

# The plant availability of phosphorus from thermo-chemically treated sewage sludge ashes as studied by $^{33}\text{P}$ labeling techniques

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## Abstract

**Aims** Phosphorus resources have to be managed sustainably and therefore the recycling of P from waste streams is essential. A thermo-chemical recycling process has been developed to produce a P fertilizer from sewage sludge ash (SSA) but its plant availability is unknown.

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**Methods** Two SSA products prepared with either  $\text{CaCl}_2$  (SSACa) or  $\text{MgCl}_2$  (SSAMg) as chemical reactant during the thermal treatment were mixed with three soils previously labeled with  $^{33}\text{P}$ . Reference treatments with water-soluble P added at equal amounts of total P were included. The transfer of P from SSACa and SSAMg to *Lolium multiflorum* or P pools of sequentially extracted soil-fertilizer incubations were quantified.

**Results** The shoot P uptake from SSAMg was higher than from SSACa. For SSAMg the relative effectiveness compared to a water-soluble P fertilizer was 88 % on an acidic and 71 % on a neutral soil but only 4 % on an alkaline soil. The proportion of P derived from the fertilizer in the plant and in the first two extraction pools of soil-fertilizer incubations were strongly correlated, suggesting that it is sufficient to conduct an incubation study to obtain robust information on plant P availability.

**Conclusions** We conclude that under acidic to neutral conditions SSAMg presents an appropriate alternative to conventional P fertilizers and the dissolution of P from SSAMg seems to be governed by protons and cations in the soil solution.

**Keywords** Sewage sludge ash ·  $^{33}\text{P}$  labeling · Recycling fertilizer · Radioisotopes · Italian ryegrass · Sequential extraction

## Abbreviations

SSA	Sewage sludge ash
SA	Specific activity
Pdf seed	P derived from the seed

Pdf fertilizer	P derived from the fertilizer
Pdf soil	P derived from the soil
Pupt	Shoot P uptake
FertPRec	Fertilizer P recovery

## Introduction

In the past years the awareness on P scarcity has increased (Cordell et al. 2009). Research on sustainable agroecosystem management (Frossard et al. 2009; Richardson et al. 2011; Tiessen et al. 2011), waste management (Lamprecht et al. 2011; Mihelcic et al. 2011) as well as on resource management policy (Schipanski and Bennett 2012) has addressed the question of how to efficiently use and reuse P in an agricultural and urban context. Phosphorus balances on a global (Smit et al. 2009) and regional level (Ott and Rechberger 2012) as well as material flow analysis (Lederer and Rechberger 2010) have revealed a large potential for P recycling from waste water. However, the direct reuse of sewage sludge in agriculture has been associated with risks such as soil contamination with organic pollutants (Bright and Healey 2003), pathogens (Gerba and Smith 2005) and heavy metals (McBride 1995). It has therefore disappeared in a number of European countries (Renner 2000). In Switzerland, sewage sludge application has been banned in 2006 and sewage sludge is incinerated ever since. The combustion ashes are landfilled, which presents a yearly net loss of 8'000 t P (Lamprecht et al. 2011). Several technologies have been developed to recycle P from sewage sludge or sewage sludge combustion residues (Sartorius et al. 2012). Products obtained from such a newly developed process combining thermal treatment and chlorination to volatilize and separate heavy metals from sewage sludge ash (SSA) are studied here. Gaseous heavy metal-chloride complexes are separated with the off-gas stream (Mattenberger et al. 2010), with at least 90 % of cadmium, copper, lead and zinc being removed (Vogel and Adam 2011; Fraissler et al. 2009). The remaining inorganic P rich residue can potentially be used as a fertilizer. The P extractability and P speciation of thermo-chemically treated SSA have been studied by Kratz et al. (2010) and Vogel et al. (2011). They came to the conclusion that thermo-chemically treated SSA, depending on the chloride-donor ( $\text{CaCl}_2$ ,  $\text{MgCl}_2$ ) used, contain tribasic Ca- or Mg phosphates and P therein is highly soluble in formic and citric acid (65 % or more)

but rather insoluble in water (less than 1 %). The plant P availability of thermo-chemically treated SSA on the other hand remains largely unknown.

The amount of P taken up by a plant from a fertilizer added to the soil can be determined by means of isotopic dilution principles using radioisotopes of P (Frossard et al. 2011). If a homogenous labeling of fertilizer P is feasible, a direct labeling approach is chosen. Phosphorus derived from the fertilizer is assessed by comparing the specific activity (SA, ratio between the radioisotope and the stable isotope, e.g.  $^{33}\text{P}/^{31}\text{P}$ ) in the plant to the SA of the previously labeled fertilizer (Gallet et al. 2003; Morel and Fardeau 1989a). For complex fertilizers containing multiple P species, an indirect labeling approach is required where plant available soil P is labeled. The indirect labeling is based on the assumptions that the plant available soil P can be labeled homogeneously and that any dilution of the specific activity arises from the unlabeled P fertilizer. Hence P derived from the fertilizer is assessed by comparison of the SA in the fertilized plant to the SA of a unfertilized plant grown on the same soil (Fardeau et al. 1996). Indirect labeling with  $^{32}\text{P}$  or  $^{33}\text{P}$  has been used to investigate the plant P availability of rock phosphate (Zapata and Axmann 1995), sewage sludge (Frossard et al. 1996), compost (Sinaj et al. 2002) and animal manure (Oberson et al. 2010). An obstacle when using indirect labeling to trace fertilizer P into plants is the contribution of unlabeled seed P to the P uptake of the plant, which must be carefully accounted for (Brookes 1982; Pypers et al. 2006).

Sequential chemical extractions operationally describe P forms based on a extraction procedure. They have been used to investigate the transformation of fertilizer P within soils and to understand its availability to plants (Condrón and Goh 1989). An often used procedure is the one developed by Hedley et al. (1982) and adapted by Tiessen and Moir (2007). It has also been combined with direct labeling with  $^{33}\text{P}$  or  $^{32}\text{P}$  to e.g., assess the effect of fertilizer inputs or cropping and tillage systems on the P dynamics in soils (Bühler et al. 2002; Bünenmann et al. 2004; Daroub et al. 2000). To our knowledge, it has not yet been applied along with indirect labeling to study the fate of fertilizer P in sequential extraction pools of soil-fertilizer mixtures.

The aim of this study was to assess the plant P availability of two thermo-chemically treated SSA on three soils with a gradient in pH and available soil P. A growth trial with Italian ryegrass (*Lolium multiflorum*) was conducted to quantify the amount of P taken up

from two differently produced SSA while properly addressing the question of seed P translocation to the shoot. In addition, a soil-fertilizer incubation experiment was conducted to study the fate of P from thermo-chemically treated SSA as affected by the different soil chemical properties. To address these objectives  $^{33}\text{P}$  labeling techniques were applied and the P availability of thermo-chemically treated SSA was evaluated relative to a water-soluble P source.

## Material and methods

### Production and description of thermo-chemically treated sewage sludge ash and description of soils

Sewage sludge ash was collected from the electro-filter of a Swiss sewage sludge mono-incineration plant near Lucerne, Switzerland, in a catchment area with communal, iron precipitated sewage sludge. SSA was mixed with technical grade  $\text{CaCl}_2$  or  $\text{MgCl}_2$  at rate of  $150 \text{ g Cl}_2 \text{ kg}^{-1}$  SSA. The SSA-chloride mixture was pelletized by tumble growth aggregation and subsequently loaded to a directly heated rotary furnace. Heavy metal removal from SSA was achieved by thermal treatment at  $950 \text{ }^\circ\text{C}$  with a mean residence time of 30 min. For a detailed description of the experimental set up we refer to Mattenberger et al. (2008). The furnace was operated in a batch mode treating  $9 \text{ kg}$  SSA-chloride mixture at a time. Thermo-chemically treated SSA was discharged from the furnace, cooled down at ambient temperature and milled to a mean particle size  $<100 \text{ }\mu\text{m}$ . The SSA fertilizer products are denoted according to the chlor donor used for the thermo-chemical treatment (SSACa or SSAMg). Table 1 shows the elemental

composition and physical characteristics of SSACa and SSAMg.

Three soils with a gradient in pH and available and total P were chosen (Table 2). The “neutral moderate P” soil was a Mollic Fluvisol (FAO classification) from Heitenried, Switzerland, and the “alkaline moderate P” soil was a Calcaric Cambisol (FAO classification) from Gütighausen, Switzerland. Both moderate P soils were under long-term arable land use. The “acidic low P” soil was collected under an ash-alder (*Fraxinus excelsior*-*Alnus glutinosa*) forest in Lindau, Switzerland. Soil samples were taken at a depth of 0–20 cm, sieved at 5 mm and air-dried. Two weeks prior to the start of the experiments, the soils were irrigated to a water holding capacity (WHC) of 50 % and pre-incubated at  $18 \text{ }^\circ\text{C}$ .

### Growth trial: phosphorus uptake by plants from thermo-chemically treated sewage sludge ash

To study the shoot P uptake from thermo-chemically treated SSA by plants, a pot experiment with the following fertilizer treatments was conducted: No P fertilization (NoP), SSACa, SSAMg and water-soluble P (WSP,  $\text{KH}_2\text{PO}_4$  in aqueous solution). For all treatments the shoot P uptake by plants was assessed using indirect labeling with  $^{33}\text{P}$ . For the purpose of methodological control, WSP was additionally studied using direct labeling (WSPdir).

