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Nitrogen and phosphorus uptake from isotope-labeled fertilizers by sorghum and soil microorganisms

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Abstract

On nutrient-depleted Lixisols from Burkina Faso, nutrient acquisition by crops and soil microbes mainly relies on the limited amounts of mineral and organic fertilizers applied by small-scale farmers. The objective of this study was to determine simultaneously the uptake of nitrogen (N) and phosphorus (P) contained in organic and mineral fertilizers by sorghum [*Sorghum bicolor* (L.) Moench] and soil microbial biomass. Double ^{15}N and ^{33}P direct and indirect labeling techniques were applied in a pot experiment to determine the contributions of different fertilizers to sorghum N and P uptake during 52 d of growth. In parallel, soil respiration, available, and microbial N and P were tracked in an incubation experiment. Sorghum derived 83–90% of P from fertilizers. Nitrogen from cattle manure was poorly available, contributing only 20% of the N taken up by sorghum. Water-soluble mineral fertilizers increased soil N and P availability, resulting in the highest total N and P uptake by sorghum from fertilizers and soil among all treatments. The application of cowpea [*Vigna unguiculata* (L.) Walp.] residues induced microbial N and P immobilization, reducing sorghum N and P uptake to the level of the non-fertilized treatment. The use of double ^{15}N and ^{33}P labeling elucidated the impact of fertilizers on soil nutrient pools. The low plant N/P ratio suggested N limitation for sorghum in the manure treatment. Cowpea residues were inefficient for sorghum nutrition, but they increased soil microbial nutrient pools. This study gives insights on the potential effects of legume residues used as green manure to build soil fertility.

Abbreviations: 0N0P, non-fertilized control; 0N1P, water-soluble mineral phosphorus fertilizer alone; 1N0P, water-soluble mineral nitrogen fertilizer alone; 1N1P, water-soluble mineral nitrogen and phosphorus fertilizer; CO_2–C, carbon from microbial respiration; DLT, direct labeling technique; DM, dry matter; ILT, indirect labeling technique; L-1N1P, labeled water-soluble mineral nitrogen and phosphorus fertilizer; LY-Cowp, labeled young cowpea residue; Manure, cattle manure; M-Cowp, mature cowpea residue; N_{dff}, nitrogen derived from fertilizer; N_{dfs}, nitrogen derived from soil; P_{dff}, phosphorus derived from fertilizer; P_{dfs}, phosphorus derived from soil; Resin P, soil phosphorus extractable using anion exchange resin; SMB, soil microbial biomass; WHC, water holding capacity; Y-Cowp, young cowpea residue.

1 | INTRODUCTION

The use of nitrogen (N) and phosphorus (P) fertilizers has played an important role in increasing global food production in the past decades (Erisman, Sutton, Galloway, Klimont, & Winiwarter, 2008). In developing countries, however, farmers with limited financial resources often apply little or no mineral fertilizer in their small-scale farming systems (Vitousek et al., 2009), relying on the availability of native soil nutrients and nutrient recycling from organic residues. These low nutrient inputs associated with erratic rainfall distributions and pest attacks contribute to the generally low productivity of crops.

In west-central Burkina Faso, cereals such as sorghum [*Sorghum bicolor* (L.) Moench] are generally produced in monocrop or combined with legumes such as cowpea [*Vigna unguiculata* (L.) Walp.] and groundnut (*Arachis hypogaea* L.]. The dominant soil types of this area are Lixisols characterized by low organic C content (<10 g kg^{-1}), low total N (<1 mg kg^{-1}), and low available P (Bationo, Kihara, Vanlauwe, Waswa, & Kimetu, 2007). In these cropping systems, most crop residues are removed from the fields for livestock feeding, building, or simply burnt for cooking (Andrieu et al., 2015; Smith et al., 2015). In addition, due to the low availability of organic inputs and/or the high cost of water-soluble mineral fertilizers (Chianu, Chianu, & Mairura, 2012; Sanchez et al., 1997), smallholder farmers apply very little or no mineral fertilizer. Under these conditions, nutrient management is mainly based on the use of local organic resources such as manure, household waste, compost, or crop residues that are not used for livestock feeding and other purposes.

Soil microorganisms play a key role in the recycling of nutrients contained in soil organic matter and applied organic amendments (Azcon-Aguilar & Barea, 2015; Zechmeister-Boltenstern et al., 2015). Easily available organic C is the first limiting factor for soil microbial growth and activity, with subsequent limitations by available N or P, or both (Traoré et al., 2016). In the case of crop residues applied to the soil, the net release of nutrients through microbial mineralization depends on their chemical properties, namely the C/N and C/P ratios (Janssen, 1996; Umrit & Friesen, 1994), and the N and P availability in the soil (Alamgir, McNeill, Tang, & Marschner, 2012; Chen et al., 2014). The application of organic inputs may lead to an immobilization of available nutrients from the soil into the soil microbial biomass (SMB) (Bünemann, Bossio, Smithson, Frossard, & Oberson, 2004), inducing a competition for those nutrients between microorganisms and plants.

Mineral and organic inputs differ in their N and P fertilizing value. Accurate estimates of the uptake of N and P added by different fertilizers and knowledge on their incorporation into soil pools are essential for better nutrient management. The labeling techniques using ^{15}N (stable) and ^{33}P (radioactive) isotopes allow assessing the proportions of N and P taken up by plants and SMB from the soil and from applied nutrient sources (Barraclough, 1995; Di, Cameron, & McLaren, 2000; Frossard et al., 2011; Oberson et al., 2010; Vanlauwe, Sanginga, & Merckx, 1998). The direct labeling technique (DLT) consists of labeling the studied nutrient in the fertilizer. The incorporation of the nutrient into the different soil pools and its uptake by the plant is then tracked. The indirect labeling technique (ILT) is used when it is difficult to homogeneously label the fertilizer, for instance in the case of complex fertilizers like manure (Douxchamps et al., 2011; Oberson et al., 2010). In the case of the ILT, the soil-available nutrient pool is labeled, and the fate of the non-labeled fertilizer is then followed using the principle of isotopic dilution (Morel & Fardeau, 1989b; Douxchamps et al., 2011). A central assumption of the ILT is a homogeneous labeling of the plant-available soil nutrient pool, so that any dilution of the label observed in the fertilized treatment compared with the non-fertilized reference treatment is assigned to nutrient release from the applied fertilizer (Fardeau, Guiraud, & Marol, 1996; Hood-Nowotny, 2008).

Isotopic techniques have been used to determine the fate of N or P added with mineral and/or organic fertilizers both in temperate (Armstrong et al., 2015) and in tropical soils (Bado, Bationo, Lompo, Cescas, & Sedogo, 2007; Douxchamps et al., 2011). In temperate Australian soils, Armstrong et al. (2015) concurrently studied the use efficiency of water-soluble mineral P and N by wheat using the ^{15}N and ^{33}P direct double labeling technique. Soil moisture had more impact on the recovery of P than of N, both from mineral fertilizer, highlighting the differences in the plant uptake of N and P and the need to follow the two nutrients simultaneously when they are applied in combination. However, there is no study addressing simultaneously the availability of N and P from organic inputs to a growing crop and the fate of both nutrients in Lixisols, although N and P are the two main limiting nutrients for

Core Ideas

- Mineral fertilizer induced the highest sorghum N and P uptake from fertilizer and soil.
- Cowpea residues resulted in microbial immobilization of added and mineral soil N and P.
- N/P ratios suggested more microbial P than N limitation in organic amendment treatments.

crops grown on these soils. The use of ^{15}N and ^{33}P double labeling allows such a dual-element approach and could potentially provide a better understanding of the mechanisms involved in the N and P supply to plants and to the SMB on nutrient-depleted Lixisols.

