The recycling potential of phosphorus in secondary resources

Resirkuleringspotensialet til fosfor i sekundære ressurser

Philosophiae Doctor (PhD) Thesis

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SUMMARY

Mineable rock phosphate is a limited resource. Replacing mineral phosphorus (P) fertiliser with P-rich secondary resources is one way to manage P more efficiently. The Norwegian potential to replace mineral P fertiliser with total P in secondary resources was analysed here using substance flow analysis. The results obtained were integrated with data on P plant-availability in secondary resources and showed that, theoretically, plant-available P in manure alone could fulfil the Norwegian demand for P fertiliser. However, P in manure is inefficiently utilised due to the geographical segregation of animal husbandry and arable farming, which contributes to considerable P over-application to agricultural soil. In Norway, agriculture and aquaculture drive P consumption and losses at similar levels, and the amount of P in fish excrement and feed losses from off-shore aquaculture pens (fish sludge) is of the same order of magnitude as P in manure. Fish sludge is currently not collected or utilised, but lost to coastal marine waters. All other secondary resources represent relatively small amounts of P, but may still be important regionally. Political incentives are thus needed in current regulations to efficiently close P cycles.

To achieve P recycling in practice, it is essential to know the relative agronomic efficiency (RAE) of secondary P products compared with mineral fertiliser. Nine secondary P products were analysed here: Two biomass ashes, meat bone meal, fish sludge, catering waste, two food waste-based digestate products, dairy manure and chicken manure. The RAE of these secondary products studied in a bioassay with ryegrass (*Lolium multiflorum*) varied widely, partly depending on soil pH. Fertilisation effects were mainly attributable to the solubility of the inorganic P species contained in the secondary products. Combining sequential chemical fractionation and non-destructive speciation methods revealed that P was mainly present as calcium phosphates of differing solubility. Further analysis showed that microbial and physicochemical soil processes induced by the secondary P products studied were of little overall importance for total P uptake in barley (*Hordeum vulgare*). Based on the results obtained, two chemical extraction methods for predicting the RAE of secondary products with unknown fertilisation effects are suggested: At soil pH <6.5, RAE should be predicted by the fraction of inorganic P in the secondary product (% of total P) that is extractable in H_2O. At soil pH >6.5, RAE should be predicted by the fraction of inorganic P (% of total P) that is extractable in 0.5 M $NaHCO_3$ (Olsen P).

SAMMENDRAG

Fosfatstein er en ikke-fornybar ressurs. Å erstatte fosfor (P) i mineralgjødsel med P-rike sekundære ressurser er en måte for å forbedre forvaltningen av P. For å kvantifisere den totale mengden P i sekundære ressurser i det norske matvaresystemet ble materialstrømsanalyse anvendt. Resultatene fra materialstrømsanalysen ble integrert med data på plantetilgjengelighet av P i sekundære ressurser. Studien viste at husdyrgjødsel alene inneholder tilstrekkelig plantetilgjengelig P til å dekke det norske behovet for P-gjødsel. P-utnyttelsen er imidlertid dårlig selv om husdyrgjødselen blir tilbakeført til landbruksjord. Dette skyldes kanaliseringspolitikken som over tid har resultert i en konsentrasjon av husdyrproduksjon på Vestlandet og kornproduksjon på Østlandet. Dette bidrar til en betydelig akkumulering av P i landbruksjord. Norsk landbruk og oppdrettsnæring bruker og taper omtrent like mye P, og totalmengden P i fiskeslam (ekskrementer og fôrrester fra oppdrettsanlegg) er i samme størrelsesorden som P i husdyrgjødsel. Fosfor i fiskeslam blir per i dag ikke utnyttet, men forsvinner i havet. Alle andre sekundære ressurser representerer små P-mengder, men kan likevel være betydningsfulle ressurser regionalt. Det er derfor et behov for sterkere politiske insentiver i dagens regelverk for å lukke P-kretsløpet.

Den relative gjødseleffekten (RAE) av sekundære P-produkter sammenlignet med mineralgjødsel, må være kjent for å kunne realisere effektiv resirkulering av P i praksis. Ni sekundære P-produkter ble analysert: To biomasseasker, kjøttbeinmel, fiskeslam, cateringavfall, to biorester basert på matavfall, storfegjødsel og hønsegjødsel. De sekundære produktene som ble studert i et potteforsøk med raigras (*Lolium multiflorum*), hadde en varierende RAE delvis avhengig av jordas pH. Gjødseleffektene kunne i stor grad forklares med løseligheten av de uorganiske P-forbindelsene i de sekundære produktene. Gjennom sekvensiell kjemisk fraksjonering og ikke-destruktive karakteriseringsmetoder ble det klart at P hovedsakelig foreligger som en kompleks blanding av uorganiske P-forbindelser, fortrinnsvis kalsiumfosfater med ulik løselighet. Effektene av de studerte sekundære P-produktene på mikrobielle og fysisk-kjemiske jordprosesser hadde liten betydning for totalt P-opptak i bygg (*Hordeum vulgare*). Det anbefales to kjemiske ekstraksjonsmetoder for å predikere RAE for sekundære produkter med ukjente gjødseleffekter. Hvis pH er <6.5, anbefales det å predikere RAE med andelen av uorganisk P (% av total P) i det sekundære produktet som er løselig i H_2O. Hvis pH er >6.5, anbefales det å predikere RAE med andelen av uorganisk P (% av total P) som er løselig i 0.5 M $NaHCO_3$ (Olsen P).