For the indirect labeling, aliquots of  $700 \text{ g}$  of soil dry matter (DM) were carefully mixed with carrier free  $^{33}\text{P}$ -orthophosphate using  $1.4 \text{ MBq kg}^{-1}$  soil. The soil was transferred into sealed pots and kept at  $18 \text{ }^\circ\text{C}$  for 7 days to reach near-equilibrium conditions for  $^{31}\text{P}$  and  $^{33}\text{P}$  in the soil. Pots containing the same amount of unlabeled soil for WSPdir were prepared and kept under the same conditions. The soils were then mixed with the respective fertilizers at a rate of  $50 \text{ mg P kg}^{-1}$  soil and filled

**Table 1** Composition and physical characteristics of thermo-chemically treated sewage sludge ash (SSA). SSA thermo-chemically treated with  $\text{CaCl}_2$  (SSACa) or  $\text{MgCl}_2$  (SSAMg)

SSA	Si <sup>a</sup> ( $\text{g kg}^{-1}$ )	Al <sup>a</sup> ( $\text{g kg}^{-1}$ )	Fe <sup>a</sup> ( $\text{g kg}^{-1}$ )	Mg <sup>a</sup> ( $\text{g kg}^{-1}$ )	Ca <sup>a</sup> ( $\text{g kg}^{-1}$ )	K <sup>a</sup> ( $\text{g kg}^{-1}$ )	P <sup>a</sup> ( $\text{g kg}^{-1}$ )	Crystallinity <sup>b</sup> (%)	Specific surface <sup>c</sup> ( $\text{m}^2 \text{ g}^{-1}$ )
SSACa	122	47	145	9.3	162	5.0	59	53	2.0
SSAMg	130	49	153	54.3	98	15.0	62	44	2.2

<sup>a</sup> XRF measurement after  $\text{Li}_2\text{B}_4\text{O}_7/\text{LiBO}_2$  fusion (PANalytical Axios Advanced)

<sup>b</sup> Determined by Rietveld refinement of XRD spectra (X’Pert PRO PANalytical diffractometer)

<sup>c</sup> BET measurement (Coulter SA3100)

**Table 2** Selected characteristics of the three soils used in the growth trial with Italian ryegrass and the incubation experiment

Soil characteristic	Soil		
	“Acidic low P”	“Neutral moderate P”	“Alkaline moderate P”
pH <sup>a</sup>	4.5	6.5	8.2
CEC (cmol <sup>+</sup> kg <sup>-1</sup> ) <sup>b</sup>	28	21	46
Base saturation (%) <sup>b</sup>	37	64	100
E <sub>1min</sub> (mg P kg <sup>-1</sup> ) <sup>c</sup>	2.1	8.2	13.1
C <sub>p</sub> (mg P l <sup>-1</sup> ) <sup>c</sup>	0.03	0.43	0.39
r1/R <sup>c</sup>	0.15	0.53	0.30
Inorganic P (mg P kg <sup>-1</sup> ) <sup>d</sup>	119	360	752
Organic P (mg P kg <sup>-1</sup> ) <sup>d</sup>	187	362	374
Clay (g kg <sup>-1</sup> ) <sup>e</sup>	226	160	289
Silt (g kg <sup>-1</sup> ) <sup>e</sup>	417	256	446
Sand (g kg <sup>-1</sup> ) <sup>e</sup>	357	584	265
OM (g kg <sup>-1</sup> ) <sup>f</sup>	50	23	27
Total C (g kg <sup>-1</sup> ) <sup>g</sup>	31	14	36
Total N (g kg <sup>-1</sup> ) <sup>g</sup>	2.0	1.3	2.2
WHC <sub>max</sub> (g H <sub>2</sub> O kg <sup>-1</sup> ) <sup>h</sup>	730	493	623

<sup>a</sup> pH in H<sub>2</sub>O (Forschungsanstalten Agroscope 2004)

<sup>b</sup> Exchangeable with BaCl<sub>2</sub> (Forschungsanstalten Agroscope 2004)

<sup>c</sup> P exchangeable within 1 min (E<sub>1min</sub>), concentration of water extractable P (C<sub>p</sub>) and proportion of radioactivity remaining in the soil solution after one minute of exchange (r1/R) determined by isotopic exchange kinetics (Fardeau et al. 1996), P availability limits plant growth when E<sub>1min</sub> is lower than 5 mg P kg<sup>-1</sup> (Gallet et al. 2003a)

<sup>d</sup> Inorganic and organic P obtained using the ignition/extraction method of Saunders and Williams (1955)

<sup>e</sup> Gravimetric measurement (Forschungsanstalten Agroscope 2004)

<sup>f</sup> Organic matter, oxidation with K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> (Forschungsanstalten Agroscope 2004)

<sup>g</sup> CN elemental analysis (Thermo-Scientific FlashEA 1112)

<sup>h</sup> Water holding capacity (WHC), maximal soil saturation with H<sub>2</sub>O without external pressure

back into pots. The SA of WSPdir was 45 Bq μg P<sup>-1</sup>. Each fertilizer treatment was replicated three times. Directly after the fertilization, 2 g of Italian ryegrass seeds (*Lolium multiflorum* var. Gemini) were sown and pots were placed in the greenhouse. Growing conditions were set to 14-hours photoperiod (light intensity of maximum ≈500 μmol m<sup>-2</sup> s<sup>-1</sup> artificial light, metal halide lamps with blue-orientated light), 65 %

atmospheric humidity and mean temperatures of 22 °C during day and 14 °C during night. Pots were watered daily to 60 % WHC and randomized twice a week. Three days after germination and following each cut, plants were given a P free nutrient solution (320 mg N, 280 mg K, 100 mg Ca, 100 mg Mg, 100 mg S kg<sup>-1</sup> soil and adequate amounts of Fe, B, Mn, Cu, Mo and Zn). The Italian ryegrass was cut 33, 61 and 97 days after sowing, the plant material was dried at 45 °C for 48 h and the above ground DM yield was recorded. The dried plant material was milled (sieve <2 mm) and a subsample of 400 mg was ashed at 550 °C for 6 h and extracted with 3 ml of hot 14.4M HNO<sub>3</sub>. Phosphorus in the diluted filtrate (0.2 μm pore size) was determined colorimetrically (Ohno and Zibilske 1991). <sup>33</sup>Phosphorus beta-emission in the labeling solutions and the plant extracts was measured by liquid scintillation counting (TRI-CARB 2500 TR, liquid scintillation analyzer, Packard Instruments, Meriden, CT). The measurements were checked for quenching effects due to matrix effects and the values were corrected for radioactive decay by means of the half-life time of the radionuclide. Internal standards of defined radioactivity were included regularly and for each run of the instrument.

Growth trial: estimation of seed phosphorus contribution

In an additional pot experiment the contribution of seed P to the plant shoot P uptake was estimated. The chosen approach was first described by Brookes (1982) and adapted by Pypers et al. (2006). Italian ryegrass (*Lolium multiflorum*, var. Gemini) was grown in a P-free medium receiving different rates of WSP labeled with <sup>33</sup>P in order to alter the plants physiological dependence on the P in the seed. Sealed pots containing 1 kg of washed coarse sand (0.8–1.2 mm particle diameter) were amended with a solution containing carrier free <sup>33</sup>P-orthophosphate and KH<sub>2</sub>PO<sub>4</sub> at a rate of 1.6, 3.1, 6.3, 12.5 and 25 mg P kg<sup>-1</sup> sand and with a specific activity of 130, 65, 33, 16 and 8 Bq μg<sup>-1</sup>P. Treatments were replicated three times per fertilization rate. Then Italian ryegrass was sown at a density of 2 g seeds per pot. The growing conditions, the fertilization with P-free nutrient solution and the handling of plants were identical to the above-described growth trial except for the watering regime. Pots were watered daily to 200 g H<sub>2</sub>O kg<sup>-1</sup> sand. Plants were harvested and plant samples were prepared and analyzed as described above.

### Soil-fertilizer incubation study: fate of phosphorus from thermo-chemically treated sewage sludge ash in the soil

In order to investigate the effect of soil chemical properties on the dissolution and distribution of thermo-chemically treated SSA in the soil, SSACa and SSAMg as well as soil-fertilizer mixtures were sequentially extracted. Soil-fertilizer incubations were sampled at 1, 8, 15, 49 and 91 days after incubation. The soil-fertilizer incubation study included the following fertilizer treatments: NoP, SSACa, SSAMg, WSP (KH<sub>2</sub>PO<sub>4</sub> in aqueous solution) and WSPdir. For the indirect labeling, an aliquot of 25 g of soil DM was labeled with 12.3 MBq kg<sup>-1</sup> soil of carrier free <sup>33</sup>P-orthophosphate and placed in a plastic bag. Unlabeled soil portions for the WSPdir treatment were prepared accordingly. Seven days later the fertilizers were mixed into the soil at a rate of 100 mg P kg<sup>-1</sup> soil. For WSPdir, P was labeled with a specific activity of 49.7 Bq μg<sup>-1</sup> P. The two times higher P application rate than in the growth trial was chosen in order to accurately quantify the recovery of P added with the fertilizer despite the high total P content of the two moderate P soils. The plastic bags were sealed and kept in the dark at 20 °C. Three replicates per fertilizer treatment and sampling time were prepared.

