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Valorization of calcium phosphite waste as phosphorus fertilizer: Effects on green manure productivity and soil properties

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ABSTRACT

The potential to use calcium phosphite (*Ca-Phi*) as phosphorus (P) fertilizer may represent an effective recycling of P-containing by-products. A greenhouse experiment was conducted to investigate the effect of *Ca-Phi* (38 kg P ha^{-1}) on soil properties and the growth parameters of four green manure species in clay and sandy soils using *Ca-Phi*, *TSP* (triple superphosphate) and *control* (no fertilization) as treatments. Eight weeks after sowing, we measured aboveground biomass yield, phosphite (Phi) concentration in plant biomass, different soil P pools as well as microbial biomass nutrients. Compared to *control*, the addition of *Ca-Phi* did not negatively affect green manure yield, except for lupine (*Lupinus albus* L.) in clay soil. The Phi concentration in plant biomass varied across species and soil type with a maximum concentration of about 400 mg Phi kg^{-1} for mustard (*Brassica juncea* L.) in clay soil. Compared to *control*, *TSP* and *Ca-Phi* fertilization had a similar effect on different P pools and microbial biomass nutrients (C, N and P) although the response was soil–type dependent. In the sandy soil, after *Ca-Phi* addition the amount of available P (P_{NHCO3}) increased to the same extent as in the *TSP* treatment (i.e. around 6 mg P kg^{-1}) suggesting that *Ca-Phi* was, at least partly, oxidized. In the clay soil with high P fixing capacity, *Ca-Phi* promoted higher P_{NaHCO3} than *TSP* likely due to different solubility of chemical P forms. Additional studies are however required to better understand soil microbial responses and to quantify the P agronomical efficiency for the following crop under *Ca-Phi* fertilization.

1. Introduction

Plant production in agricultural systems is closely dependent on phosphorus (P), which is widely recognized as an important macronutrient (Marschner, 2012). However, P fertilizers are produced from mineral rocks whose economically viable reserves could be depleted in the next decades (Childers et al., 2011; Gilbert, 2009). Such a scenario suggests the necessity to implement innovative practices in order to recycle P-containing wastes (Koppelaar and Weikard, 2013). In Switzerland, for example, the amount of P-containing waste (i.e. 9600 t) is higher than the annual amount of P provided by fertilizers (i.e. 4200 t) (Mayer et al., 2019). In the case of calcium phosphite (*Ca-Phi*), a byproduct of hypophosphite and phosphine production, around 300 t P are annually discharged in Switzerland. The possibility to recycle *Ca-Phi* as P fertilizer in agriculture systems could be then a valuable option to optimize the P cycle.

Phosphite (Phi) *per se* cannot be used as fertilizer because this form of P is nutritionally inefficient (Gómez-Merino and Trejo-Téllez, 2015; Ratjen and Gerendás, 2009). Phi is stored in the cell vacuoles of plants similarly to phosphate (Pi), but it cannot be metabolized as a P source (Lambers and Plaxton, 2018; Ratjen and Gerendás, 2009; Smillie et al., 1989). Phi prevents the plant biochemical mechanisms responsible of P starvation, subsequently leading to negative impact on plant growth (Lambers et al., 2006; Scott et al., 2004; Ticconi et al., 2001). Several decades ago, MacIntire et al. (1950) observed a toxic effect for different crops (millet, red clover, ryegrass and soybean) fertilized with *Ca-Phi* whereas a beneficial effect was observed for the subsequent crops (alfalfa, ryegrass and soybean), a result that was ascribed to Phi oxidation

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in the soil. Indeed, Phi can be oxidized to Pi by soil microorganisms (Adams and Conrad, 1953; White and Metcalf, 2007) and, consequently, it may be a valuable P source for crops even if the concept of using Phi oxidation as Pi source for a subsequent crop has been neglected. In fact, Phi oxidation is the only way to upgrade Phi as P source for crops considering that Phi oxidation does not naturally occur in plants (Ouimette and Coffey, 1989; Pandeya et al., 2018; Ratjen and Gerendás, 2009). Since abiotic reaction kinetic is very slow, microbial activity is the main driver of Phi oxidation (McDonald et al., 2001; White and Metcalf, 2007). The ability of certain microbial strains to oxidize Phi to Pi is an ancestral trait in life evolution on Earth (Schink and Friedrich, 2000), thus Phi-oxidizing microorganisms are likely present in soils (Stone and White, 2012). On the other hand, Phi can still be toxic for some microorganisms (e.g. *Phytophtora infestans*) and, for this reason, it is widely used as fungicide (Hardy et al., 2001; Lobato et al., 2010). Therefore, Phi addition to soil can favor microbes that can use Phi oxidation as source of P and/or use Phi as source of energy through an array of enzymes, namely alkaline-phosphatase, C–P lyase or Phi-dehydrogenase (Costas et al., 2001; Gómez-Merino and Trejo-Téllez, 2015; Poehlein et al., 2013; Yang and Metcalf, 2004). After Phi addition, soil microorganisms must adapt to the suddenly increased amount of Phi in soil so that a time lag from two weeks to four months before Phi oxidation have been reported depending on experimental conditions (Ouimette and Coffey, 1989; Stoven et al., 2007).