The aim of this study was to determine the uptake of N and P from organic and mineral fertilizers by sorghum and to understand the incorporation of N and P added with these fertilizers into available and microbial nutrient pools of a Lixisol. For this purpose, we used ^{15}N and ^{33}P double labeling techniques. We expected that the ratio of N and P taken up by sorghum from the different types of fertilizers would vary more broadly than the N/P ratio of these fertilizers because of their differing N and P availability to crops, and because of the microbial immobilization induced by the concomitant addition of C with the organic fertilizers.

2 | MATERIALS AND METHODS

2.1 | Overview on the experiments and treatments

We carried out a pot and an incubation experiment in parallel, both for 52 d, to assess the uptake of N and P by sorghum from applied organic fertilizers and the incorporation of N and P into the SMB and soluble nutrient pools. The fertilizer treatments were: labeled and non-labeled young cowpea plant material produced under controlled conditions in the greenhouse (LY-Cowp and Y-Cowp, respectively), mature cowpea residues collected in a farmer's field (M-Cowp), cattle manure collected in a cow-shed (Manure), labeled and non-labeled mineral N and P produced from water-soluble salts (L-1N1P and 1N1P), and a non-fertilized control (0N0P). The amount of N added was fixed to 75 mg N kg^{-1} soil for all treatments as done by Broadbent and Nakashima (1967). The P doses varied according to the P contents of the different organic fertilizers (Table 1). Both pot and incubation experiments had completely randomized designs, with four replicates for each treatment.

In the pot experiment, the labeled inputs were applied to non-labeled soil (direct labeling technique, DLT), whereas non-labeled inputs were applied to ^{15}N and ^{33}P labeled soil (indirect labeling technique ILT). The ^{15}N and ^{33}P labeled soil was also used in the 0N0P control treatment. Two reference treatments, 1N0P and 0N1P, were included to calculate the proportions of N and P in sorghum derived from the fertilizers (nitrogen derived from fertilizer, N_{dff}, and phosphorus derived from fertilizer, P_{dff}) using the ILT. The pot experiment was done with pots filled with 2 kg (dry weight equivalent) of soil. Two seeds of the sorghum variety Sariasso 14 were sown in each pot. Pots were kept in a greenhouse with day and night temperature between 25 and 22 °C, 12 h light, and 70% air humidity. The sorghum seeds contained on average 0.46 mg N $grain^{-1}$ at a $\delta^{15}N$ natural abundance of around 7‰, and about 80 μg P $grain^{-1}$. After addition of the fertilizers, a volume of 11.4 ml kg $soil^{-1}$ of N and P free Hoagland nutrient solution was added to all treatments including the non-fertilized 0N0P, to avoid any other nutrient limitation. This nutrient solution added in mg kg^{-1} soil 4.3 K, 2.2 Ca, 1.1 Mg, 0.1 Fe, and 1.4 S, and in μg kg^{-1} soil 6.1 B, 2.4 Mn, 2.6 Zn, 0.7 Cu, and 1.1 Mo. The water content of the soil was kept at 60% water holding capacity (WHC) by an addition of distilled water as required based on daily weighing. The aboveground biomass of the sorghum plants was harvested after 52 d, at the stage of seven fully developed leaves. The harvested biomass was dried at 55 °C for 5 d, weighed, and ground before analysis.

The incubation experiment included the same treatments as the pot experiment, but the non-labeled inputs were applied to non-labeled soil (i.e., there was no ILT). The equivalent of 600 g of dry soil preconditioned as for the pot experiment was amended separately for each of the four replicates of each treatments like in the pot study. Then, an amount equivalent to 100 g of dry soil was used for the measurement of the CO_2 emissions. The remaining 500-g portions were incubated to study the changes in N and P pools. To this end, microbial C and N, dissolved N and extractable P from the anion exchange resin were measured periodically. Soils were incubated in a dark room constantly held at 25 °C.

2.2 | Soil conditioning and labeling

The soil was a ferric Lixisol (FAO, 2006) sampled in a farmer's field located close to the agricultural research station of Saria in the Centre West of Burkina Faso (12°16′ N, 2°9′ W, elevation 300 m). It had a sandy loam texture with abundant presence of small stones, which were removed prior to the experiment by sieving through a 5-mm mesh. According to the farmer, the field was cropped continuously for more than 30 yr. During the last 5 yr before the soil sampling, sorghum and cowpea were continuously grown in mixed cropping without any mineral or organic fertilizer input. The 0-to-15-cm layer of the soil was sampled in January 2013 during the dry season at a water content of 5 g water kg^{-1} of dry soil.

The soil had a pH in water of 5.8, a total C of 5.5 g kg^{-1}, total N of 295 mg kg^{-1}, 136 mg kg^{-1} of total P determined after digestion with concentrated H_2SO_4 and H_2O_2 (Anderson & Ingram, 1993), and 0.18 mg kg^{-1} of available P determined with anion exchange resin extraction (Kouno, Tuchiya, & Ando, 1995).

TABLE 1 Characteristics of the mineral fertilizers and organic inputs and quantities of total C, N, and P added to the soil in the pot and incubation experiments

Amendments properties	LY-Cowp	Y-Cowp	M-Cowp	Manure	L-1N1P	1N1P
Total C, mg g^{-1} [a]	414	415	417	191	–	–
Total N, mg g^{-1} [a]	17.5	17.5	18.5	16.4	–	–
Total P, mg g^{-1} [b]	2.11	1.63	1.28	3.93	–	–
Mass C/N ratio	23.7	23.7	22.6	11.7	–	–
Mass C/P ratio	196	255	326	49	–	–
Mass N/P ratio	8.3	10.7	14.5	4.2	8.3	8.3
P_{resin} (in % of total P)	69	55	36	50	100	100
^{15}N atom excess, % [c]	9.11	–	–	–	2.13	–
Specific activity, kBq $mg\ P^{-1}$	202	–	–	–	246	–
Rates of nutrients added to the soil						
Total dry matter, g kg^{-1} soil	4.3	4.3	4.1	4.6	–	–
C, g kg^{-1} soil	1.78	1.78	1.70	0.88	–	–
N, mg kg^{-1} soil	75	75	75	75	75	75
P, mg kg^{-1} soil	9.1	7.0	5.2	18.0	9.1	9.1

Note. LY-Cowp, labeled young cowpea; Y-Cowp, non-labeled young cowpea; M-Cowp, mature cowpea; Manure, cattle manure; L-1N1P, labeled mineral N and P fertilizer; 1N1P, non-labeled mineral N and P fertilizer.

[a]Total C and total N determined using a chemical elements analyzer Vario PYRO cube, Elemetar, on finely milled and encapsulated samples.

[b]Total P determined on samples burned at 550 °C for 5 h, followed by a dilution of the ashes in fuming HNO_3 and measurement of the P concentration in the extracts by the Malachite green method.

[c]The ^{15}N atom excess measured using a chemical elements analyzer Vario PYRO cube, Elemetar, coupled with a mass spectrometer IsoPrime 100, Manchester, on finely milled and encapsulated samples.

Before the experiments, the soil was progressively moistened during a first incubation to mimic the restart of soil microbial activity at the beginning of the rainy season. Firstly, the soil water content was brought to 97 g kg^{-1}, which is equivalent to 45% of its WHC by adding distilled water. Then, the soil was incubated in the dark at about 22 °C for 12 d. Afterward, the soil was separated into two portions. The portion for ILT was labeled with ^{15}N and ^{33}P, whereas the other portion remained unlabeled.