At the day of the sampling an aliquot of 2 g of dry soil was weighed into a 50 ml centrifuge tube and a sequential chemical extraction adapted from Tiessen and Moir (2007) was performed. For the first step two anion-exchange membranes were used (BDH 55164 2S, 2 cm×6 cm, HCO<sub>3</sub><sup>-</sup> form, elution with 0.5 M HCl). The following steps consisted of extractions with 0.5 M NaHCO<sub>3</sub> (step 2), 0.1 M NaOH (step 3) and 1 M HCl (step 4). Each extraction lasted 16 h whereby samples were shaken horizontally at 160 rpm. After each step samples were centrifuged at 6870×g and the supernatant was filtered (0.2 μm pore size). After step 4 the residual soil-fertilizer sample was vacuum dried at 50 °C and a subsample of 0.2 g was transferred into a glass vial for a total digestion with 30 % H<sub>2</sub>O<sub>2</sub>, Se, Li<sub>2</sub>SO<sub>4</sub> and concentrated H<sub>2</sub>SO<sub>4</sub> at 360 °C for 170 min on a heating block (Parkinson and Allen 1975). The same sequential procedure was applied to SSACa and SSAMg but

starting the procedure with 50 mg of substrate only and transferring the complete residue to glass vials for the last step.

Inorganic P and total P in the extracts were determined colorimetrically by flow analysis (Skalar San++ System) according to ISO 15681-2:2003 using an integrated UV digestion and hydrolysis procedure. Organic P was calculated by the difference between total and inorganic P. We present only total P since the organic P was not affected by the fertilizer treatments and since for the counting of <sup>33</sup>P beta-emission no distinction between inorganic and organic P was undertaken. Total <sup>33</sup>P beta-emission was measured as described above.

Calculations for the indirect and direct labeling with <sup>33</sup>P

In the trial with Italian ryegrass grown on soil, the total P taken up by plant shoots (Pupt<sub>plant</sub>, mg P kg<sup>-1</sup> soil) is the sum of P derived from the seed (Pdf seed, mg P kg<sup>-1</sup> soil), the soil (Pdf soil, mg P kg<sup>-1</sup> soil) and the fertilizer (Pdf fertilizer, mg P kg<sup>-1</sup> soil):

$$\text{Pupt}_{\text{plant}} = \text{Pdf seed} + \text{Pdf soil} + \text{Pdf fertilizer}. \quad (1)$$

The P derived from the seed was assessed by growing Italian ryegrass on sand, where two sources contributed to the shoot P uptake of the plant:

$$\text{Pupt}_{\text{plant}} = \text{Pdf seed} + \text{Pdf fertilizer}. \quad (2)$$

Equation (1) and (2) were resolved using the principles for direct and indirect labeling techniques reviewed in Frossard et al. (2011). For the direct labeling, the Pdf fertilizer was calculated the following:

$$\text{Pdf fertilizer} = \frac{\text{SA}_{\text{plant}}}{\text{SA}_{\text{fert}}} \times \text{Pupt}_{\text{plant}} \quad (3)$$

where SA<sub>plant</sub> is the specific activity (<sup>33</sup>P/<sup>31</sup>P, Bq μg<sup>-1</sup> P) in the plant amended with a labeled fertilizer and SA<sub>fert</sub> the specific activity of the respective fertilizer.

When an indirect approach was followed, the Pdf fertilizer in the plant was calculated using Eq. (4):

$$\text{Pdf fertilizer} = \left( 1 - \frac{\text{SA}_{\text{plant+P}}}{\text{SA}_{\text{plantNoP}}} \times K \right) \times \text{Pupt}_{\text{plant+P}} \quad (4)$$

by comparing the specific activity in the fertilized plant (SA<sub>plant+P</sub>) to the specific activity in the plant receiving



no P fertilization ( $SA_{\text{plantNoP}}$ ). A factor K was added to account for the contribution of P taken up from the seed:

$$K = \frac{\left( \frac{\text{Pdf seed}_{\text{plant+P}}}{P_{\text{upt}}_{\text{plant+P}}} \right)}{\left( \frac{\text{Pdf seed}_{\text{plantNoP}}}{P_{\text{upt}}_{\text{plantNoP}}} \right)} \quad (5)$$

The Pdf seed was obtained from the seed P contribution experiment. It revealed that the Pdf seed depends on the P status of the plant. The underlying functions describing the relation between the Pdf seed and the shoot P uptake for cut 1 and 2 are presented below (Fig. 1). In order to calculate the Pdf seed and to obtain the factor K, the shoot P uptake of plants grown on soil was inserted into these functions. For cut 3, K was assumed to be 1 (Frossard et al. 1994).

The fertilizer P recovery (FertPRec, %) in the plant was calculated by comparing the Pdf fertilizer to the amount of P applied with the fertilizer ( $P_{\text{fert}}$ , mg P  $\text{kg}^{-1}$  soil):

$$\text{FertPRec} = \frac{\text{Pdf fertilizer}}{P_{\text{fert}}} \times 100. \quad (6)$$

The relative effectiveness (%) of SSA fertilizers was calculated by comparing their Pdf fertilizer to the Pdf fertilizer of the WSP source added at the same fertilization rate:

$$\text{Relative effectiveness} = \frac{\text{Pdf fertilizer}_{+P}}{\text{Pdf fertilizer}_{\text{WSP}}} \times 100. \quad (7)$$

In the following, the above-defined parameters are either presented individually for each cut of Italian ryegrass or cumulated. In the absence of a plant, the same calculation principles were applied to each extraction pool of the sequentially extracted samples from the soil-fertilizer incubation. However, the non-existence of a seed P source results in Eq. (8) for the direct and Eq. (9) for the indirect labeling:

$$\text{Pdf fertilizer} = \frac{SA_{\text{pool}}}{SA_{\text{fert}}} \times P_{\text{pool}} \quad (8)$$

$$\text{Pdf fertilizer} = \left( 1 - \frac{SA_{\text{pool+P}}}{SA_{\text{poolNoP}}} \right) \times P_{\text{pool+P}} \quad (9)$$

where  $P_{\text{pool}}$  (mg P  $\text{kg}^{-1}$  soil) is the total P content in a given extraction pool.

## Statistics

Data sets were analyzed using variance analysis (two-way ANOVA) testing the factor soil (S), fertilizer treatment (F) and their interaction ( $S \times F$ ). Directly labeled treatments were excluded from the variance analysis and results are presented independently including the standard deviation of replicates. Prior to the analysis, data was visually checked for normal distribution by means of normal probability charts and, if indicated, log transformed to obtain normal distribution. Since variance homogeneity (Hartley's Fmax statistic) was not always given a rather conservative significance level for post-hoc multiple comparisons was chosen ( $\alpha=0.01$ ). Multiple comparisons were performed applying Tukey's HSD test using a pooled variance term within a soil or within an extraction pool of a soil. Statistical analyses were performed with aabel 20/20 Data Vision software v. 2.0 (Gigawiz Ltd. Co., Tulsa, USA).

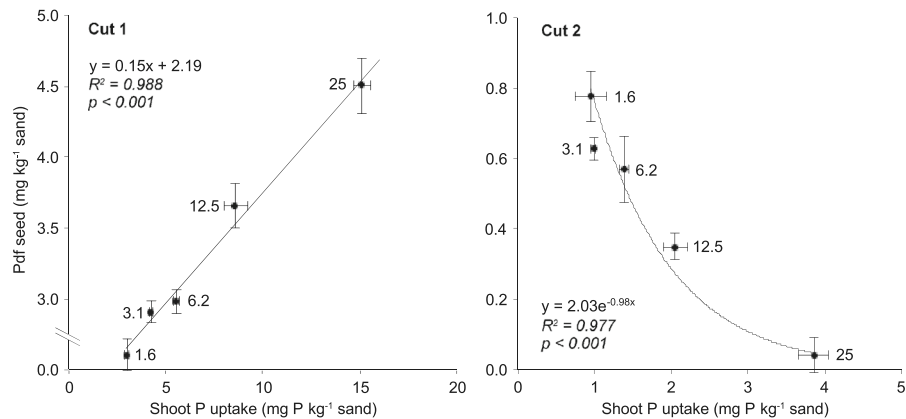
## Results

### Growth trial: estimation of seed phosphorus contribution

The cumulated shoot DM production for two cuts of Italian ryegrass grown on sand and fertilized with different rates of WSP (1.6 to 25 mg P  $\text{kg}^{-1}$  sand) ranged from 4.4 to 6.2 g  $\text{kg}^{-1}$  sand and increased with increasing fertilization rate (data not shown). A third regrowth failed due to severe P deficiency at the lowest two P fertilization rates.