Although Phi oxidation efficiency of isolated microbial strains, including soil microbes, has been already investigated (Casida Jr, 1960; Metcalf and Wolfe, 1998), only one study has clarified the capability of microflora to oxidize Phi in different soil types (Stone and White, 2012). In addition, the few studies that investigated Phi oxidation rate in a single soil type mainly focused on the persistence of soluble Phi in order to assess the fungicide effect on Phi concentration in plant biomass or on microbial activity and feeding activity of mesofauna (Ouimette and Coffey, 1989; Stoven et al., 2007). These experiments were carried out using fungicides containing Phi in a chemical form that was highly soluble in water (e.g. potassium phosphonate, potassium dihydrogenphosphite or Fosetyl-Al). The effect of *Ca-Phi* on soil P pools, especially plant available forms of P (i.e. P_{NaHCO3}), has never been investigated. How soil properties influence the Pi availability after Phi oxidation is unknown. In addition, no study investigated the effect of crop species diversity on Phi oxidation. Therefore, understanding how different plant species influence microbial properties in response to *Ca-Phi* fertilization in different soil types is crucial to test if *Ca-Phi* can be used as source of Pi. This is particularly relevant in crop rotations where green manure (GM) crops fertilized with Phi are used to provide Pi to the subsequent commercial crops through the stimulation of Phi oxidation by soil microorganisms. The benefits of GM application between two commercial crops are well known for their capacity to enhance soil physical, chemical and biological properties, including P availability, even though GM crops do not necessarily provide enough available P to meet the nutrient requirement of the crops (Fageria, 2007; Garg and Bahl, 2008; Wittwer et al., 2017). Thus, a combination of mineral P fertilization and GM crops could have an overall effect to maximize the crop yields.

By taking into account the role of taxonomic diversity of green manure crops (i.e. four different species) and soil type (i.e. a clay and a sandy soil), the potential of *Ca-Phi* to be valorized as a P fertilizer was investigated by comparing *Ca-Phi* with *TSP*. The objectives of the study were to assess if and to what extent *Ca-Phi* addition can affect (i) the aboveground biomass productivity and correspondent Phi concentration in green manure; (ii) the amount of mineral, organic and available P in soil; (iii) the microbial biomass nutrients.

2. Material and methods

2.1. Experimental design

A greenhouse pot experiment was carried out at Agroscope-Changins (Nyon, Switzerland). Three fertilization treatments, namely *Ca-Phi*, *TSP* (used as reference fertilizer) and *0P* (*control* without P addition), were compared. The crop rotation was GM crops followed by the maize. GM crops were supplied with 38 kg P ha^{-1} in forms of *Ca-Phi* and *TSP*, as of Swiss Fertilizer Recommendations of maize (Sinaj et al., 2017). *TSP* granules were powdered using a Retsch BB50 jaw crusher to enhance P release and dispersion within soil collected at two Swiss Federal Research Stations (Changins and Cadenazzo). The experiment was performed on clay and sandy soils with different physico-chemical characteristics (Table 1). We selected two types of agricultural Swiss soils that are, as the majority of Swiss agriculture soils, not deficient in P. The selection of these two soil types will consequently avoid any potential toxic effects of Phi as reported for soils deficient in P (Avila et al., 2011; Barrett et al., 2004; Schroetter et al., 2006). To calculate the amount of P fertilizer during soil preparation, an incorporation depth of 20 cm was considered, soil water content was measured using a moisture analyzer and soil apparent density reported in Gallet et al. (2003) was used. Amounts of 21.3 and 21.5 mg P kg^{-1} dry soil of P fertilizer powders (*Ca-Phi* or *TSP*) were thoroughly mixed with clay and sandy soils, respectively. Pots (volume 9.3 l, diameter 27 cm, height 24.3 cm) were filled with a similar soil volume corresponding to 9.5 kg of clay soil and 9.3 kg of sandy soil.

Four GM crops were selected with contrasted root morphologies and strategies for P uptake, namely oat (*Avena sativa* L.), mustard (*Brassica juncea* L.), lupine (*Lupinus albus* L.) and pea (*Pisum sativum* L.). Oat has the highest root length and root area among the studied species (Wendling et al., 2016). Lupine and pea are legumes with a high efficiency to scavenge soil P, with different strategies (Graham and Vance, 2003; Moraghan, 1993). Lupine is a non-mycorrhizal species forming root clusters (i.e. proteoid roots) to mobilize P that is sparingly soluble, whereas pea is a mycorrhizal species and has a higher specific root length than lupine (Erman et al., 2009; Nuruzzaman et al., 2005; Wamberg et al., 2003). Mustard is a non-mycorrhizal species with a pivotal root system (Tester et al., 1987), known to promote rhizobacteria that enhance P nutrition and can be used as P accumulator (Delorme et al., 2000; Kumar et al., 2013). Also, pea and lupine exude higher amount of carboxylate enhancing sparingly soluble mineral P (Neumann and Römheld, 1999) more than oat (Nuruzzaman et al., 2005). Overall, pea and lupine are more effective in mobilizing sparingly soluble P compared to oat, which is by contrast, more effective to draw P from the soil solution (Maltais-Landry, 2015). According to previous field experiments (Wendling et al. 2016, 2017), the plant densities were 5, 20, 25 and 7 plants per pot for lupine, oat, mustard and pea respectively.

Table 1
Mean chemical properties (n = 3) of clay and sandy soils with coefficient of variance (%) in parenthesis.

Soil properties	Clay soil	Sandy soil
pH (H_2O)	7.8 (3)	5.8 (0)
Clay (g kg^{-1})	291.0 (4)	61.8 (3)
Sand (g kg^{-1})	281.5 (6)	518.8 (5)
C_{Org} (g kg^{-1})	18.7 (3)	15.6 (10)
N_{Tot} (g kg^{-1})	2.2 (0)	1.5 (2)
C/N	7.7 (2)	8.2 (12)
P_{Min} (mg kg^{-1})	411.3 (3)	1032.7 (5)
P_{Org} (mg kg^{-1})	258.3 (4)	241.0 (18)
P_{NaHCO3} (mg kg^{-1})	29.3 (5)	50.1 (5)
CEC ($cmol_c$ kg^{-1})	14.3 (9)	6.8 (3)
K_{exch} ($cmol_c$ kg^{-1})	0.43 (3)	0.25 (15)
Ca_{exch} ($cmol_c$ kg^{-1})	37.1 (1)	4.8 (2)
Mg_{exch} ($cmol_c$ kg^{-1})	0.99 (13)	0.75 (1)

To ensure optimal photosynthetic conditions for plant growth, daily temperature was maintained between 18 °C and 25 °C. The natural daylight was supplemented with high-pressure sodium lamps (400 W m^2) from 6 a.m. to 8 p.m. when light intensity dropped below 250 W m^{-2}. Pots were manually watered to keep a constant soil moisture content (i.e. 70%–80% of the field capacity). Pots were relocated every three weeks to avoid potential bias related to greenhouse heterogeneity.