The soil was labeled by adding a small quantity of N (0.25 mg N kg^{-1} dry soil) as an aqueous solution of $(NH_4)_2SO_4$ containing 60 atom% of ^{15}N (Sigma-Aldrich Chemie GmbH). The same quantity of unlabeled $(NH_4)_2SO_4$–N was mixed into the non-labeled portion of soil, also in aqueous solution. An amount of 6 mg kg^{-1} of labile C (corresponding to about 0.1% of the total soil C content) was added at the same time in the form of glucose solution to stimulate the integration of a part of the ^{15}N into the soil microbial pool so as to promote homogeneous soil N labeling (Douxchamps et al., 2011). These additions brought the soil water content from 45 to 50% of the WHC (108 g water kg^{-1} soil). The solutions were carefully mixed into the soil and then incubated for another 12 d.

After the second period of incubation, a second and last addition of 0.25 mg N kg^{-1} of soil was done as a solution of $(NH_4)_2SO_4$ containing 60 atom% of ^{15}N for the labeled soil and as unlabeled $(NH_4)_2SO_4$–N for the non-labeled soil. In total, the N added corresponded to about 0.2% of the total soil N content. Simultaneously with the addition of the second dose of N, the ^{15}N-labeled soil was labeled with ^{33}P by thoroughly mixing into the soil a solution containing carrier-free ^{33}P orthophosphate (Hartmann Analytics) at the rate of 3.3 MBq kg^{-1} soil. After these additions, which brought the soil to 55% WHC corresponding to 119 g water kg^{-1} soil, the soil was incubated for another 8 d before starting the experiments.

2.3 | Preparation of the organic and mineral fertilizers

The labeled and non-labeled young cowpea material (LY-Cowp and Y-Cowp, respectively) were produced in hydroponics. Cowpea seeds were pre-germinated. At the stage of two well developed tri-foliate leaves, the seedlings were transferred into two separate hydroponic boxes. Each box contained 22 L of N and P free Hoagland nutrient solution (Hoagland & Arnon, 1950). The total amount of P (149 mg P) was added with an aqueous solution of KH_2PO_4 at the beginning of the hydroponic production, and half of the total N amount (1,518 mg N) was added as NH_4NO_3 at the beginning and the second half after 10 d. The doubly labeled (^{15}N and ^{33}P) cowpea was produced by adding 85.1 MBq of carrier-free ^{33}P orthophosphate (Hartmann Analytics) and N in the form of 10% ^{15}N enriched NH_4NO_3 to one box. The level of water in the boxes was corrected daily by adding reverse osmosis water. At harvest, which took place after 4 wk of growth in hydroponics, the cowpea plants were at the stage of five to six fully developed leaves and about 20 to 25 cm height. After the harvest, cowpea shoots were dried at 55 °C for 4 d and ground (< 0.1 mm) using an ultra-centrifuge mill (ZM 200, Retsch, Haan, Germany) for analysis. After the first week of cowpea growth, a small loss of nutrient solution from the labeled box was noticed. The correction of this loss induced a small increase of P concentration in the labeled hydroponic solution, which resulted in a slightly higher total P content in the labeled cowpea. The labeled and non-labeled young cowpea plants were separately coarsely ground (max 2 mm) to facilitate their application.

The mature cowpea residues (M-Cowp) with a C/N ratio wider than that of the young cowpea (Table 1) were obtained from a farmer who had collected them from his field after harvest of the grains and stored dry for use as animal fodder. These mature cowpea residues were also ground, similarly to the young cowpea.

The cattle manure (Manure) which was stored in a pit during the dry season in a barn of the Saria research station was sampled in 2010. This manure came from by cattle feed by crop residues (cereal straw and legume haulms). The sample was air-dried and sieved at 2 mm before application. This manure had a high total P content compared with the cowpea residues (Table 1).

Mineral N and P fertilizers were prepared in separate aqueous solutions of 75 mg N ml^{-1} and 9.05 mg P ml^{-1}, produced from $(NH_4)_2SO_4$ and KH_2PO_4, respectively. The solution of labeled N was prepared from 10 atom% ^{15}N mineral $(NH_4)_2SO_4$ solution, which was diluted in a non-labeled mineral $(NH_4)_2SO_4$ solution, resulting in 2.13 atom% ^{15}N (Table 1). At the starting day of the experiments, a part of the non-labeled mineral P solution was labeled by adding a carrier-free ^{33}P orthophosphate (Hartmann Analytics) in order to reach a specific activity of 246 kBq mg^{-1} P (Table 1).

2.4 | P and N analyses of plants and fertilizers

The total N content of non-labeled samples (cattle manure, mature cowpea residues, and cowpea seeds) was measured on ball-milled samples using a CNS analyzer (FlashEA 1112/MAS200 package, Thermo-Finnigan). The total N content and atom% ^{15}N excess of ^{15}N labeled samples (sorghum biomass and labeled cowpea) were measured using a chemical element analyzer (Vario PYRO cube,

Elementar), coupled with a mass spectrometer (IsoPrime100). The ^{15}N atom% excess of the mineral fertilizer N solution was also measured using the same instrument on 50 µl of the liquid sample containing approximately 30 µg of N.

The total P content of all organic materials was determined after incineration of 200 mg of ball-milled samples at 550 °C for 16 h. The resulting ashes were dissolved in 2 ml of fuming HNO_3 (67%), and the volume was made up to 50 ml with distilled water. The P concentration of the extracts was determined by colorimetry with malachite green (Ohno & Zibilske, 1991). The radioactivity of the ^{33}P in the extract of the labeled samples was measured using a liquid scintillation counter (TRI CARB 2500 TR, Packard) after addition of 5 ml of a scintillation liquid (Ultima Gold AB, Packard). The radioactivity was calculated back to the beginning of the experiment to correct for radioactive decay.

2.5 | Measurement of soil respiration

Soil respiration was determined by capturing the CO_2 emitted in 20 ml of 0.2 M NaOH, followed by precipitation with $BaCl_2$ and back-titration with HCl (Alef, 1995). The CO_2 emission was measured at 3, 5, and 10 d after the beginning of the incubation, and then weekly until Day 52.

2.6 | Measurement of soil-available and microbial P

Anion exchange resin-extractable and microbial P were determined at 4, 25, 46 and 52 d after the beginning of the soil incubation by the fumigation extraction method of Kouno et al. (1995), using hexanol as fumigant (Bünemann, Marschner, McNeill, & McLaughlin, 2007). Resin extractable P in non-fumigated samples (P_{resin}) was used as an indicator of available inorganic soil P. Microbial P (P_{mic}) was calculated as the difference between resin extractable P in fumigated samples (P_{fum}) and non-fumigated samples (P_{resin}), after correction for P sorption, exactly as described by Schneider et al. (2017).

The radioactivity of the ^{33}P eluted from the resin membranes of the treatments LY-Cowp and L-1N1P was measured by liquid scintillation as described above for plant extracts. The recovery of ^{33}P in P_{mic} ($^{33}P_{mic}$) was calculated by correcting the radioactivity released during the fumigation by the proportion of ^{33}P recovered ($^{33}P_{rec}$) from a ^{33}P spiked non-fumigated sample as described by Bünemann, Smithson, Jama, Frossard, and Oberson (2004) and Schneider et al. (2017).

2.7 | Measurements of soil microbial C and N and dissolved N

Microbial C (C_{mic}) and microbial N (N_{mic}) were determined after 46 d of incubation using the chloroform fumigation-extraction method of Vance, Brookes, and Jenkinson (1987) with moist soil equivalent to 10 g soil dry matter. Total organic C and N concentrations in the extracts were measured with a Total Organic Carbon and Total Nitrogen analyzer (Formacs SERIES, Skalar). The total dissolved N measured in the non-fumigated samples, composed in unknown proportions of mineral and soluble organic forms of N, was used as a proxy of soil-available N. The microbial C (and N) were calculated as the difference in dissolved C (and N) between fumigated and non-fumigated samples without using conversion factors, because they were not determined on these soils (i.e., as chloroform-labile C and N).