The average amount of P added with 2 g of seeds was 6.2 mg P  $\text{kg}^{-1}$  sand. Figure 1 shows the relationships between the shoot P uptake and Pdf seed established for cut 1 and 2. These relationships were used to estimate Pdf seed for the indirect and direct labeling in Eqs. (1) and (5) at cut 1 and 2. For the third cut, the contribution of seed P was assumed to be negligible. For the first cut the relation between shoot P uptake and Pdf seed was linear and positive. The plants with the lowest fertilizer addition took up 2.6 mg P  $\text{kg}^{-1}$  sand and seed derived P accounted for 85 % of the shoot P uptake. For the plants with the highest fertilization rate, the transfer of seed P to the shoots accounted for 29 % of the shoot P uptake and Pdf seed was 4.5 mg P  $\text{kg}^{-1}$  sand.

For the second cut the relationship between shoot P uptake and Pdf seed was negative. At the lowest P



**Fig. 1** Relation between the shoot P uptake and the seed P contribution (Pdf seed) for the two cuts of Italian ryegrass grown on sand and fertilized with different rates of water soluble P labeled with  $^{33}\text{P}$ . The relation was used to estimate the P derived

from the seed (Pdf seed) in plants grown on soil. Total seed P was  $6.2 \text{ mg P kg}^{-1} \text{ sand}$ . Numbers next to experimental points indicate the fertilization rate ( $\text{mg P kg}^{-1} \text{ sand}$ ). Error bars present standard deviations of three replicates

fertilization rate 81 % of the P in the shoots or  $0.8 \text{ mg P kg}^{-1} \text{ sand}$  was derived from the seed, while at the highest rate plants hardly contained any seed P. For the sum of the two cuts, Pdf seed in the shoots amounted to 53 % and 73 % of the P added by seeds resulting in a cumulated Pdf seed of  $3.4 \text{ mg P kg}^{-1} \text{ sand}$  for the lowest and  $4.5 \text{ mg P kg}^{-1} \text{ sand}$  for the highest fertilization rate.

Growth trial: phosphorus uptake by plants from thermo-chemically treated sewage sludge ash

The cumulated shoot DM productivity of Italian ryegrass ranged from  $6.6$  to  $12.6 \text{ g kg}^{-1} \text{ soil}$  and was lowest on the “acidic low P” soil and highest on the “alkaline moderate P” soil. A response to P fertilization was only observed on the “acidic low P” soil, where productivity was higher with SSAMg and WSP than with NoP treatment (Table 3).

Irrespective of the cut and the fertilizer treatment, the shoot P concentration of Italian ryegrass grown on the “acidic low P” soil was  $<2 \text{ mg P g}^{-1} \text{ shoot DM}$  i.e., below the reported range for non P limiting conditions (Bergmann 1993; Whitehead 2000). On the other two soils, the shoot P concentrations were significantly higher and within the optimal range for plant growth (data not shown).

The cumulated shoot P uptake ranged from  $6.4$  to  $27.4 \text{ g P kg}^{-1} \text{ soil}$  (Table 3). On the “acidic low P” soil and the “neutral moderate P” soil it was significantly affected by the fertilizer treatments and the plants

fertilized with SSAMg and WSP showed a higher shoot P uptake than the plants of the NoP treatment.

The SA in the shoots of Italian ryegrass was influenced by the soil and the fertilizer treatment at each cut (Fig. 2). The SA of the NoP treatment on all soils increased from cut 1 to 2, most distinctively on the “acidic low P” soil. For the same soil, at the first cut, the SA of plants fertilized with SSACa or SSAMg was significantly higher than the SA of NoP plants. On the other hand, at cut 3, the SA of all fertilized plants was lower than in the NoP treatment. For the two “moderate P” soils none of the fertilized plants had a significantly higher SA than the plants of the NoP treatment.

The estimated seed P contribution for the “acidic low P” soil, on average, amounted to  $4.0 \text{ mg P kg}^{-1}$  and was slightly lower than for the other soils (Fig. 3). The average Pdf seed was  $5.1 \text{ mg P kg}^{-1} \text{ soil}$  on the “neutral moderate P” soil and  $4.8 \text{ mg P kg}^{-1} \text{ soil}$  on the “alkaline moderate P” soil. Within a soil the Pdf seed remained unaffected by the fertilizer treatments.

The average cumulated Pdf soil was  $2.9 \text{ mg P kg}^{-1} \text{ soil}$  for the “acidic low P” soil,  $12.2 \text{ mg P kg}^{-1} \text{ soil}$  for the “neutral moderate P” soil and  $14.8 \text{ mg P kg}^{-1} \text{ soil}$  for the “alkaline moderate P” soil. It did not differ between the fertilizer treatments. Nevertheless, on the “acidic low P” soil, a trend towards a higher Pdf soil for SSACa and SSAMg fertilized plants was observed when comparing it to the Pdf soil of the NoP and the WSP fertilization treatment.

The cumulated Pdf fertilizer on the “acidic low P” soil was significantly higher for SSAMg and WSP than

**Table 3** Shoot dry matter yield (DM), P taken up by the shoots (Shoot P uptake) and fertilizer P recovery (FertPRec) cumulated for three cuts of Italian ryegrass and the effectiveness relative to the water-

soluble P (relative effectiveness, %) of sewage sludge ash (SSA) based fertilizers on soils with contrasting pH and P availability

Soil/Fertilizer	DM (g kg <sup>-1</sup> soil)	Shoot P uptake (mg P kg <sup>-1</sup> soil)	FertPRec (%)	Relative effectiveness (%)
“Acidic low P”				
NoP	4.6 a	6.4 a	na	na
SSACa	5.8 ab	8.3 a	2.3 a	14 a
SSAMg	7.8 b	15.7 b	14.1 b	88 b
WSP	8.1 b	14.4 b	16.1 b	100 <sup>a</sup>
Pooled SD	0.9	0.9	1.8	11.6
WSPdir	7.9±0.5	14.2±0.9	16.2±0.5	nd
“Neutral moderate P”				
NoP	7.5	16.4 ab	na	na
SSACa	7.7	18.2 bc	4.6 a	24.1
SSAMg	8.6	23.4 c	13.6 ab	71.2
WSP	8.8	27.4 c	19.1 b	100 <sup>a</sup>
Pooled SD	0.9	2.1	3.3	20.9
WSPdir	8.8±1.1	28.3±1.3	16.6±0.8	nd
“Alkaline moderate P”				
NoP	8.5	21.0	na	na
SSACa	8.1	20.9	2.3 a	18.3
SSAMg	8.4	20.7	0.5 a	4.0
WSP	8.5	25.1	12.6 b	100 <sup>a</sup>
Pooled SD	0.5	1.4	1.9	15.1
WSPdir	7.8±0.4	23.1±0.7	10.4±0.8	nd
Source of variation ( <i>p</i> -value) indirect labeling				
Soil (S)	<0.001	<0.001	<0.001	0.002
Fertilizer (F)	<0.001	<0.001	<0.001	<0.001
S × F	ns	0.028	0.014	<0.001

The P fertilizer treatments included no P fertilization (NoP), SSA thermo-chemically treated with CaCl<sub>2</sub> (SSACa) or MgCl<sub>2</sub> (SSAMg) and water soluble P (WSP). The FertPRec was determined by labeling either the soil (indirect labeling) or WSP (direct labeling, WSPdir) with <sup>33</sup>P. The fertilization rate was 50 mg P kg<sup>-1</sup> soil. Letters indicate differences between fertilizer treatments within a soil for the indirect labeling (Tukey's HSD,  $\alpha=0.01$ ). Pooled standard deviation for the indirect labeling (pooled SD). Standard deviation for the direct labeling ( $\pm$ ); Not significant (*ns*); Not applicable (*na*); Not determined (*nd*)