In total 96 pots were included in this study resulting from a combination of four GM crops, three fertilization treatments, two soil types, and four replicates. In addition, in order to isolate the "direct" effect of P fertilization on soil microorganisms, three replicates of bare soil (without crops) were incubated in the same greenhouse conditions for each fertilization treatment totaling 18 pots for both soils, i.e. 2 soil types × 3 fertilization treatments × 3 replicates. These bare soil pots will be hereafter referred as incubated soils, whereas the pots with GM crops will be referred as cropped soils.

2.2. Aboveground biomass sampling and phi concentration measurement

Eight weeks after sowing, i.e. during the flowering period, aboveground biomass of GM crops was harvested and weighted to obtain the fresh aboveground biomass. A subsample of 20 g was oven-dried (55 °C for 72 h) to estimate the water content. The dried biomass was ground using a Retsch rotor mill to analyze the Phi content. For this purpose, the QuPPE European reference method (Anastassiades et al., 2015), developed to analyze pesticides residues in plants, was applied. Briefly, 0.5 g of dry and ground aboveground biomass was added to 5 ml methanol HPLC grade and acidified with formic acid (CH_2O_2, 1% vol/vol), vortexed two times for 30 s within a 5 min period and centrifuged at 4500 rpm for 5 min. Finally, the supernatant was injected onto a Thermo Hypercarb column (100 × 2.1 mm, granulometry = 3 μm) in order to measure the Phi concentration using a LCMS-MS device (Waters Acquity H-Class/TQ-S Micro). If measured Phi value was between the limit of detection (2 mg kg^{-1}) and the limit of quantification (5 mg kg^{-1}), a concentration value of 5 mg kg^{-1} was assigned.

2.3. Soil sampling and physico-chemical analysis

At the end of the GM cultivation period, four soil cores (2.5 cm diameter) were sampled along the entire thickness of each pot, sieved (2 mm mesh size) and thoroughly mixed. About 100 g of fresh soil was immediately stored in a cold chamber (4 °C) for microbial biomass analysis and the remaining soil was air-dried before storing for chemical analysis.

Soil texture analysis was obtained using the pipette method (five fractions, NFX 31 107). Organic carbon (C_{Org}) was determined based on sulfochromic oxidation (NF ISO 14235). Soil pH was measured in water using a soil:solution ratio of 1:5 (NF ISO 10390). Cation exchangeable capacity and exchangeable K, Ca and Mg were determined using a Thermo Radial ICAP 6000 Series ICP-OES (Thermo Fisher Scientific, Freemont, CA, USA) after ammonium acetate extraction (NFX 31–108). Total soil N (N_{Tot}) was analyzed by dry combustion using an elemental analyzer (Thermo, flash 2000, USA) (NF ISO 13878). Total P (P_{Tot}) was measured using a molybdate colorimetric method (Murphy and Riley, 1962) following an extraction using 0.25 g of soil in 5 ml of hydrofluoric acid (40%) and 1.5 ml of $HClO_4$ (65%) (NFX 31–147). Organic P (P_{Org}) was measured according to Saunders and Williams, (1955). Mineral P (P_{Min}) was estimated by subtracting P_{Org} from P_{Tot}. Available P (P_{NHCO3}) was estimated after sodium bicarbonate (Na–HCO_3) extraction (Olsen, 1954) and measured according to Murphy and Riley (1962) (NF ISO 11263).

2.4. Microbial biomass nutrients

Soil microbial C (C_{mic}), N (N_{mic}) and P (P_{mic}) were estimated using the chloroform fumigation extraction (Vance et al., 1987). Total C and N of fumigated and non-fumigated samples were analyzed using a TOC/TN auto analyzer (Shimadzu analyzer TOC-V CPH + TNM-1) after (1:10) 0.5 M K_2SO_4 extraction. Phosphorus was measured by a colorimetric method using a sulfomolybdic reagent following (1:20) 0.5 M $NaHCO_3$ (pH 8.5) extraction (Murphy and Riley, 1962; Olsen, 1954). Values of C_{mic}, N_{mic} and P_{mic} were estimated according to the coefficient factors k_C, k_N and k_P, respectively, 0.45, 0.54 and 0.40 (Jenkinson et al., 2004).

2.5. Statistical analysis

All statistical analyses were carried out using R 3.01 (Team, 2013). Normality data distribution was checked with shapiro.test function available in the *stats* package. The condition of independence was not met for the entire set of data. Adjusted R squared (adj. R^2) and significance for soil type, fertilization treatment and plant species effects were computed for the entire data set and for each soil type. Data were generally modeled separately for each soil type considering that soil type effect was systematically high and significant and that interaction between effects of soil type and fertilization or GM crops were frequently significant. If species effect was not significant, fertilization treatment was tested using the Tukey test with the function tukeyHSD (*stats* package) or using pairwise permutation test with the function pairwisePermutationTest (*rcompanion* package) if data did not satisfy normality condition. If species effect was significant and if interaction between species and treatment was not, mixed models were carried out with species effect as random factor and fertilization treatment as fixed factor using the lme function (*nlme* package). In case the interactions between fertilization type and GM crops were significant, the role of GM crops was modeled separately for each fertilization treatment. Finally, effect of fertilization treatments on microbial biomass nutrients were computed using the means of *control* treatment as reference values.