The ^{15}N atom excess in the extracts of fumigated and non-fumigated samples was determined using the oxidation–diffusion method as described in Zemek, Frossard, Scopel, and Oberson (2018). The ^{15}N atom excess in the SMB (% excess$^{15}N_{mic}$) was then calculated using the ^{15}N mass balance as described in Douxchamps et al. (2011).

2.8 | Calculations

Using the direct labeling technique (DLT), the proportions of N and P derived from fertilizer (%P_{dff} or %N_{dff}) were calculated for all compartments (plant uptake, soil microbial N and P, dissolved N, and soil-available P) by dividing the isotopic composition (IC) of the compartment (IC_{comp}) by the IC of the fertilizer (IC_{fert}) (Barraclough, 1995; Morel & Fardeau, 1989a) as expressed in Equation 1, with the IC being the specific activity of P in the case of the %P_{dff} or the ^{15}N atom excess in the case of the %N_{dff}.

Proportion of nutrient derived from fertilizer

$$(\%P_{dff} \text{ or } \%N_{dff}) = \frac{IC_{comp}}{IC_{fert}} \times 100 \quad (1)$$

Using the indirect labeling technique (ILT), the proportions of N and P derived from the fertilizer were calculated following Equation 2 (Barraclough, 1995; Morel & Fardeau, 1989a), with the IC of the compartment receiving the non-labeled fertilizer (IC_{comp}) and the IC of a plant grown in a control treatment without the studied fertilizer (IC_0).

Proportion of nutrient derived from fertilizer

$$(\%P_{dff} \text{ or } \%N_{dff}) = 1 - \frac{IC_{comp}}{IC_0} \times 100 \quad (2)$$

TABLE 2 Sorghum shoot dry matter (DM) yield, N and P concentrations and N and P uptake in sorghum aboveground biomass after application of mature cowpea residues (M-Cowp), young cowpea (Y-Cowp), cattle manure (Manure), non-labeled mineral N and P (1N1P), fertilized controls 1N0P, 0N1P, and non-fertilized control 0N0P in a pot study with a ferric Lixisol

Treatments	Yield	N concentration	P concentration	N uptake	P uptake
	g DM kg^{-1} soil	g kg^{-1} DM		mg kg^{-1} soil	
0N0P	0.19 c[a]	18.5 c	0.62 c	3.5 d	0.11 c
1N0P	0.20 c	35.6 a	0.74 c	7.1 c	0.15 c
0N1P	1.08 b	8.0 d	0.88 bc	8.6 bc	0.94 b
Manure	1.79 b	6.3 d	1.07 ab	11.4 b	1.91 a
M-Cowp	0.13 c	20.4 cd	0.70 c	2.7 d	0.10 c
Y-Cowp	0.14 c	24.0 b	1.12 a	3.3 d	0.15 c
1N1P	2.54 a	20.4 cd	0.82 bc	49.1 a	2.00 a
SEM	0.22	1.4	0.08	1.0	0.09
Significance	***	***	***	***	***

Note. SEM, standard error of the mean; $n = 4$.

[a]In each column, values with different letters are statistically different according to Tukey's HSD test.

***Significant at $p < .001$.

For P, the IC_0 was the specific activity of P in the plants of treatment 1N0P, whereas for N it was the ^{15}N atom excess of the plants of the 0N1P treatment.

Seed N and P are also sources of nutrients that could bias the calculation of the plant N_{dff} and P_{dff} using the ILT by inducing a supplementary dilution of the ^{15}N atom excess and the ^{33}P activity of the plants growing on labeled soil, including the 0N1P and 1N0P treatments used as control. The seed N and P were therefore considered in the calculation by assuming 100% of seed N and P translocated into the shoots (see the Supplemental Material).

For both techniques (DLT and ILT), the amounts of N and P derived from the fertilizers (N_{dff} and P_{dff}) in each compartment were calculated by multiplying the $\%N_{dff}$ and the $\%P_{dff}$, respectively, with the total amount of N and P of the compartment. The recovery of N and P in a compartment was calculated by dividing the N_{dff} and the P_{dff} by the amount of N and P applied with the fertilizer.

2.9 | Statistical analyses

Statistical analyses were performed using the software SPSS Statistics 17.0. Given the completely randomized design of the experiments with four replicates for each treatment, analyses of variance (ANOVA) using the general linear model were performed after verification of the homogeneity of the variances and the normal distribution of the data. The effects of treatments alone on each parameter were tested with one-way ANOVA followed by a post hoc comparison with Tukey's HSD test. For parameters that were measured several times during the soil incubation, the effect of treatments and incubation time (day of incubation) were tested with two-way ANOVA.

3 | RESULTS

3.1 | Sorghum shoot production and N and P uptake

The shoot dry matter yield (DM) varied between 0.1 and 2.5 g kg^{-1} soil (Table 2). The smallest sorghum shoot yield was obtained with the cowpea treatments (M-Cowp, Y-Cowp) and in 0N0P and 1N0P, with no significant difference between them. The mineral N and P fertilizer (1N1P) treatments produced the highest sorghum biomass. Even though N and P concentrations showed a different order of treatments than dry matter yield, N and P uptake both varied over a broad range. The N uptake was greatest with the 1N1P treatment (49.1 mg N kg^{-1} soil), whereas only 3 mg kg^{-1} of N were taken up with cowpea residue and 11 mg kg^{-1} with manure (Table 2). The P uptake in the treatments 1N1P and manure (1.9–2 mg P kg^{-1} soil) was greater than in both cowpea residue treatments (M-Cowp, Y-Cowp) and in 0N0P and 1N0P, for which values ranged between 0.10 and 0.15 mg P kg^{-1} soil (Table 2).

Mineral and organic fertilizers resulted in high proportions of N in the sorghum that were derived from fertilizer ($\%N_{dff}$), which were greater than 50%, except for manure, which contributed 20% of sorghum N (Table 3). However, because of the low N uptake by sorghum, the amount of N derived from fertilizer was less than 2.5 mg kg^{-1} soil for all organic N fertilizers. In contrast, more than 30 mg kg^{-1} soil of fertilizer N was taken up in the 1N1P treatment.

TABLE 3 Nitrogen and P uptake of sorghum shoots from fertilizers (N_{dff}, P_{dff}), soil (N_{dfs}, P_{dfs}), and sorghum N and P recoveries from applied mature cowpea residues (M-Cowp), labeled and non-labeled young cowpea (LY-Cowp and Y-Cowp), cattle manure (Manure), labeled and non-labeled mineral N and P (L-1N1P and 1N1P) in a pot study with a ferric Lixisol

Labeling technique	Treatments	N_{dff}	N_{dff}	N_{dfs}	N recovery	P_{dff}	P_{dff}	P_{dfs}	P recovery
		%	mg kg^{-1} soil		% of added N	%	mg kg^{-1} soil		% of added P
Indirect labeling	0N1P					82.6 a	0.78 c	0.16 b	8.6 c
	1N0P	80.8 a[a]	5.3 c	1.3 c	7.1 c				
	Manure	20.4 c	2.2 cd	8.5 b	2.9 c	89.9 a	1.72 a	0.19 b	9.6 c
	M-Cowp	54.7 b	1.3 d	0.9 c	1.7 c	49.0 b	0.05 d	0.05 b	1.0 d
	Y-Cowp	81.0 a	2.3 cd	0.5 c	3.0 c	54.6 b	0.09 d	0.06 b	1.2 d
	1N1P	78.5 a	38.2 a	10.4 ab	51.0 a	82.2 a	1.63 a	0.36 a	18.1 a
Direct labeling	LY-Cowp	52.3 b	2.7 cd	2.3 c	3.6 c	56.3 b	0.14 d	0.11 b	1.6 d
	L-1N1P	74.a	30.5 b	10.7 a	40.7 b	78.3 a	1.23 b	0.34 a	13.6 b
SEM		4.2	4.2	2.82	0.86	3.8	0.13	0.03	1.2
Significance		***	***	***	***	***	***	***	***

Note. SEM, standard error of the mean; $n = 4$. Results were obtained using the scenario of 100% of seed N and P translocated to the plant.