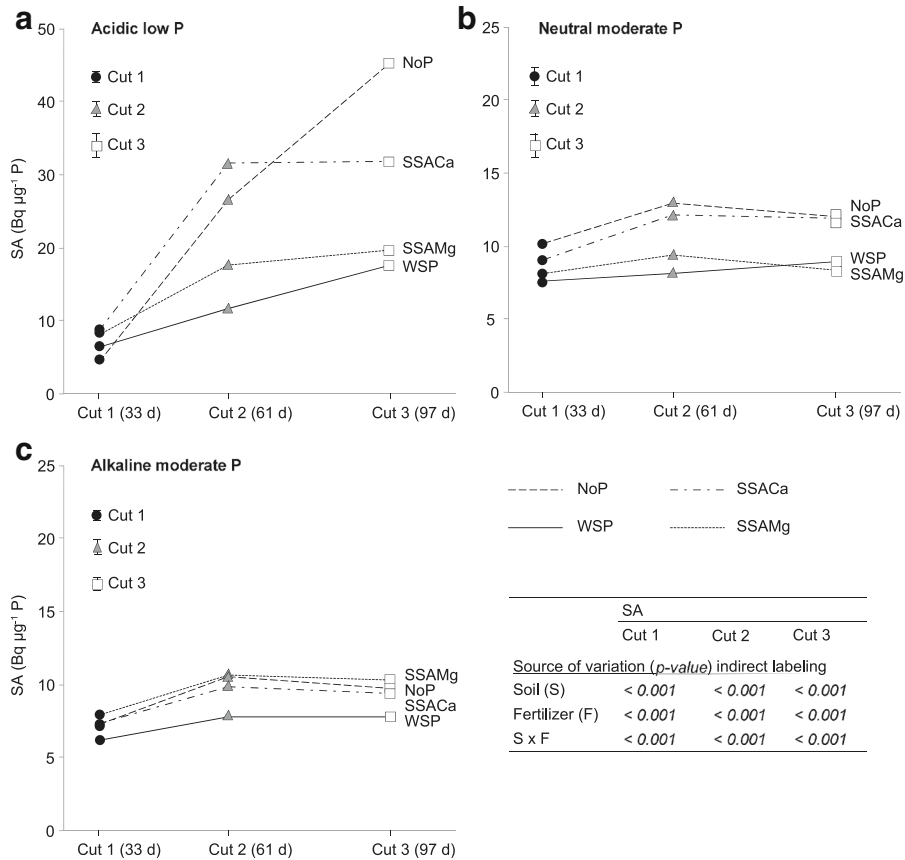
<sup>a</sup> by definition set to 100 %

for SSACa (Fig. 3). It ranged from 1.1 to 8.0 mg P kg<sup>-1</sup> soil. The relative effectiveness was 14 % for SSACa and 88 % for SSAMg (Table 3). On the “neutral moderate P” soil, the Pdf fertilizer was higher with WSP (9.5 mg P kg<sup>-1</sup> soil) than with SSACa (2.3 mg P kg<sup>-1</sup> soil) and the Pdf fertilizer with SSAMg was intermediate. The relative effectiveness of SSACa was 24 % and the one of SSAMg was 71 %. On the “alkaline moderate P” soil, the Pdf fertilizer with SSACa and SSAMg was lower than with WSP and the Pdf fertilizer for all

treatments ranged from 1.1 to 6.3 mg P kg<sup>-1</sup> soil. The relative effectiveness was 18 % for SSACa and 4 % for SSAMg.

The fertilizer P recovery ranged from 0.5 to 19.1 % of the total P amendment (Table 3). So far we abstained from comparing WSP and WSPdir because no differences were observed. However the variability of Pdf fertilizer, fertilizer P recovery and Pdf soil was generally lower for the direct than for the indirect labeling (Fig. 3, Table 3).





**Fig. 2** Specific activity (SA, Bq  $\mu\text{g}^{-1}$  P) before taking into account the contribution of unlabeled seed P in the shoots of Italian ryegrass grown on  $^{33}\text{P}$  labeled soils with contrasting pH and P availability. The P fertilizer treatments included no P fertilization

(NoP), sewage sludge ash (SSA) thermo-chemically treated with  $\text{CaCl}_2$  (SSACa) or  $\text{MgCl}_2$  (SSAMg) and water soluble P (WSP); the pooled standard deviation for each soil and cut is displayed in the legend

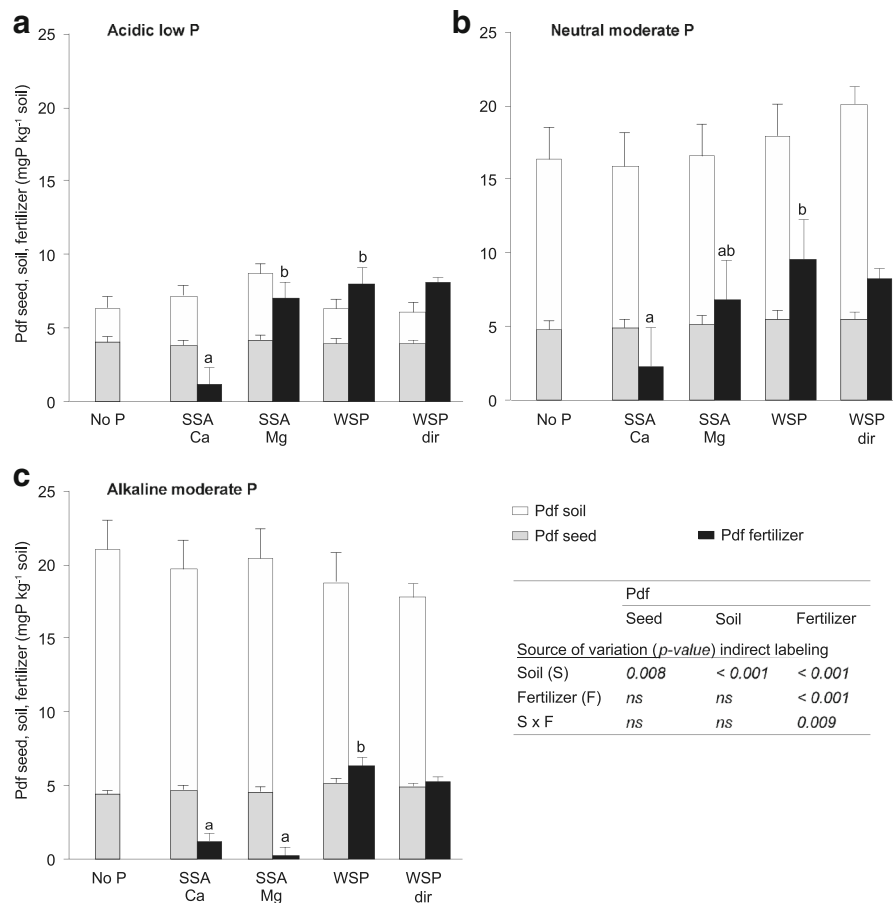
Soil-fertilizer incubation study: fate of phosphorus from thermo-chemically treated sewage sludge ash in the soil

For the sequential extraction of the SSA products only, the proportion of P extractable by resin accounted for 3 % of total P in SSACa and for 17 % of total P in SSAMg (Table 4). For SSAMg, additional 6 % of the total P were extractable with  $\text{NaHCO}_3$ . The major part of P in SSA was found to be HCl extractable accounting for 94 % of total P in SSACa and for 74 % of total P in SSAMg, while very little P was extracted during the NaOH step. The proportion of residual P was 3 % for both SSA and the sum of all P fractions was in accordance with the total P determined by X-ray fluorescence analysis (Table 1).

Regarding the sequential extraction of soil-fertilizer incubations, no significant differences between time

points were observed neither for the size of the P pools nor for their specific activity (data not shown). Therefore averaged values over the entire incubation period are presented. On the “acidic low P” soil less than 2 mg P  $\text{kg}^{-1}$  soil was found to be resin extractable in the NoP treatment (Table 5). The addition of SSAMg increased the resin pool by 39 mg P  $\text{kg}^{-1}$  soil and the addition of WSP led to an increase of 49 mg P  $\text{kg}^{-1}$  soil. A significant increase following the addition of SSAMg and WSP was also observed for the  $\text{NaHCO}_3$  and the NaOH pool. In addition, SSAMg led to an increase of 22 mg P  $\text{kg}^{-1}$  soil of the HCl extractable pool. The addition of SSACa increased the HCl extractable P pool by 70 mg P  $\text{kg}^{-1}$  soil and it was the only pool affected by this fertilizer treatment.

The “neutral moderate P” soil and the “alkaline moderate P” soil contained 26 and 33 mg P  $\text{kg}^{-1}$  soil of resin extractable P when unfertilized (NoP). On the “neutral



**Fig. 3** Phosphorus derived from the fertilizer, soil and seed (Pdf fertilizer, Pdf soil, Pdf seed, mg P kg<sup>-1</sup> soil) in the above ground plant material of three cuts of Italian ryegrass fertilized with thermo-chemically treated sewage sludge ash (SSA) and water soluble phosphate as assessed by either labeling the available soil P (indirect labeling) or the fertilizer (direct labeling) with <sup>33</sup>P. Sewage sludge ash thermo-chemically treated with CaCl<sub>2</sub>

(SSACa) or MgCl<sub>2</sub> (SSAMg). Water soluble P (WSP) and directly labeled WSP (WSPdir). Fertilization rate is 50 mg P kg<sup>-1</sup> soil. Error bars present pooled standard deviations within a soil for the indirect labeling and standard deviations for the direct labeling technique. Letters indicate significant differences between fertilizer treatments within a soil for the Pdf fertilizer (Tukey's HSD,  $\alpha=0.01$ ); Not significant (*ns*)

moderate P" soil the incubation with SSAMg increased the resin extractable pool by 20 mg P kg<sup>-1</sup> soil and the incubation with WSP by 49 mg P kg<sup>-1</sup> soil. The WSP treatment also increased the NaHCO<sub>3</sub> extractable pool, while SSACa and SSAMg led to a significant increase of 98 and 59 mg P kg<sup>-1</sup> soil of the HCl extractable pool, respectively.

For the "alkaline moderate P" soil WSP was the only treatment affecting the resin extractable pool as illustrated by an increase of 46 mg P kg<sup>-1</sup> soil. In contrast SSACa and SSAMg increased the HCl extractable pool by about 100 mg P kg<sup>-1</sup> soil, suggesting that the P added with SSACa and SSAMg was entirely incorporated into this pool.