3. Results

3.1. Soil chemical properties

Initial soil properties for C_{Org}, N_{Tot}, pH and CEC were higher in clay soil than in sandy soil whereas P_{Min} and P_{NaHCO3} were lower in clay soil than in sandy soil (Table 1). At the end of the experiment, no significant effects of *Ca-Phi* treatment were found for C_{Org} and N_{Tot} in the incubated clay soil compared to *control* conditions (Table 2). Instead, for cropped clay soil, a significant decrease of C_{Org} and N_{Tot} was observed with *TSP* and *Ca-Phi* treatments for all GM crops (Table 2), while crop effect was not significant (Table 3). To a lesser degree, the C_{Org} also decreased in the cropped sandy soil with *Ca-Phi* and *TSP* treatments similarly for each GM crop (i.e. no interaction between effects of P fertilization and species, Tables 2 and 3). For N_{Tot}, the interaction between P fertilization and GM crop effect was significant in cropped sandy soils (Table 3). For leguminous (lupine and pea), *Ca-Phi* decreased N_{Tot} compared to *TSP* and no fertilization treatment. For non-leguminous (oat and mustard), N_{Tot} was higher for *TSP* compared to *Ca-Phi* and *control*.

In the incubated clay soil, the *Ca-Phi* treatment increased P_{NaHCO3} compared to the *control* treatment while no significant effect of *TSP* treatment was observed (Table 2). For cropped clay soil, an interaction between P fertilization and GM crops was found for P_{Min} (Table 3). *Ca-Phi* and *TSP* decreased P_{NaHCO3} and increased P_{Org} compared to *control* conditions for all the GM crops (Table 2), except for lupine. Cropped pots with lupine and fertilized with *Ca-Phi* had lower P_{Org} ($p < 0.001$) and higher P_{NaHCO3} ($p < 0.05$) compared to the other GM crops. Crop impact (i.e. incubated soil – cropped soil) affected P_{NaHCO3} in the clay soil following the order: *Ca-Phi* > *TSP* > *0P*. In the incubated as well as in the cropped sandy soils, P_{NaHCO3} increased in the *Ca-Phi* and *TSP* treatments compared to the *control* treatment, whereas no effect was observed for P_{Org} (Table 3).

Table 2
Fertilization treatment (*Ca-Phi* vs *TSP* vs *0P-control*) effects on chemical properties of incubated soils (IS, n = 9), cropped soils (CS, n = 48), sandy soil cropped with leguminous (i.e. lupine and pea, n = 24) and without (i.e. mustard and oat, n = 24), clay soil cropped with lupine (n = 12) or without (n = 36) (variance coefficient in %).

Chemical properties		Clay			Sand		
		Ca-Phi	TSP	0P	Ca-Phi	TSP	0P
C_{Org} (g kg^{-1})	IS	18.5 (3) a	17.4 (2) b	18.7 (2) a	15.6 (4)	15.6 (2)	15.6 (3)
	CS	16.0 (5) c	16.8 (4) b	18.3 (7) a	15.1 (2) b†	14.9 (2) c†	15.5 (4) a†
N_{Tot} (g kg^{-1})	IS	2.06 (2) a	2.01 (1) b	2.06 (0) a	1.41 (1)	1.45 (0)	1.44 (3)
	CS	1.85 (2) c	1.94 (1) b	2.00 (5) a	–	–	–
	leguminous	–	–	–	1.33 (5) b	1.42 (1) a	1.43 (4) a
	non leguminous	–	–	–	1.34 (2) b	1.40 (2) a	1.29 (4) c
P_{Min} (mg kg^{-1})	IS	427.0 (4)	434.0 (2)	425.7 (1)	1054.0 (1)	1051.7 (1)	1032.7 (0)
	CS	–	–	–	1025.5 (1)	1018.8 (1)	1014.4 (3)
	lupine	427.5 (3)	429.8 (1)	422.3 (1)	–	–	–
	without lupine	406.8 (3) b	423.9 (3) a	403.8 (3) b	–	–	–
P_{Org} (mg kg^{-1})	IS	246.7 (4)	239.0 (3)	241.0 (1)	243.7 (2)	241.7 (4)	250.3 (5)
	CS	–	–	–	234.8 (3)	237.4 (7)	235.6 (5)
	lupine	228.0 (6) b	245.9 (2) a	235 (1) ab	–	–	–
	without lupine	250.1 (5) a	249.7 (6) a	234.6 (6) b	–	–	–
P_{NaHCO3} (mg kg^{-1})	IS	39.7 (2) a	30.9 (3) b	33.5 (8) b	58.4 (2) a	57.9 (2) a	50.7 (1) b
	CS	–	–	–	54.4 (3) a	52.9 (4) b	47.9 (3) c
	lupine	31.7 (8) a	26.2 (9) b	33.2 (4) a	–	–	–
	without lupine	26.2 (2) b	25.6 (6) b	31.6 (7) a	–	–	–

Table 3
Adjusted R^2 and significance of the effects of soil type, fertilization treatment and species on plant and soil properties for cropped soils (n = 96) and separately for each soil type (n = 48). Significant interactions between effects of soil type and crops or of fertilization and crops are indicated in bold-italic.