[a]In each column, values with different letters are statistically different according to Tukey's HSD test.

***Significant at $p < .001$.

Interestingly, this value dropped to about 5 mg N kg^{-1} in the 1N0P treatment, where no P was applied. The highest recovery of the added N was obtained with the 1N1P treatments (more than 40%), whereas for the organic N fertilizers, less than 4% of the added N was recovered in the sorghum shoot biomass because of the low amounts of sorghum N derived from organic fertilizer (Table 3).

Using the ILT, the $\%P_{dff}$ ranged from 49% in mature cowpea to about 90% in manure (Table 3). The P recoveries ranged from 1% of P added with cowpea residues to 18% of P added with water-soluble P in the 1N1P treatment (Table 3). The treatment order of the P recoveries did not follow the order of amount of P derived from fertilizer, because different P doses had been added with the different fertilizers (Table 1).

3.2 | CO_2–C emission during soil incubation

Organic fertilizers significantly increased CO_2–C emissions compared to the mineral 1N1P fertilizer, especially during the first few days of incubation (Figure 1). The highest daily CO_2–C emission was recorded on the third day. All treatments showed a continuous decrease of CO_2–C emissions during the incubation period. A rapid decline in C emission between Day 3 and 5 was especially observed with Y-Cowp. The greatest CO_2–C emission rates were observed for the treatments Y-Cowp and M-Cowp, with 213 and 73 mg C kg^{-1} day^{-1} during the first 3 d of incubation.

3.3 | Soil microbial and resin-extractable P after addition of the fertilizers

Cowpea residues significantly increased microbial P (Figure 2a). The highest values of microbial P (P_{mic}) (>8 mg P kg^{-1} soil) were measured on the fourth day after addition of the labeled and non-labeled young cowpea, followed by the mature cowpea treatment with 4.3 mg kg^{-1} soil of microbial P (Figure 2a, Table 4). Afterward, microbial P decreased in these treatments, but stayed relatively constant between 3.5 and 4 mg P kg^{-1} soil until the end of the incubation. In the control 0N0P treatment, microbial P stayed unchanged between 1.4 and 1.7 mg P kg^{-1} soil over the entire incubation period, and the treatments 1N1P and manure presented similar microbial P values of about 3 mg P kg^{-1} soil on the fourth day and about 2 mg P kg^{-1} soil at the end of the incubation (Figure 2a, Table 4). The labeled young cowpea (LY-Cowp) treatments resulted in higher amounts of microbial P derived from the soil than the L-1N1P treatment. The recovery in the SMB of the P added by LY-Cowp decreased over the incubation time,

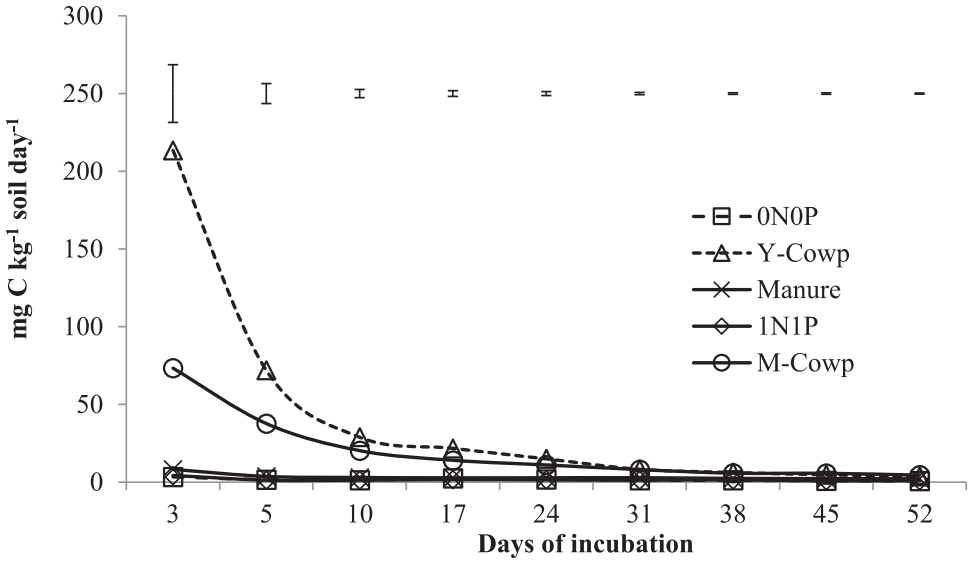

FIGURE 1 Evolution of daily C mineralization during 52 d of soil incubation after addition of young cowpea (Y-Cowp), mature cowpea residues (M-Cowp), cattle manure (Manure), non-labeled mineral fertilizer (1N1P), and a non-fertilized control (0N0P) in a soil incubation study with a ferric Lixisol; $n = 4$. Error bars are standard error of the means. Symbols of a given treatment are connected to facilitate identification of treatments