Regarding the Pdf fertilizer and Pdf soil, extraction pools beyond the NaHCO<sub>3</sub> extraction step are not presented because these pools were associated with a low specific activity and high variability. For all three soils the Pdf soil of the two first extraction pools was not affected by the fertilizer treatment (Fig. 4). Hence Pdf fertilizer corresponded to the increase in extraction pool size following the fertilizer application as presented above.

The fertilizer P recovery for the sum of the resin and NaHCO<sub>3</sub> pool was highest for WSP and it was approximately 60 % irrespective of the soil (Table 6). SSAMg showed a decreasing fertilizer P recovery from the "acidic low P" soil to the "alkaline moderate P" soil and the effectiveness relative to WSP was 77 % on the "acidic

**Table 4** Phosphorus (mg P kg<sup>-1</sup>) in the pools of the sequential extraction of thermo-chemically treated sewage sludge ash (SSA)

SSA	Sequential extraction steps					
	Resin	NaHCO <sub>3</sub>	NaOH	HCl	Residual	Sum
SSACa	2.0	0.2	<0.1	56.8	1.6	60.8
SSAMg	11.0	3.7	0.36	46.9	1.6	63.5
Pooled SD	0.1	0.4	<0.1	1.3	0.3	1.5
Source of variation ( <i>p</i> -value)						
SSA	<0.01	<0.01	<0.01	<0.01	<i>ns</i>	<i>ns</i>

SSA thermo-chemically treated with CaCl<sub>2</sub> (SSACa) or MgCl<sub>2</sub> (SSAMg). Pooled standard deviation (pooled SD); Not significant (*ns*)

low P” and 32 % on the “alkaline moderate P” soil. The fertilizer P recovery of SSA Ca was generally lowest, with an effectiveness relative to WSP of 15 % to 28 %.

## Discussion

Importance and validation of seed phosphorus contribution and use of <sup>33</sup>P labeling

For the first cut of the Italian ryegrass grown on sand, the Pdf seed in the shoots increased with increasing P fertilization rate and shoot P uptake. This is in accordance with Brookes (1982) who established a similar relationship for the dry matter production of Italian ryegrass. Under non P limited conditions plants grow more vigorously and may take up higher amounts of P from any available sources including the seed. Brookes (1982) also suggested that there is a physiologically determined limit of P uptake from the seed and therefore assumed, without measuring it, an inverse relationship between the Pdf seed in the shoots and the P fertilization rate for the following cuts. This agrees with our results. Nevertheless one could argue that plants grown on sand do not necessarily mimic the plant response and internal use of seed P of plants grown on soil and that therefore the proposed estimation of seed P contribution might not be adequate. In this study, however, the shoot P uptake of plants grown on sand and soil were within a similar range. Additionally, effects of depletion during the first regrowth probably occurred to the same extent for both substrates because of a similar decrease in P uptake from cut 1 to cut 2. On the sand cultures the average shoot P uptake of cut 2 amounted to 26 % of the uptake of cut 1.

In the pot trials with soil, the average shoot P uptake of cut 2 equaled 31 % of the shoot P uptake of cut 1.

The SA in the above ground plant material of Italian ryegrass grown on the “acidic low P” soil (Fig. 2) illustrates the importance of seed P consideration in P limited soils. The increasing SA of the NoP treatment with progressive cuts and the higher SA in the fertilized treatments when compared to the NoP treatment can only be explained by the contribution of seed P and it is in accordance with earlier observations by Morel and Fardeau (1989b). Without taking into account the seed as an additional unlabeled P source, the determination of Pdf fertilizer with the indirect labeling technique would have failed on the “acidic low P” soil. The seed P contribution was less critical for the “moderate P” soils because Pdf seed accounted for less than 25 % of the total shoot P uptake by plants, while it was up to 50 % on the “acidic low P” soil.

Alternative methods to correct for seed P contribution have been discussed. Truong and Pichot (1976) proposed a grass with low seed P reserves. Bühler et al. (2003) and Frossard et al. (1994) only considered the third and successive cuts for the determination of isotopically exchangeable soil P as measured by the specific activity in plants (L-value) supposing that the amount of P derived from the seed would then be negligible. Oberson et al. (2010) determined the P uptake from animal manure by deriving a seed P correction from the difference in Pdf fertilizer between a directly and indirectly labeled fertilizer treatment. The latter approach was used to validate the estimation of seed P in the present study. Since we did not find differences in the Pdf fertilizer of WSP and WSPdir (Fig. 3), we have further evidence that the applied seed P estimation was adequate, at least for the WSP treatment.

The use of <sup>33</sup>P labeling was not only essential for the estimation of the seed P contribution but also e.g., for the growth trial where on the “alkaline moderate P” soil the absence of a response in DM productivity and shoot P uptake to the P fertilization made the use of a labeling approach indispensable. The non-isotopic difference method (increase in shoot P uptake of a fertilized plant when compared to the NoP treatment) would have failed. Furthermore, the difference method would have led to a higher estimate of the Pdf fertilizer of SSACa and SSAMg on the “acidic low P” as illustrated by a trend for a higher Pdf soil in the SSA fertilized plants when compared to the unfertilized control treatment. We assume that the higher Pdf soil was a mere effect of

**Table 5** Total P (mg P kg<sup>-1</sup> soil) in the pools of the sequential extraction, pH and total exchangeable aluminum (Al<sub>ex</sub>, mg Al kg<sup>-1</sup> soil) in soil-fertilizer incubations using soils with contrasting pH and P availability

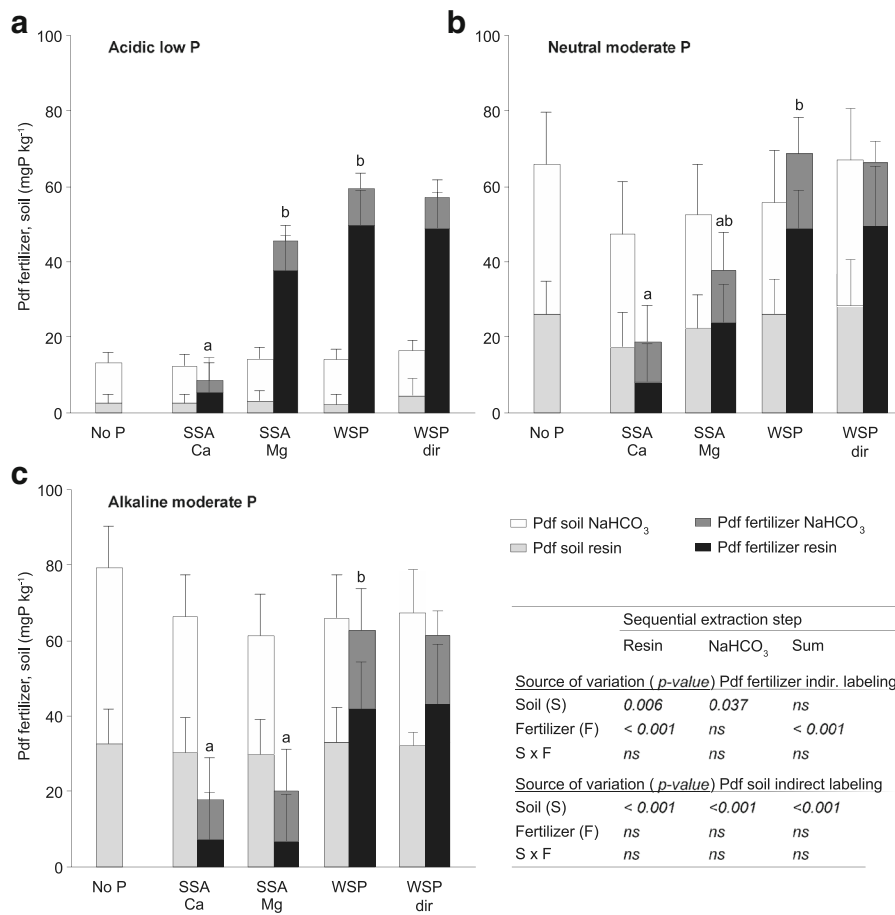
Soil/Fertilizer	Sequential extraction steps						pH <sup>a</sup>	Al <sub>ex</sub> <sup>a</sup>
	Resin	NaHCO <sub>3</sub>	NaOH	HCl	Residual	Sum		
<b>“Acidic low P”</b>								
NoP	2 a	11 a	113 a	5 a	197	328 a	3.53 a	191 b
SSACa	8 a	13 a	116 a	75 c	211	424 b	3.63 b	127 a
SSAMg	41 b	19 b	142 b	27 b	214	443 b	3.61 b	148 a
WSP	51 b	22 b	147 b	7 a	219	445 b	3.55 a	180 b
Pooled SD	10	5	14	5	25	33	0.03	9.5
WSPdir	43±10	21±4	156±13	6±1	194±27	419±31	<i>nd</i>	<i>nd</i>
<b>“Neutral moderate P”</b>								
NoP	26 a	40 a	354	122 a	237	779 a	4.38 a	7.2 c
SSACa	26 a	40 a	355	220 c	254	896 b	4.44 ab	4.5 b
SSAMg	46 b	44 ab	368	181 b	255	897 b	4.64 b	1.9 a
WSP	75 c	49 b	370	135 a	259	888 b	4.32 a	5.3 bc
Pooled SD	8	12	39	18	31	55	0.08	0.8
WSPdir	79±12	50±14	391±37	130±14	261±26	921±50	<i>nd</i>	<i>nd</i>
<b>“Alkaline moderate P”</b>								
NoP	33 a	48	188	475 a	409	1151	7.13 a	<0.5
SSACa	37 a	46	188	579 b	417	1267	7.24 b	<0.5
SSAMg	37 a	45	184	567 b	435	1266	7.31 b	<0.5
WSP	79 b	53	200	491 a	445	1269	7.20 ab	<0.5
Pooled SD	9	16	26	40	73	77	0.05	<0.5
WSPdir	75±13	53±15	212±20	514±45	444±77	1293±70	<i>nd</i>	<i>nd</i>
Source of variation ( <i>p</i> -value) indirect labeling								
Soil (S)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Fertilizer (F)	<0.001	0.033	0.042	<0.001	<i>ns</i>	<0.001	<0.001	<0.001
S × F	0.003	<i>ns</i>	<i>ns</i>	0.007	<i>ns</i>	<i>ns</i>	0.002	<0.001