	Clay & Sand (n = 96)			Clay (n = 48)		Sand (n = 48)	
Effect	Soil type	Fertilization	Crops	Fertilization	Crops	Fertilization	Crops
Aboveground biomass	***0.52******	***0.00***	***0.30*****	***0.12****	***0.49*****	0.00	0.86***
Phi concentration	0.00	–	0.20*	–	0.64**	–	0.93***
C_{Org}	0.45***	0.16***	0.00	0.51***	0	0.15**	0.12*
N_{Tot}	0.93***	0.00	0.00	0.47***	0.06	***0.18****	***0.10****
N_{Tot} leguminous	–	–	–	–	–	0.44**	0.00
N_{Tot} Non leguminous	–	–	–	–	–	0.62**	0.00
P_{Min}	0.99***	0.00	0.00	***0.21****	***0.15****	0.00	0.07
P_{Min} without lupine	–	–	–	0.34***	0.00	–	–
P_{Org}	0.05*	0.03	0.00	0.13*	0.04	0.00	0.00
P_{NaHCO3}	0.92***	0.00	0.00	0.58***	0.08	0.73***	0.03
C_{mic}	0.84***	0.00	0.00	***0.12****	***0.27*****	0.17**	0.00
C_{mic} without oat and lupine	–	–	–	0.31***	0.00	–	–
N_{mic}	0.61***	0.00	0.03	0.04	0.31***	0.00	0.09
P_{mic}	0.47***	0.00	0.05*	0.05	0.05	0.09*	0.23**

Linear models are significant at $p < 0.05$ (*), $p < 0.01$ (**) or $p < 0.001$ (***).

3.2. Green manure productivity and phosphite content

Overall (i.e. n = 96), aboveground productivity was affected by soil type and plant species, whereas fertilization treatment did not show any significant effect (Table 3). Aboveground GM productivity was 38% higher on sandy soil compared to clay soil. Also, it was 44% higher for pea and lupine compared to oat and mustard (Fig. 1). Because an interaction between soil type and plant species was found (Table 3), soil type effect was investigated separately for each GM crop. An effect of fertilization treatment was observed for the clay soil due to a decrease of aboveground productivity of lupine and oat with *Ca-Phi* fertilization compared to control and *TSP* treatments, respectively (Table 2; Fig. 1a and d). In addition, a positive effect of *TSP* compared to control was found for oat aboveground productivity on sandy soil (Fig. 1a).

For the *Ca-Phi* treatment, a lower Phi concentration in aboveground biomass was detected in lupine and a higher concentration in mustard compared to the other two GM crops in the clay soil (Fig. 2), while pea showed a higher Phi concentration compared to the other GM crops in the sandy soil (Fig. 2). Phi concentration in lupine and oat biomass did not differ by soil type, whereas it was significantly different for pea and mustard (Fig. 2). In addition, no relationship was found between aboveground productivity and Phi concentration for species in both soil types.

3.3. Soil microbial properties

Overall (n = 96) the microbial biomass C_{mic}, N_{mic} and P_{mic} were primarily affected by soil type, whereas plant species and fertilization treatment had a limited effect only within each soil type (Table 3). Compared to the *control* treatment, no differences were found for C_{mic} and N_{mic} with *Ca-Phi* or *TSP* fertilization, whereas P_{mic} decreased in the *Ca-Phi* treatment in incubated soils (Fig. 3). In the cropped clay soil, an interaction between P fertilization and GM crops was found (Table 3). In fact, *Ca-Phi* decreased the C_{mic} compared to *control* condition to higher extent for oat compared to other GM crops, whereas no decrease was observed for lupine. For mustard and pea in clay soil as well as for all GM crops cropped in sandy soil (Fig. 3, Table S1), *Ca-Phi* and *TSP* decreased C_{mic} compared to the *control*. In addition, N_{mic} and P_{mic} decreased in clay soil with *Ca-Phi* fertilization, with the exception of lupine. For sandy soil, *TSP* treatment increased P_{mic} compared to the *control* condition.