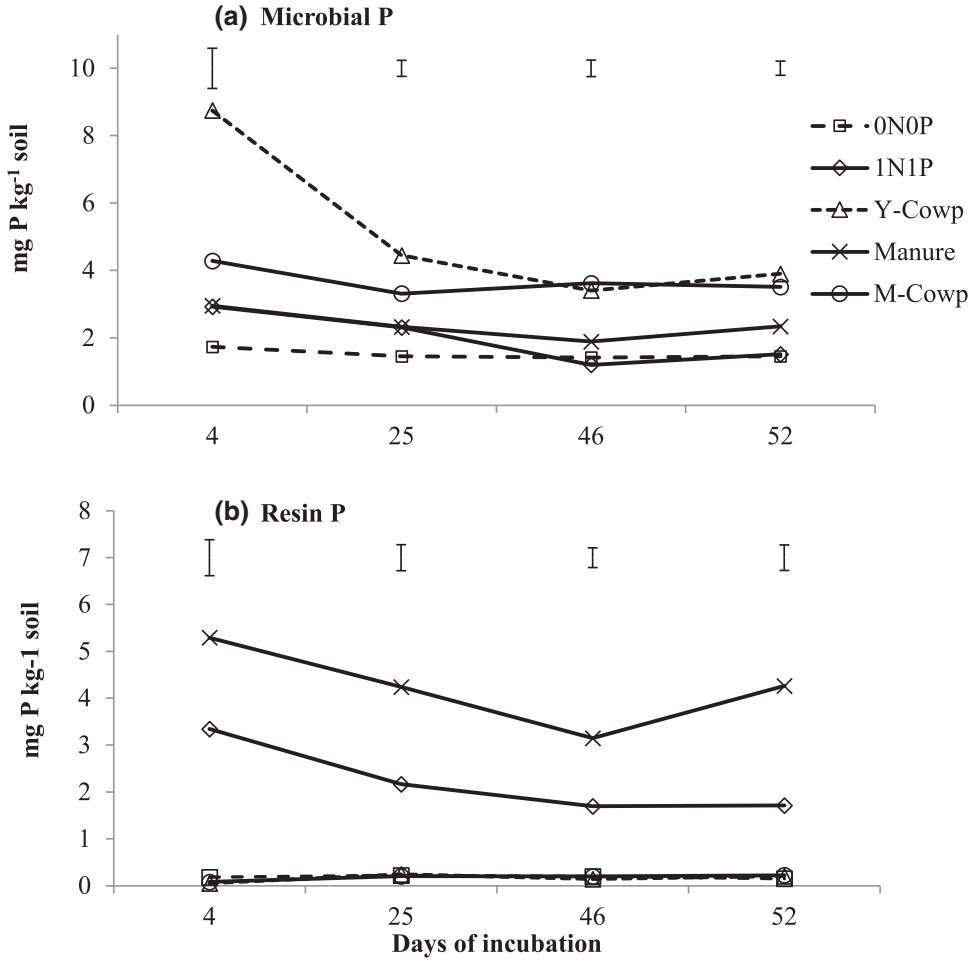

FIGURE 2 Evolution of soil microbial P (a) and resin-extractable P (b) after addition of young and mature cowpea residues (Y-Cowp and M-Cowp), cattle manure (Manure), mineral fertilizer (1N1P), and in the non-fertilized control (CON) in a soil incubation study with a ferric Lixisol; $n = 4$. Error bars are standard error of the means. Symbols of a given treatment are connected to facilitate identification of treatments

TABLE 4 Evolution of microbial P and resin extractable P, of microbial and resin P derived from soil and fertilizer (P_{dff}, P_{dfs}), recovery of added P in microbial P and resin extractable P during 52 d soil incubation after addition of labeled mineral fertilizer (L-1N1P) and labeled young cowpea (LY-Cowp) in a soil incubation study with a ferric Lixisol

Treatment	Day of incubation	Microbial P	Microbial P_{dff}	Microbial P_{dfs}	Resin P	Resin P_{dff}	Resin P_{dfs}	P recovery in microbial P	P recovery in resin P
		mg kg^{-1} soil						% added P	
L-1N1P	4	4.3 b[a]	1.17 bc	3.2 b	2.09 a	1.94 a	0.14 b	13.0 bc	21.5 a
	25	2.6 c	0.91 bcd	1.7 cd	1.91 ab	1.27 b	0.64 a	10.1 bcd	14.0 b
	46	1.6 d	0.58 cd	1.0 d	1.54 b	0.90 c	0.64 a	6.4 cd	9.9 b
	52	1.4 d	0.32 d	1.0 d	1.84 ab	1.19 bc	0.65 a	3.5 d	13.2 b
LY-Cowp	4	11.1 a	7.00 a	4.1 a	0.03 c	0.02 d	0.02 b	77.5 a	0.2 c
	25	3.5 cd	1.65 b	1.8 c	0.21 c	0.14 d	0.07 b	18.2 b	1.5 c
	46	3.8 b	1.60 b	2.2 c	0.28 c	0.15 d	0.13 b	17.6 b	1.7 c
	52	3.6 cd	1.43 b	2.2 c	0.43 c	0.26 d	0.17 b	15.8 b	2.9 c
SEM		0.4	0.24	0.2	0.16	0.12	0.05	2.7	1.3
ANOVA sources of variation	Treatment	***	***	***	***	***	***	***	***
	Day	***	***	***	NS	***	***	***	***
	Treatment × Day	***	***	**	**	***	***	***	***

Note. SEM, standard error of the mean; $n = 4$. NS, nonsignificant.

[a]In each column, values with different letters are statistically different according to Tukey's HSD test.

**Significant at $p < .01$. ***Significant at $p < .001$.

from 7 mg P kg^{-1} soil (75% of the P added) to 1.4 mg P kg^{-1} soil (16% of the P added) (Table 4). The recovery of the fertilizer P in microbial P was much higher for LY-Cowp than for the L-1N1P treatment in which the P recovered in the SMB decreased slowly during the incubation (1.2–0.3 mg P kg^{-1} soil, representing a recovery of 13–3.5% of added P; Table 4).

Manure addition resulted in the highest soil resin P during the incubation, varying from 5.3 mg P kg^{-1} soil on Day 4 to 3 mg P kg^{-1} at the end of the incubation (Figure 2b). A decrease in resin P from 3.3 to 1.7 mg P kg^{-1} was observed in 1N1P. Lowest resin P values were measured in the non-amended control and the cowpea treatments, all with constant values close to 0.2 mg P kg^{-1}. The amount of resin P derived from the soil was higher in the L-1N1P treatment than in YL-Cowp (Table 4). The recovery of the fertilizer P in the resin P pool was higher in L-1N1P than L-Cowp, varying from 1.9 mg P kg^{-1} (21% of the P added) on the fourth day to 1.2 mg P kg^{-1} (13%) at the end of incubation. In contrast, less than 3% of the P added in LY-Cowp was recovered in resin P during the whole incubation period (Table 4).

3.4 | Soil microbial N and dissolved N as affected by the fertilizers

Soil microbial N and dissolved N at Day 46 of the incubation showed the same trends as microbial and resin P (Table 5). Microbial biomass C was greater in soils that had received organic fertilizers than in the mineral 1N1P treatment. Cowpea residues resulted in somewhat greater microbial N values than manure amendment. The direct labeling revealed a greater microbial immobilization of N added with LY-Cowp than with L-1N1P, with 8% of the N added with LY-Cowp recovered in the microbial N pool compared with 2% for L-1N1P. As expected, the mineral treatments 1N1P resulted in the highest contents of dissolved N, with 78 mg N kg^{-1} soil. At the same time, the recovery of the fertilizer N in the dissolved N pool was also much higher for L-1N1P (92%) than for LY-Cowp (12%).

4 | DISCUSSION

4.1 | Effects of organic and mineral fertilizers on soil microorganisms, N, and P dynamics and their supply to sorghum

Phosphorus addition alone in the mineral 0N1P treatment increased sorghum biomass production more than N addition alone in the 1N0P treatment with higher P uptake in 0N1P and similar N uptake between the two treatments (Table 2). This suggests that P was limiting

TABLE 5 Soil dissolved N, and microbial C and N measured 46 d after application of mature cowpea residues (M-Cowp), non-labeled and labeled young cowpea (Y-Cowp and LY-Cowp), cattle manure (Manure), non-labeled and labeled mineral fertilizer (1N1P) and non-amended control (0N0P), microbial and dissolved N derived from labeled fertilizers and soil (N_{dff} and N_{dfs}), and N recovery in microbial and dissolved N in a soil incubation study with a ferric Lixisol

Treatments	Microbial C	Microbial N	Microbial N_{dff}	Microbial N_{dfs}	Dissolved N	Dissolved N_{dff}	Dissolved N_{dfs}	N recovery in microbial N	N recovery in dissolved N
	mg kg^{-1} soil							% added N	
0N0P	45.4 bc[a]	5.1 bc	–	–	19.4 b	–	–	–	–
Manure	68.2 ab	3.5 c	–	–	26.0 b	–	–	–	–
M-Cowp	66.8 ab	6.1 c	–	–	25.1 b	–	–	–	–
Y-Cowp	73.1 a	7.6 b	–	–	21.3 b	–	–	–	–
1N1P	23.2 cd	4.8 bc	–	–	75.8 a	–	–	–	–
LY-Cowp	75.9 a	12.6 a	6.0 a	6.7 a	18.9 b	9.1 b	9.9 a	8.0 b	0.1 b
L-1N1P	14.7 d	4.7 bc	1.1 b	2.6 b	78.1 a	69.3 a	8.8 a	1.9 a	0.9 a
SEM	7.9	1.2	0.3	0.5	2.6	0.6	0.3	0.3	0.01
Significance	***	***	***	**	***	***	NS	***	***

Note. SEM, standard error of the mean; $n = 4$. NS, nonsignificant.