The P fertilizer treatments included no P fertilization (NoP), sewage sludge ash (SSA) thermo-chemically treated with CaCl<sub>2</sub> (SSACa) or MgCl<sub>2</sub> (SSAMg) and water soluble P (WSP). Soil P was labeled with <sup>33</sup>P (indirect labeling) except for WSPdir where the fertilizer was labeled (direct labeling). The fertilization rate was 100 mg P kg<sup>-1</sup> soil. Letters indicate differences between fertilizer treatments within a soil for the indirect labeling (Tukey’s HSD, α=0.01). Pooled standard deviation for the indirect labeling (pooled SD). Standard deviation for the direct labeling (±); variability measures were derived after pooling the data of five sampling dates 1, 8, 15, 49, and 91 after incubation, except for pH and Al<sub>ex</sub> (see footnote a); Not significant (*ns*); Not determined (*nd*)

<sup>a</sup> Determined with 1M KCl, S:L 1:2.5, 8 days after incubation

liming since the soil-fertilizer incubations revealed that the addition of SSACa and Mg increased the soil pH and led to a significant reduction of the exchangeable aluminum in the soil (Table 5). The liming potential of SSA is well known and has been described by Bierman et al. (1995) and Dann et al. (1989). The implications of the liming effect and of the lack of a response to P fertilization became most obvious when comparing the relative effectiveness of SSACa and SSAMg assessed by the isotopic and the non-isotopic approach (supplementary

Table 2). On the “acidic low P soil” the non-isotopic approach for SSA based fertilizers resulted in a higher relative effectiveness than the isotope method. The underlying assumption that fertilized and unfertilized treatments take up the same amount of P from the soil was violated because e.g., fertilizers facilitated plant growth or affected soil P availability by other effects than P supply (Morel and Fardeau 1990b). On the two “moderate P” soils the relative effectiveness estimated by the non-isotopic method was generally lower than for the



**Fig. 4** Phosphorus derived from the fertilizer and soil (Pdf fertilizer, Pdf soil, mg P kg<sup>-1</sup>) in the two first pools of the sequential extraction of soil-fertilizer incubations with thermo-chemically treated sewage sludge ash (SSA) and water soluble phosphate as assessed by either previously labeling the available soil P (indirect labeling) or the fertilizer (direct labeling) with <sup>33</sup>P. Sewage sludge ash thermo-chemically treated with CaCl<sub>2</sub> (SSACa) or MgCl<sub>2</sub>

(SSAMg). Water soluble P (WSP) and directly labeled WSP (WSPdir). Fertilization rate is 100 mg P kg<sup>-1</sup> soil. Error bars present pooled standard deviations within a soil for the indirect labeling and standard deviations for the direct labeling technique. Letters indicate significant differences between fertilizer treatments within a soil for the Pdf fertilizer for the sum of the two extraction pools (Tukey’s HSD, α=0.01); Not significant (ns)

isotopic approach suggesting that P from the fertilizer partially substituted available soil P.

When comparing direct and indirect labeling, disadvantages of the indirect labeling approach are that a usually large P pool is labeled and that an unfertilized control plant (NoP) is required for the assessment of the Pdf fertilizer. This can lead to a high variability as it has been reported for N studies (Douxchamps et al. 2011). In this study, however, a higher variation for the indirect than for the direct labeling was only observed in the growth trial, while for the soil-fertilizer incubation study variation was generally high. The latter can be explained by the relatively small subsample used for the sequential extraction

compared to pots where roots have explored nearly the whole soil volume and where any inhomogeneous distribution of fertilizer P had fewer consequences.

#### Phosphorus availability of thermo-chemically treated sewage sludge ashes

The cumulated fertilizer P recovery of WSP in Italian ryegrass after three cuts amounted from 12 to 19 % of the applied P. This is in the range of the recovery for water-soluble P fertilizers reported in other isotope dilution studies. After the application of water-soluble P at a rate of 87 mg P kg<sup>-1</sup> soil and four cuts of English ryegrass,



**Table 6** Fertilizer P recovery (FertPRec, %) and effectiveness relative to the water-soluble P (relative effectiveness, %) in the two first pools of the sequential extraction of soil-fertilizer incubations using soils with contrasting pH and P availability

Soil/Fertilizer	FertPRec			Relative effectiveness
	Resin	NaHCO <sub>3</sub>	Resin + NaHCO <sub>3</sub>	Resin + NaHCO <sub>3</sub>
<b>“Acidic low P”</b>				
SSACa	5.2 a	3.4	8.6 a	14.5 a
SSAMg	37.7 b	7.8	45.5 b	76.7 b
WSP	49.8 b	9.5	59.3 b	100 <sup>a</sup>
Pooled SD	9.3	4.4	9.9	16.7
WSPdir	49.0±9.8	8.2±3.8	57.2±10.0	<i>nd</i>
<b>“Neutral moderate”</b>				
SSACa	8.3 a	10.3	18.6 a	27.2
SSAMg	24.0 ab	13.8	37.8 ab	55.2
WSP	48.9 b	19.6	68.5 b	100 <sup>a</sup>
Pooled SD	10.2	10.0	15.8	23.1
WSPdir	50.1±20.2	12.8±3.3	62.9±16.6	<i>nd</i>
<b>“Alkaline moderate P”</b>				
SSACa	7.2 a	10.6	17.8 a	28.3
SSAMg	6.9 a	13.3	20.2 ab	32.2
WSP	41.8 b	20.9	62.8 b	100 <sup>a</sup>
Pooled SD	12.4	11.0	20.9	14.5
WSPdir	44.2±14.1	16.7±4.9	60.91±6.4	<i>nd</i>
Source of variation ( <i>p</i> -value) indirect labeling				
Soil (S)	0.006	0.037	<i>ns</i>	0.042
Fertilizer (F)	<0.001	<i>ns</i>	<0.001	<0.001
S × F	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>

The P fertilizer treatments included sewage sludge ash (SSA) thermo-chemically treated with CaCl<sub>2</sub> (SSACa) or MgCl<sub>2</sub> (SSAMg) and water soluble P (WSP). The FertPRec was determined by labeling either the soil (indirect labeling) or WSP (direct labeling, WSPdir) with <sup>33</sup>P. Fertilization rate was 100 mg P kg<sup>-1</sup> soil. Letters indicate differences between fertilizer treatments within a soil for the indirect labeling (Tukey’s HSD,  $\alpha=0.01$ ). Pooled standard deviation for the indirect labeling (pooled SD). Standard deviation for the direct labeling ( $\pm$ ); Not significant (*ns*); Not determined (*nd*)

<sup>a</sup>By definition set to 100 %

Frossard et al. (1996) found a fertilizer P recovery of 9 % on a loamy soil with a  $E_{1min}$  of 19 mg P kg<sup>-1</sup> and a recovery of 12 % on clayey soil with a  $E_{1min}$  of 10 mg P kg<sup>-1</sup> soil. In a pot experiment with English ryegrass by Morel and Fardeau (1989a), the fertilizer recovery for water-soluble P ranged from 10 to 25 % and decreased with increasing rate of P application. Even though the fertilizer P recovery as well as the relative effectiveness of a fertilizer compared to a reference fertilizer may depend on the experimental conditions, the similar fertilizer P recoveries for WSP allow a comparison of the relative effectiveness of SSA based fertilizers with other P sources studied in other works. The relative effectiveness of