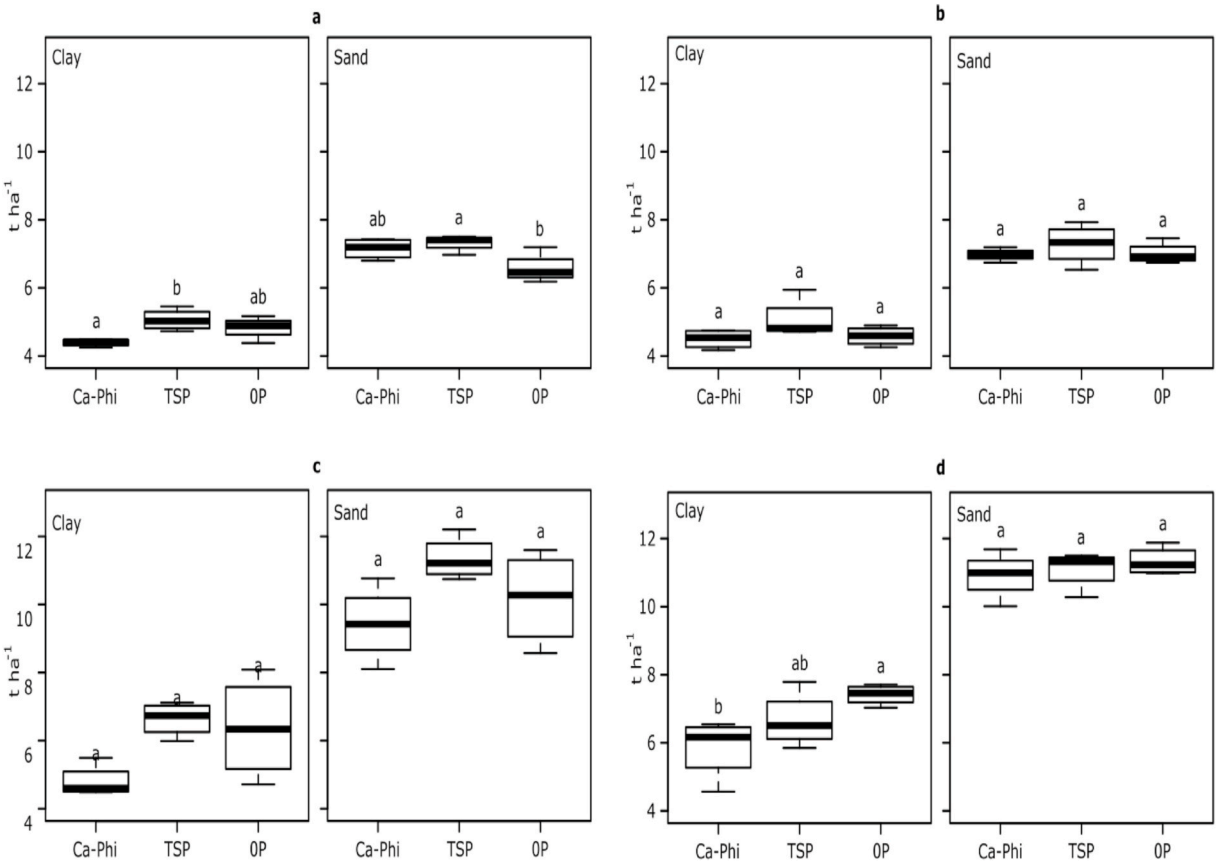

Fig. 1. Boxplot of aboveground biomass productivity (oven dry weight) for oat (a), mustard (b), pea (c) and lupine (d) in clay and sandy soils (n = 12).

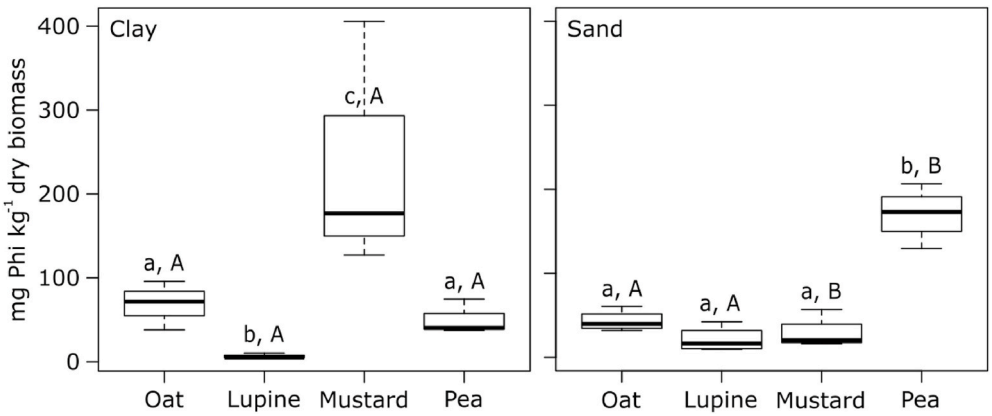

Fig. 2. Boxplot of phosphite (Phi) concentration in aboveground biomass of GM crops after calcium phosphite (*Ca-Phi*) fertilization (n = 4). Different letters indicate significant differences ($p < 0.05$) between GM crops within the same soil type (lower case) and between soil types for the same GM crop (upper case).

4. Discussion

4.1. Soil properties

The higher concentration of P_{NaHCO3} in sandy soil after *Ca-Phi* and *TSP* addition compared to clay soils (Table 2) is likely due to the lower P fixing capacity of sandy soils (Arai and Sparks, 2007; Beauchemin and Simard, 1999; Sinaj et al, 1997, 2001). The high initial values of P_{NaHCO3} in our studied soils (Table 1) may have decreased the availability of P sorption sites and, therefore, led to a strong response of GM crops to *Ca-Phi* and *TSP* fertilization (Barrett et al., 2004).

Compared to *control* conditions, the lack of any significant effect of *TSP* fertilization on P_{NaHCO3} in incubated clay soil seems to suggest that P was quickly released from *TSP* and subsequently adsorbed onto the soil solid phase to be, eventually, incorporated into the P_{Min} pool (Hesterberg, 2010). This result seems in line with Morais and Gatiboni (2015) reporting a fast increase of P_{NaHCO3} during the first thirty days after *TSP* addition and a return to initial concentration values after sixty days due to P sorption onto soil particles. In contrast, P from *Ca-Phi* was slowly released since it is neither water, nor $NaHCO_3$ soluble. The slow P release from *Ca-Phi* can partly explain the increase of P_{NaHCO3} after eight weeks of crop growth compared to *TSP* and *control*. A slow P release is advisable for P fertilizer since it can enhance the P agronomic efficiency (Roger et al., 2016). Overall, the crop impact (i.e. incubated soil – bare soil) resulted in a higher depletion of available P with *TSP* and *Ca-Phi* compared to *control* treatment (Table 2), suggesting that a higher

