viii: Necessary data to calculate C/P ratio

Name	Weight (dry sample)(g/pot)	Amount P (mg/pot)	mg C/pot	C/P
aBO1	27,15	31,732	11163,66	351,81
aBO2	24,764	43,645	10181,08	233,27
aBO3	21,124	22,712	8684,59	382,38
aBO4	20,134	29,372	8277,57	281,81
aBA1	32,224	51,252	13393,19	261,32
aBA2	27,204	41,951	11306,74	269,52
aBA3	30,954	84,456	12865,35	152,33
aBA4	28,074	59,050	11668,34	197,60
aBD1	27,134	50,573	11360,94	224,65
aBD2	19,644	46,043	8224,89	178,64
aBD3	14,114	39,105	5909,50	151,12
aBD4	11,814	38,415	4946,49	128,76
aBR1	31,544	93,689	12953,43	138,26
aBR2	30,364	62,409	12468,87	199,79
aBR3	32,034	37,598	13154,65	349,88
aBR4	21,434	35,595	8801,80	247,28
aTO1	2,734	9,882	1102,29	111,54
aTO2	4,014	12,460	1618,36	129,88
aTO3	3,574	10,516	1440,96	137,02
aTO4	4,224	12,931	1703,03	131,70
aTA1	3,824	10,571	1391,76	131,66
aTA2	6,244	21,036	2272,52	108,03
aTA3	7,244	22,923	2636,48	115,01
aTA4	5,464	20,824	1988,64	95,50
aTD1	3,234	11,628	1264,23	108,72
aTD2	2,084	6,445	814,67	126,40
aTD3	1,334	4,584	521,48	113,76
aTD4	1,874	5,595	732,58	130,94
aTR1	3,404	9,034	1300,47	143,96
aTR2	3,644	5,952	1392,15	233,89
aTR3	4,214	8,961	1609,92	179,65
aTR4	4,404	12,898	1682,51	130,44
aPO1	17,524	36,739	6547,40	178,21
aPO2	9,914	16,803	3704,12	220,44
aPO3	11,134	18,614	4159,94	223,49
aPO4	7,144	13,014	2669,18	205,10
aPA1	21,214	77,297	8146,24	105,39
aPA2	15,914	59,325	6111,02	103,01
aPA3	18,744	71,922	7197,75	100,08
aPA4	15,464	60,187	5938,22	98,66
aPD1	8,024	23,044	3031,36	131,54

Name	Weight (dry sample)(g/pot)	Amount P (mg/pot)	mg C/pot	C/P
aPD2	12,144	42,592	4587,85	107,72
aPD3	13,984	51,690	5282,98	102,21
aPD4	10,334	44,659	3904,05	87,42
aPR1	12,914		4959,37	
aPR2	2,604	3,448	1000,02	290,04
aPR3	13,964	44,310	5362,60	121,02
aPR4	8,934	30,294	3430,93	113,25
cBO1	22,854	22,972	9719,45	423,10
cBO2	21,374	24,263	9090,03	374,64
cBO3	22,114	18,342	9404,74	512,73
cBO4	17,004	20,330	7231,54	355,70
cBA1	23,044	41,063	9564,06	232,91
cBA2	22,914	38,404	9510,11	247,63
cBA3	22,074	22,926	9161,48	399,61
cBA4	21,134	18,059	8771,34	485,71
cBD1	27,854	23,582	11622,25	492,85
cBD2	28,124	25,451	11734,91	461,09
cBD3	27,174	35,700	11338,52	317,61
cBD4	26,674	33,025	11129,89	337,01
cBR1	23,484	17,899	9948,97	555,83
cBR2	21,634	21,552	9165,22	425,27
cBR3	20,464	28,740	8669,55	301,66
cBR4	24,504	30,741	10381,09	337,70
cTO1	2,574	6,324	1060,92	167,77
cTO2	3,964	9,036	1633,83	180,82
cTO3	6,654	21,746	2742,57	126,12
cTO4	3,774	11,990	1555,52	129,74
cTA1	6,884	18,401	2830,03	153,80
cTA2	5,954	13,804	2447,70	177,32
cTA3	7,144	19,281	2936,91	152,32
cTA4	4,734	17,169	1946,16	113,36
cTD1	7,274	54,257	2944,59	54,27
cTD2	5,614	18,296	2272,60	124,21
cTD3	8,864	27,134	3588,24	132,24
cTD4	6,634	20,741	2685,51	129,48
cTR1	8,354	20,823	3453,47	165,85
cTR2	6,884	17,685	2845,79	160,92
cTR3	6,884	22,785	2845,79	124,90
cTR4	6,844	16,181	2829,25	174,85
cPO1	7,954	8,614	3124,33	362,72
cPO2	9,254	10,702	3634,98	339,64
cPO3	7,244	11,881	2845,45	239,50
cPO4	5,034	9,459	1977,36	209,06

Name	Weight (dry sample)(g/pot)	Amount P (mg/pot)	mg C/pot	C/P
cPA1	8,244	9,797	3174,82	324,07
cPA2	14,534	21,076	5597,14	265,56
cPA3	14,084	29,491	5423,84	183,92
cPA4	9,334	18,939	3594,59	189,80
cPD1	19,744	38,944	7712,38	198,04
cPD2	14,844	16,718	5798,35	346,84
cPD3	13,474	22,788	5263,20	230,96
cPD4	13,714	27,152	5356,95	197,29
cPR1	10,014	15,136	3885,83	256,72
cPR2	10,184	19,724	3951,80	200,35
cPR3	6,524	10,074	2531,57	251,29
cPR4	6,084	8,945	2360,83	263,94

The name is the code of the pot explained in table 5 in section 2.2 (experimental setup), which represents the treatment applied in each pot