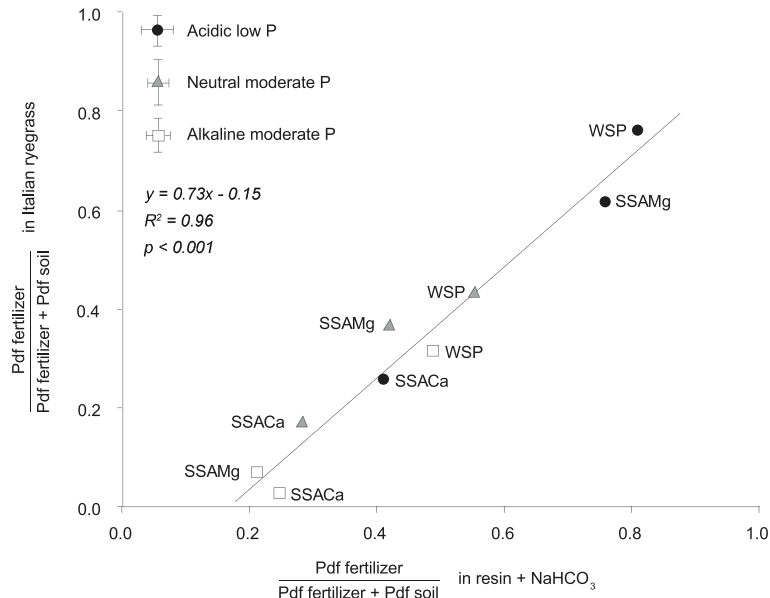
SSAMg under acidic to neutral soil conditions is in the range of effectiveness reported for animal manure, compost or sewage sludge. For Italian ryegrass grown on a loamy silt, Oberson et al. (2010) revealed an effectiveness of 60 to 85 % for manure P relative to water-soluble P, both added at a rate of 30 mg P kg<sup>-1</sup> soil. The P availability from different composts to white clover was described by Sinaj et al. (2002). The relative effectiveness of compost derived thereof ranged from 37 to 79 % and was on average 49 % on a sandy acidic and 64 % on a clayey calcareous soil. In the above described study by Frossard et al. (1996) the effectiveness of P from digested and undigested sewage sludge ranged from 76 to 85 % on

the loamy soil and from 62 to 78 % on the clayey soil. On the “alkaline moderate P” soil the relative effectiveness of SSAMg was lower than for the above reported P fertilizers and similar to the effectiveness of SSACa. Under alkaline conditions and for SSACa in general, the relative effectiveness of SSA based fertilizers can be compared to the effectiveness of rock phosphate or residues from the incineration of meat-and-bone meal ash. In a pot study with maize applying no  $^{33}\text{P}$  labeling techniques, the effectiveness of these two P substrates was approximately 5 to 45 % relative to triple super phosphate (Cabeza et al. 2011). In the same study, the relative effectiveness of a heavy metal depleted SSA (of unknown characteristics) was approximately 15 % on an acidic sandy and 40 % on a neutral loamy soil. This is in agreement with our results obtained for SSACa but contradicts the high relative effectiveness of SSAMg on the “acidic low P” and “neutral moderate” P soil.

While the relative effectiveness may level out some effects of experimental conditions such as e.g., duration of an experiment, it depends on the application rate. Barrow (1985) highlights that, in situations where the marginal response to fertilizers is not constant for different application rates, the comparison of two fertilizers applied

at the same rate, referred to as “vertical” comparison, may differ from the mineral fertilizer equivalent obtained by a “horizontal” comparison. He proposes the establishment of a response curve with different application rates of one fertilizer to assess the equivalency to another fertilizer by comparing their response. The mineral fertilizer equivalent in % of P applied with SSACa and SSAMg was generally lower than the relative effectiveness (supplementary Table 2). However, it followed the same trend, underlining a high fertilizer value of SSAMg and decreasing values for both SSACa and SSAMg with increasing soil pH.

The proportion of Pdf fertilizer in the plant illustrates the effect of soil chemical properties on plant P availability of SSA and the different availabilities of SSACa and SSAMg. In Fig. 5 the ratio between the Pdf fertilizer and the sum of Pdf fertilizer and Pdf soil is displayed against the corresponding ratio in the resin and  $\text{NaHCO}_3$  extractable pool in the soil-fertilizer incubation study. This correlation is in agreement with the current interpretation of the inorganic P availability reported for these two pools. Inorganic P in the resin and  $\text{NaHCO}_3$  extractable pool is indeed considered to be readily soluble or easily exchangeable and weakly associated with soil surfaces. It is therefore likely to be mobilized and taken up by the



**Fig. 5** Relationship between the Pdf fertilizer (as proportion of the sum of Pdf fertilizer and Pdf soil) in the above ground plant material harvested with three cuts of Italian ryegrass and in the two first pools of the sequential extraction of soil-fertilizer incubations (resin,  $\text{NaHCO}_3$ ) as assessed by labeling the available soil P with  $^{33}\text{P}$  (indirect labeling). Sewage sludge ash (SSA) thermo-

chemically treated with  $\text{CaCl}_2$  (SSACa) or  $\text{MgCl}_2$  (SSAMg) and water soluble P (WSP). “Acidic low P” soil (black circles), “neutral moderate P” soil (grey triangles) and “alkaline moderate P” soil (white dots), the pooled standard deviation for each soil and parameter is displayed in the legend

plant during growth. In fact both extraction pools have been successfully related to soil P uptake by plants (Brookes et al. 1983; Sibbesen 1983). The deviation of the slope from 1 in Fig. 5 is presumably due to the higher fertilization rate in the soil-fertilizer incubation experiment ( $100 \text{ mg P kg}^{-1} \text{ soil}$ ) than in the growth trial ( $50 \text{ mg P kg}^{-1} \text{ soil}$ ). Nevertheless, we might as well discuss the effect of soil chemical properties or SSA characteristics on the plant P availability by explaining the fate of fertilizer P in the soil-fertilizer system in the absence of a plant. Morel and Fardeau (1991) showed that in fact no plant is needed to assess the contribution of applied P to plant nutrition since the amount of diammonium phosphate taken up by English ryegrass was well predicted by an isotopic exchange procedure applied to fertilized soils.

The fertilizer P recovery in the resin and  $\text{NaHCO}_3$  pool of the soil-fertilizer incubations with SSACa and SSAMg was generally higher than the respective proportion found when SSACa and SSAMg were extracted alone. This indicates that in the soil further dissolution of P from SSACa and SSAMg took place. Since no significant differences were found for the time points we assume that the dissolution proceeded fast and a new equilibrium between soil and fertilizer P phases was established within few days.

For SSACa, the fertilizer P recovery in the resin and  $\text{NaHCO}_3$  pool was below 20 % for all soils. For SSAMg it ranged from 46 to 20 % from the “acidic low P” to the “alkaline moderate P” soil. For WSP the fertilizer P recovery remained the same irrespective of the soil. This shows that the dissolution of P phases in SSACa and SSAMg was strongly affected by soil chemical properties and that it was generally lower for SSACa than for SSAMg. Both observations can be explained by the P speciation and physical properties of the respective SSA. The sequential extraction of SSACa and SSAMg revealed a high proportion of P associated with Ca. This is in accordance with crystallographic observations by Peplinski et al. (2009) and Adam et al. (2009) who detected chlorapatite ( $\text{Ca}_5(\text{PO}_4)_3\text{Cl}$ ) in SSACa and combinations of chlorapatite and the Mg containing phosphates standfieldite ( $\text{Ca}_4\text{Mg}_5(\text{PO}_4)_6$ ) and farringtonite ( $\text{Mg}_3(\text{PO}_4)_2$ ) in SSAMg. Similar to apatite in rock phosphate, the dissolution of these minerals might be controlled by the supply of protons and by the P and cation concentration in the soil solution (Hedley et al. 1995; Morel and Fardeau 1990a; Rajan et al. 1996). This explains the decreasing plant P availability of SSAMg with increasing pH, soil P availability or base saturation as these

parameters gradually change from the “acidic low P” to the “alkaline moderate P” soil (Table 1). Furthermore, we suggest that the higher plant P availability of SSAMg than SSACa was related to a higher solubility of Mg phosphate species and a lower crystallinity of SSAMg (Table 1). While little is known about the solubility of standfieldite and farringtonite, the degree of crystallinity is known to be an important parameter for mineral dissolution (Harley and Gilkes 2000).

The fertilizer P recoveries in the resin and  $\text{NaHCO}_3$  extraction pool (Table 6) and the significant pool size changes following a fertilizer application in the sequentially extracted soil-fertilizer incubations (Table 5) further suggest, that 82 to 91 % of P in SSACa remained as rather insoluble Ca phosphate and did not become available within a time period relevant for plant growth. For SSAMg between 55 to 80 % of the added P might have either become strongly sorbed to Fe, Al or soil organic surfaces or entered the HCl extractable pool. For WSP, 32 to 41 % of the readily soluble P was presumably transformed into less labile P.

## Conclusions

The plant P availability of SSAMg was higher than for SSACa because of a probable lower thermo-dynamic stability of the contained P phases and/or because of its lower crystallinity. The dissolution of P from thermo-chemically treated SSA was influenced by chemical soil properties: at an acidic to neutral pH and at low to moderate soil P availability, SSAMg proved to be an efficient P fertilizer and its fertilizer P recovery in the plant and in the plant available P pools of soil-fertilizer incubations was comparable to the recovery of WSP. The resulting effectiveness relative to WSP was 88 % (“acidic low P” soil) and 71 % (“neutral moderate P” soil). The relative effectiveness of both SSA based fertilizer strongly decreased with increasing soil pH. For Italian ryegrass, a soil-fertilizer incubation study revealed to be powerful and sufficient to predict the plant P availability of fertilizers. However, isotope dilution techniques are indispensable to quantify the effective transfer of fertilizer P to plants, in particular in situations where the basic requirements for the non-isotopic method are not met.

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