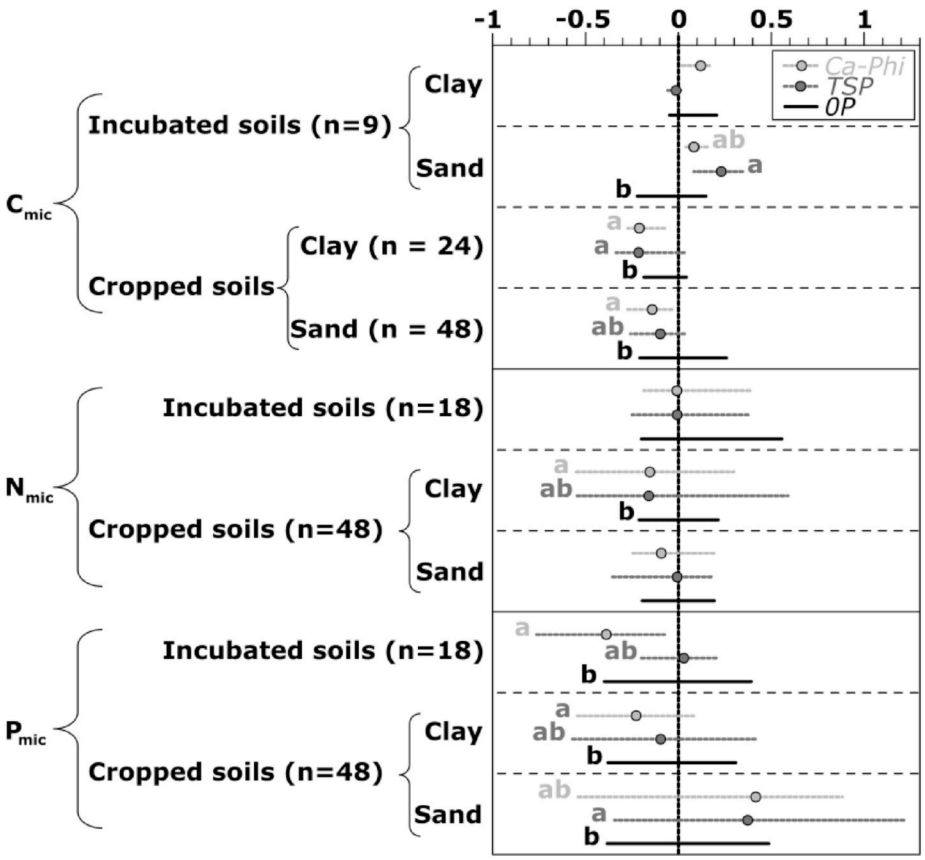

Fig. 3. Effect of fertilization treatments (*Ca-Phi* and *TSP*) as compared to *control* (*0P*) for microbial biomass nutrients (C_{mic}, N_{mic} and P_{mic}) for incubated soils (n = 18) and cropped soil (n = 48 or n = 24 for C_{mic} that included only pea and mustard in order to avoid interaction between GM crops and P fertilization). Significant differences ($p < 0.05$) between fertilization treatments are indicated by different letters. Bold points indicate mean value for *Ca-Phi* and *TSP* fertilization treatments for all crops combined. The zero value correspond to the mean of the *control* (*0P*) and the segment encompass the minimal and maximal values of C_{mic} or N_{mic} or P_{mic}.

amount of P was mobilized in cropped pots fertilized with *TSP* and *Ca-Phi* compared to *control* treatment.

In cropped clay soils, the decrease of P_{NaHCO3} with *TSP* and *Ca-Phi*, compared to *control*, may be explained by the concomitant decrease of C_{Org} (adj $R^2 = 0.28$, $p < 0.001$, n = 60; Table 2). Indeed, organic acids can compete with P for sorption sites and therefore increase P_{NaHCO3} (Von Wandruszka, 2006). Also, the increase of P_{Org} with the *TSP* treatment for all GM crops and with *Ca-Phi* treatment for oat, mustard and pea (Table 2) suggests that P was transferred from P_{NaHCO3} to P_{Org} pool as previously reported by other studies in response to mineral P addition (Ch'ng et al., 2014; Hassan et al., 2012; McLaughlin et al., 1988). Although *Ca-Phi* and *TSP* fertilization treatments decreased P_{mic} pool in cropped clay soils, the increase of P_{Org} could be the result of a higher microbial turnover (Oehl et al., 2001; Hedley et al., 1982; Stewart and Tiessen, 1987). This is in line with the decrease of C_{Org} and N_{Tot} after *Ca-Phi* and *TSP* fertilization treatments (Table 2), suggesting a potential priming effect promoting microbial decomposition of organic matter through root exudates and/or an increase of heterotrophic respiration due to a higher C and N microbial demand (Clarholm, 1985; Dendooven et al., 2015; Poeplau et al., 2016). Alternatively, the increase of P_{Org} could be also due to an abiotic process binding Pi or/and Phi with C_{Org} particles (Eichler-Löbermann et al., 2007). In contrast, P_{Org} did not increase for lupine fertilized with *Ca-Phi* whereas P_{Min} soil content was higher compared to the other GM crops (Table 2), likely due to the specific strategy of P uptake by lupine. Indeed, lupine is well known to mobilize P sparingly soluble through proteoid roots that release organic acids (Lambers et al., 2013) and, in turn, may efficiently solubilize *Ca-Phi*. In fact, the higher P_{NaHCO3} content and the lower Phi concentration in tissues of lupine compared to the other GM crops suggest that Phi oxidation was more efficient in clay soil (Fig. 2, Table 2). In the sandy soil, the decrease of N_{Tot} for leguminous fertilized with *Ca-Phi* could suggest that symbiotic process of N fixation was hindered (Table 2). However, *Ca-Phi* increased P_{NaHCO3} compared to *control* treatment, both in incubated and GM cropped soils, likely because of the Phi oxidation during plant growth as previously observed for *Ca-Phi* (Adams and Conrad, 1953). These results point out that *Ca-Phi* fertilization increased available P at least to a similar extent of *TSP* fertilization.