[a]In each column, values with different letters are statistically different according to Tukey's HSD test. Dash indicates that characteristic has not been determined.

**Significant at $p < .01$. ***Significant at $p < .001$.

sorghum growth more than N in the studied Lixisol. The Lixisols from West-central Burkina Faso are known to be strongly depleted in N and P, especially when they have been cropped for a long time without fertilizer application (Lompo et al., 2008; Mando, Bonzi, Wopereis, Lompo, & Stroosnijder, 2005) as it was the case for our soil. Earlier studies have pointed out P as the most growth limiting nutrient for crops production on Sudano–Sahelian soils (Muehlig-Versen, Buerkert, Bationo, & Roemheld, 2003; Sinaj et al., 2001). In a 3-yr mineral N and P fertilization trial on a Sahelian soil with similar properties as our soil, Fofana, Wopereis, Bationo, Breman, and Mando (2008) also found that millet [*Pennisetum glaucum* (L.) R. Br.] response to N fertilization was only obvious when P is applied. Therefore, crop fertilization strategies should first alleviate P limitation on this soil, even if both P and N are limiting for crop production (Buerkert & Hiernaux, 1998). The availability of N and P for crops is also affected by C inputs, which can increase microbial nutrient immobilization and mineralization.

4.2 | Cowpea residues

Addition of young and mature cowpea residues induced high microbial activity as revealed by CO_2–C emission, and microbial growth as indicated by microbial C and P. Moreover, the greater CO_2–C emission observed after addition of young compared with mature cowpea residues especially in the first days of incubation (Figure 1), confirms that the organic compounds contained in the mature cowpea residues are less degradable by the SMB than those in the young cowpea residues. Similar observations were made by Ha, Marschner, Bünemann, and Smernik (2007) in a study with pea residue. The increase in microbial activity after addition of cowpea residues goes along with nutrient immobilization in these treatments.

After 4 d of incubation, microbial P was greater in soils amended with young and mature cowpea residues by factors 5 and 2.5, respectively, than in the non-amended control 0N0P (Figure 2a). The labeled young cowpea treatment also revealed that the amount of microbial P derived from soil (2–4 mg P kg^{-1} soil) was higher than the resin extractable P in the non-amended control (Table 4, Figure 2b). This suggests that microorganisms took up P from soil P pools other than resin-extractable P, an observation that is in agreement with the results of Bünemann, Steinebrunner, Smithson, Frossard, and Oberson (2004), which highlighted that P from NaOH extractable soil inorganic P pool could have buffered microbial P uptake after the addition of Crotalaria residues to a Kenyan Ferralsol. The rapid decline in CO_2–C emission between Day 3 and 5 for Y-Cowp could be explained by the reduction of easily available C compounds contained in the cowpea residues.

Nitrogen immobilization in the SMB was still observed in the cowpea amended treatments after 46 d of incubation, when levels of microbial C and N were higher in soils of these treatments than in the non-amended control or the 1N1P soils (Table 5). The LY-Cowp treatment revealed that both cowpea N and soil N contributed similarly to this microbial N after 46 d of incubation. However, the contribution of cowpea N was probably greater during the first few days of incubation according to the dynamics of the SMB as suggested by the evolution of microbial P (Table 4) and daily respiration rates (Figure 1). Because of the microbial N and P immobilization after addition of cowpea residues, plant-available P was reduced to the level of the 0N0P treatment (Figure 2b). For dissolved N, there was no significant reduction of cowpea amended treatments compared with 0N0P (Table 5), but dissolved N is composed of mineral and organic N, and it does not entirely reflect the plant-available N. Nevertheless, microbial nutrient immobilization following application of cowpea residues led to a low sorghum biomass production and low N and P uptake from soil and fertilizers (Tables 2, 3, and 4).

The grinding of the cowpea plant materials facilitated their incorporation to the soil and was practical for the laboratory work. However, this probably intensified the measured processes by increasing the contact between the soil and plant material. In the field, surface application or incorporation into the soil of coarse plant material would probably result in different results.

4.3 | Cattle manure

Contrary to the cowpea residues, the cattle manure used in this study had little impact on the soil microbial activity and biomass, with a very low CO_2–C release (Figure 1). The microbial C of manure-amended soil on Day 46 was not significantly greater than in the 0N0P treatment (Table 5). The insignificant impact of manure on the SMB is also illustrated by the similar microbial P associated with the mineral 1N1P treatment. Indeed, similar amounts of resin extractable P were added with this manure as with the 1N1P mineral fertilizer (Table 1), while the manure brought a weakly degradable form (as deduced from the C emissions) of C which did not induce microbial immobilization of P (Figure 2a). Therefore, similar proportions of sorghum P were derived from manure as from the mineral 1N1P and 0N1P treatments (Table 3).

This manure hardly affected soil N dynamics and N supply to the sorghum plants (Table 3, 5). Fresh cow manures can have C/N ratios ranging from 20 to 30 (Alwaneen,

2016; Tripetchkul, Pundee, Koonsrisuk, & Akeprathumchai, 2012), whereas the manure used in our study had a C/N ratio of 12. A decrease in C/N ratio is usually observed during storage or composting (Ko, Kim, Kim, Kim, & Umeda, 2008) and coupled with the loss of the labile N compounds without adequate storage and management options (Rufino, Rowe, Delve, & Giller, 2006). The manure used in our study was sampled from a cowshed in 2010 where it had been exposed to drying off because of weather conditions. Although this corresponds to the usual practice of farmers in the area around the Saria Station, labile N compounds have probably been lost before collection of the manure. It was then air-dried and stored for almost 3 yr before being used in this experiment. However, our results remain close to on-farm conditions where manure is applied in the form of dried powder that has been stored during several months of dry season.

4.4 | Mineral fertilizer

The application of mineral 1N1P fertilizers significantly increased resin extractable P and dissolved N contents compared with the control 0N0P and the Y-Cowp and M-Cowp treatments (Figure 2b, Table 5). There was a clear response of dissolved N to mineral N addition, suggesting that it can to some degree be used as an indicator for soil-available N. The 1N1P treatments also increased microbial P without affecting microbial activity. This is in accordance with Frossard et al. (2016), who found that microorganisms in a similar nutrient depleted soil from a field experiment in the same region are highly dependent on nutrient inputs. However, microbial P in the treatment mineral 1N1P was significantly lower than after addition of labeled young cowpea residues, which brought similar amounts of N and P to the soil, but combined with C. This agrees with Traoré et al. (2016), who found in a ferric Acrisol of similar properties that SMB growth is P limited only after addition of easily available C, which is the first nutrient limiting the SMB. The lack of C input also explains the absent effect of mineral 1N1P addition on soil respiration compared with the cowpea amended treatments (Figure 1).

The increase in soil resin P not only resulted from the effect of the added mineral P but was also due to a supplementary contribution of soil P reserves, as revealed using ^{33}P-labeled mineral fertilizer. The amount of resin P derived from the soil after application of L-1N1P (about 0.6 mg P kg^{-1} soil) was greater than the resin P of the non-amended soil (about 0.2 mg kg^{-1}) (Figure 2b, Table 4). This shows that mineral P addition resulted in the release of soil P, which also explains the higher amount of sorghum P derived from soil after addition of mineral P in 1N1P and to a lesser extent in 0N1P (Table 3). The improved P nutrition also enhanced plant uptake of soil N as shown by the higher N uptake in 0N1P than in the 0N0P control (Table 2). This was probably due to a better development of the root system of the plants allowing a greater exploration and uptake of soil N in 0N1P than in 0N0P. Consequently, for the plants, the mineral 1N1P fertilizers resulted in the highest shoot biomass, N and P uptake from the soil and from the fertilizers, and the highest fertilizer N and P recoveries. On the field level, the increased use of soil N and P reserves after application of water-soluble mineral fertilizers would require nutrient budget approaches to prevent nutrient depletion and ensure the sustainability of cropping systems.