The effect of P fertilization on microbial biomass nutrients was generally smaller compared to the effect associated with soil type or GM crops (Table 3), very likely because the initial P content in both soil types was not limiting (Bünemann et al., 2004; Thirukkumaran and Parkinson, 2000). In different soil types, the amount of soil-bacteria capable to oxidize Phi varied substantially (Stone and White, 2012). In our study, *Ca-Phi* addition did not seem to affect the microbial biomass differently than *TSP* for both soil types (Fig. 3). Interestingly, no negative effect of *Ca-Phi* on C_{mic} was observed for lupine in clay soil, again supporting a singular response of this GM crop compared to the other species (Table S1). In addition, we observed a positive relationship between C_{mic} and P_{NaHCO3} in cropped and incubated clay soils for all the fertilization treatments except for lupine (adj. $R^2 = 0.32$, $p < 0.001$, $n = 45$), a result indicating a similar response of microbial biomass to both Phi and Pi addition. However, as far as we know, this is the first study reporting the Phi effect on C_{mic}, N_{mic} or P_{mic} and, therefore, there is no established benchmark to compare our results.

The impact of GM crops in affecting microbial nutrient uptake after P fertilization can be observed by comparing the effects of *Ca-Phi* and *TSP* in incubated and cropped soils (Beauregard et al., 2010). In incubated soils the decrease of P_{mic} with *Ca-Phi* fertilization suggests that microbial P uptake was somehow hindered, whereas the presence of GM crops may have favored the P microbial uptake in the sandy soil (Fig. 3), most likely in response to root exudates favoring P_{mic} (Richardson and Simpson, 2011). On the contrary, C_{mic} generally decreased in GM crops for all P additions (Fig. 3), probably in response to lower organic matter (Table 3) reducing the amount of easily decomposable C_{Org} for microbial communities (Chen et al., 2016; Paré et al., 1998; Tu et al., 2006).

4.2. Aboveground biomass productivity of green manure crop species

Aboveground biomass of the green manure crops species ranged from 4.2 to 12.2 t ha^{-1}. Other researchers reported green manure yields within the same range (Biederbeck et al., 1993; Cherr et al., 2006; Wendling et al., 2017). The effect of P fertilization on aboveground productivity was primarily related to soil type. A higher productivity was observed in the sandy soil compared to the clay soil (Fig. 1), a result in line with the more favorable physical conditions for plant growth. Indeed, on the basis of the C_{Org} to clay content ratio, an index of soil structure quality (Johannes et al., 2017), the sandy soil was characterized by a ratio of c. 1:5, a value above the threshold for optimal structural quality (i.e. 1:8), whereas the clay soil had a ratio of c. 1:17, i.e. below the threshold indicative of a poor structural state (i.e. 1:13).

The studied soils were characterized by high soil available P content (Table 1), a condition that may explain why we did not observe significant differences in GM productivity between P fertilization and *control* treatments (Fig. 1). High P availability may have also buffered the negative Phi effects that have been reported in soils with low P availability (Avila et al., 2011; Barrett et al., 2004; Schroetter et al., 2006). Barrett et al. (2004) observed an increase of Phi phytotoxicity with increasing Phi concentration in plant biomass. In contrast, we did not observe any relationship between GM productivity and Phi concentration. In addition, we observed that Phi concentration within the same GM crop might vary depending on soil type, particularly for mustard and pea (Fig. 2). This result suggests that oxidation and/or uptake of Phi depends strongly on the interactions between plant species and soil characteristics, and not necessarily on plant productivity. The only GM crop showing a significant decrease in productivity in response to *Ca-Phi* addition, compared to *control*, was lupine in the clay soil (Fig. 1). A negative effect of higher Ca availability, in light of the sensitivity of lupine to this element (De Silva et al., 1994), does not seem to play a crucial role considering that also the *TSP* treatment increased soil exchangeable Ca (Ca_{exch}, Table S1) and Ca concentration in lupine biomass (+21% and +29%, respectively, for *Ca-Phi* and *TSP* treatments; Table S2). It was previously reported that Phi interferes with the biochemical pathways of lupine by increasing the number of proteoid roots, root exudates and root enzymatic activities when P is not limiting (Gilbert et al., 2000). Even if we did not measure proteoid roots, we speculate that their stimulation by *Ca-Phi* addition would cause a decrease of aboveground biomass productivity, considering the high energy cost for their production and function (Massonneau et al., 2001).

5. Conclusions

Our study indicated that any effect of *Ca-Phi* on aboveground biomass productivity of GM crops were negligible compared to the effect due to plant species diversity and soil type. Phi concentration in aboveground GM biomass varied across species and across soil type and it was not related to GM productivity. Only a decrease of productivity for lupine on clay soil was observed with *Ca-Phi* compared to *control*. In the sandy soil, the leguminous probably fixed lower atmospheric N with *Ca-Phi* compared to other fertilization treatments. The different P pools were generally more affected by the fertilization treatments than the nutritional strategies of the selected GM crops (except for lupine fertilized with *Ca-Phi*) and the P pools were influenced differently in both soil types. However, the increase of Pi in both soils after *Ca-Phi* fertilization suggests that Phi was at least partly oxidized. Furthermore, *Ca-Phi* and *TSP* fertilization seem to have a similar impact on soil biological fertility considering that microbial biomass nutrients were generally not affected by fertilization type. Additional studies are, however, necessary to verify if GM residues produced under *Ca-Phi* fertilization can provide as much Pi as *TSP* fertilization for the following cash crop. We are aware that our results are based on a greenhouse experiment that was designed to investigate the effects of GM crops and soil type, so the transfer of these results to field conditions should be done cautiously. Overall, based on our preliminary data, it seems that the valorization of *Ca-Phi* as P fertilizer is promising.

Credit author statement

Mario Fontana: Conceptualization, Methodology, Data curation, Investigation, Visualization, Formal analysis, Writing - original draft. **Luca Bragazza**: Writing - review & editing. **Thomas Guillaume**: Writing - review & editing. **Mathieu Santonja**: Writing - review & editing. **Alexandre Buttler**: Writing - review & editing. **Saïd Elfouki**: Investigation, Formal analyses. **Sokrat Sinaj**: Project administration, Funding acquisition, Resources, Conceptualization, Methodology, Data curation, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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