4.5 | N/P ratios of soil microbial biomass and plants in response to mineral and organic fertilizers

Soil microbial N/P ratios are useful to study N or P limitations and the balance between N and P supply from fertilizers to the SMB (Griffiths, Spilles, & Bonkowski, 2012). After 46 d, the N/P ratios of the SMB in the mineral 1N1P treatment (between 7.7 and 6.9, Table 6) were close to the N/P ratio of the mineral fertilizer, despite similar microbial biomass as in the non-amended 0N0P treatment. The SMB took up N and P to meet its internal demand at the level of its biomass which is firstly limited by C. This agrees with Frossard et al. (2016), who found that N/P ratios in the SMB were strongly and positively correlated with the N/P ratios of the nutrient inputs in different fertilizer treatments of a long-term trial on a ferric Acrisol from the same location as the studied soil. This also suggests N and P limitations for the SMB in this soil (Traoré et al., 2016). The microbial N/P ratio was higher in 1N1P than 0N0P despite similar microbial biomass indicating that in the absence of biomass growth, soil microorganisms were limited by N and took up preferentially more N than P from the added mineral fertilizer. Application of the different organic amendments resulted in N/P ratios in SMB that are 2.3–8.6-fold lower than the N/P ratios of the corresponding organic amendments. This suggests that the SMB took up more P than N from the organic amendments, and that P is the first limiting nutrient when C limitation is alleviated. The low N/P ratio of SMB in the manure treatment is likely due to the high availability and uptake of P compared with N added by this manure. This also reflects a plasticity in the microbial N/P ratios depending on the availability of the nutrients added by fertilizers and organic inputs in nutrient-depleted soils (Frossard et al., 2016). The N/P ratios of the SMB depend on the measured values of microbial N and P. These values were not corrected for incomplete recovery from the soil during

TABLE 6 N/P ratios harvested in sorghum plants: N/P of total N and P uptake, and of N and P derived from fertilizers (mg N_{dff}/mg P_{dff}) and from soil (N_{dfs}/P_{dfs}) and N/P ratios of soil microorganisms in a pot study and soil incubation study with a ferric Lixisol. For the N/P ratio of the fertilizers, see Table 1

Treatments	Plant internal N/P ratios according to sources			N/P ratios of soil microorganisms at the 46th of incubation
	N/P	N_{dff}/P_{dff}	N_{dfs}/P_{dfs}	
0N0P	30.2 b[a]	–	–	3.6 b
1N0P	48.6 a	–	–	–
0N1P	9.1 e	–	–	–
Manure	5.8 e	1.3 c	46.7 a	1.9 cd
M-Cowp	28.7 bc	25.5 ab	24.4 c	1.7 d
Y-Cowp	21.5 cd	28.5 a	23.3 c	2.4 bcd
LY-Cowp	21.3 d	18.1 b	9.3 d	3.4 bc
1N1P	24.8 bcd	23.5 ab	30.9 b	6.9 a
L-1N1P	26.5 bcd	24.7 ab	31.9 b	7.7 a
SEM	2.1	3.0	2.6	0.50
Significance	***	***	***	***

Note. SEM, standard error of the mean; $n = 4$. 0N0P, non-fertilized control; 1N0P, mineral N fertilizer alone; 0N1P, mineral P fertilizer alone; Manure, cattle manure; LY-Cowp, labeled young cowpea; Y-Cowp, non-labeled young cowpea, M-Cowp, mature cowpea; L-1N1P, labeled mineral N and P fertilizer; 1N1P, non-labeled mineral N and P fertilizer.

[a]In each column, values with different letters are statistically different according to Tukey's HSD test. Dash indicates that characteristic has not been determined (for N/P ratios) or that characteristics (N_{dff}/P_{dff}, N_{dfs}/P_{dfs} ratios) are not applicable.

***Significant at $p < .001$.

extraction, because the conversion factors were not determined for the studied soil. This could have introduced a bias in the calculation of the microbial N/P ratios if K-factors would differed for P and N. Therefore, the N/P ratios measured in the SMB give an indication on the changes in microbial nutrient demand resulting from the application of the different fertilizers but should not be taken as absolute values.

Low plant N/P ratios indicate N limitation for plants and P uptake contributes more to the variation of plant N/P ratio than N uptake (Güsewell, 2004; Sadras, 2006). The full supply in N and P by the mineral 1N1P treatments resulted in a similar plant N/P ratio as in 0N0P (Table 6), indicating that in 0N0P, N, and P uptake by plants was balanced but limited by the availability of these nutrients in the soil. This is also supported with a plant N_{dfs}/P_{dfs} in 1N1P close to the plant N/P ratio in 0N0P. The very low plant N/P ratio in the manure treatment is due to its more significant P than N fertilizer effect. The manure treatment resulted in a clear N limitation of the sorghum plants as shown by the high N_{dfs}/P_{dfs} in reaction to the low N supply from manure. Although the amounts of N and P released by the cowpea amendments and taken up by the plants were very low, the N/P ratios in sorghum derived from cowpea residues were similar to those of the mineral 1N1P treatments (Table 6). This suggests that the nutrient release in these treatments was quite balanced but small due to microbial immobilization.

4.6 | Methodological considerations

The isotopic labeling techniques were required in this study for an accurate estimation of N and P uptake from the tested fertilizers. The difference method cannot identify the proportion of N or P in the plant derived from fertilizer if there is no or little increase of N or P uptake in a fertilized treatment over the zero control, as it was the case with the cowpea treatments in the pot experiment. However, the sorghum $\%N_{dff}$ measured in the young cowpea treatment using ILT was significantly higher than that measured by DLT. This might be due to the pool substitution described by Hood, N'goran, Aigner, and Hardarson (1999). In this process, the immobilization-mineralization induced by the application of the non-labeled young cowpea on the labeled soil resulted in the release of plant-available N from non-labeled N pools. This led to an isotopic dilution in addition to the N derived from the non-labeled cowpea. Indeed, soil amendment with young cowpea residues resulted in the greatest increase in soil respiration and SMB growth, which may as well have involved mineralization of soil organic matter. Since we have no DLT for the mature cowpea treatment, we cannot derive whether M-Cowp induced such a pool substitution. Because it induced a less marked response in microbial activity, we assume that it was less pronounced than with young cowpea. Still, the high percentage of sorghum N derived from young cowpea and to a lesser extent from the

mature cowpea residues was most probably overestimated. However, we assume that the N derived from manure was less overestimated because soil microbial activity (respiration) and biomass were weakly affected, suggesting little mineralization of non–^{15}N labeled soil organic matter.

6 | CONCLUSIONS

The use of the double ^{15}N and ^{33}P labeling techniques in a pot experiment and soil incubation allowed the simultaneous assessment of plant uptake of N and P added by organic and mineral fertilizers and their fate in the soil. The ratios of N and P derived from fertilizers indicated that the tested manure acted mainly as a source of P, with similar P uptake and plant N/P ratios as the treatment 0N1P. The delivery of added N and P was balanced in the mineral 1N1P treatment and resulted in higher sorghum biomass production and N and P uptake from soil reserves. The use of isotope techniques further showed that the cowpea residues enhanced microbial uptake of P and N from the soil as a consequence of the increased microbial biomass and activity. In turn, sorghum biomass and N and P uptake in soil amended with cowpea residues were similar to the non-fertilized control treatment. Further investigations are needed to understand the residual effects of legume residues applied as green manure to this Lixisol and to determine the proper rate, form and period of application of these residues in order to synchronize the release of nutrients immobilized in the SMB with the demand of the plants.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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