LIST OF PAPERS

Paper I

Hamilton HA, Brod E, Hanserud OS, Gracey EO, Vestrum MI, Bøen A, Steinhoff FS, Müller DB, Brattebø H (2015) Investigating cross-sectoral synergies through integrated aquaculture, fisheries and agriculture phosphorus assessments: A case study of Norway. Journal of Industrial Ecology: Doi:10.1111/jiec.12324

Paper II

Hamilton HA, Brod E, Hanserud OS, Müller DB, Brattebø H, Haraldsen TK. Estimating the recycling potential of secondary phosphorus resources by integrating substance flow analysis and plant-availability. Manuscript

Paper III

Brod E, Øgaard AF, Hansen E, Wragg D, Haraldsen TK, Krogstad T (2015) Waste products as alternative phosphorus fertilisers part I: Inorganic P species affect fertilisation effects depending on soil pH. Nutrient Cycling in Agroecosystems 103: 167–185

Paper IV

Brod E, Øgaard AF, Haraldsen TK, Krogstad T (2015) Waste products as alternative phosphorus fertilisers part II: Predicting P fertilisation effects by chemical extraction. Nutrient Cycling in Agroecosystems 103: 187–199

Paper V

Brod E, Øgaard AF, Krogstad T, Haraldsen TK, Frossard E, Oberson A. Drivers of phosphorus uptake by barley following secondary resource application. Accepted for publication in Frontiers in Nutrition and Environmental Sustainability

ABBREVIATIONS

AL	Norwegian standard soil test: Extraction with 0.1 M ammonium lactate and 0.4 M acetic acid adjusted to pH 3.75 (Egnér et al. 1960)
C_P	Phosphorus concentration in the soil solution
E_{1min}	Isotopically exchangeable inorganic phosphorus in soil within 1 min
ICP-OES	Inductively coupled plasma-optical emission spectroscopy
MinP	Mineral phosphorus fertiliser
NoP	No phosphorus fertiliser
P	Phosphorus
^{31}P MAS-NMR	Magic angle spinning nuclear magnetic resonance spectroscopy targeting ^{31}P
^{33}P	Radioisotope of phosphorus
Pdf	Phosphorus derived from the fertiliser, soil or seed
Pdff Resin P	Resin-extractable phosphorus derived from the fertiliser
PFA	Phosphorus flow analysis
Pi	Inorganic phosphorus
PUE	Apparent phosphorus use efficiency
RAE	Relative agronomic efficiency
Resin P	Resin-extractable phosphorus
SA	Specific activity
Secondary resource	Untreated or treated phosphorus-rich material, e.g. municipal solid waste
Secondary product	Treated phosphorus-rich material, e.g. anaerobic digestate or compost of source-separated municipal solid waste
XRD	X-ray powder diffraction

TABLE OF CONTENTS

1 INTRODUCTION

Mineable rock phosphate is a limited resource. However, industrialised agroecosystems are currently far from managing phosphorus (P) efficiently and Europe’s food production is largely dependent on imports of mined rock phosphate (van Dijk et al. 2015). The greatest reductions in P imports could be obtained by replacing mineral fertiliser with recycled P from secondary resources (Schoumans et al. 2015). In practice, P recycling is seldom an economically or agronomically viable alternative to the use of mineral P fertiliser.

Implementing political incentives in current regulations can promote P recycling. This requires decision makers to have an understanding of the relative importance of secondary resources compared with the demand for P fertiliser. Phosphorus flow analysis (PFA) is recognised as a useful tool for estimating the P recycling potential of food systems by quantifying the amount of P in secondary resources and has been applied in a range of countries (e.g. Antikainen et al. 2005; Senthilkumar et al. 2012a; Cooper and Carliell-Marquet 2013; Cordell et al. 2013; Klinglmair et al. 2015; Smit et al. 2015). Manure is often identified as the most important secondary resource in terms of total P, e.g. in Europe (Ott and Rechberger 2012; van Dijk et al. 2015). However, in Norway, export-orientated aquaculture and fishery industries have much greater economic importance than domestic agriculture. Norway was the largest per capita aquaculture producer in the world in 2011 (FAO 2011) and the production of Norwegian aquaculture is expected to increase five-fold by 2050 (DKNVS and NTVA 2012). However, the amount of P in secondary resources generated in the Norwegian aquaculture and fishery sector has so far never been quantified. Furthermore, an integrated PFA study considering agriculture, aquaculture and fisheries within the same system has not yet been conducted, despite significant cross-sectoral dependencies through plant-based feed input to aquaculture.

Although many previous studies have attempted to determine the P recycling potential of food systems by using PFA as a method, all have neglected the greatest barrier to P recycling, namely the plant-availability of P in secondary resources. Growth experiments have shown that the P fertilisation effects of secondary resources vary widely, even between production plants and batches of a particular product, and that they are usually lower than those of mineral fertiliser (e.g. Kratz et al. 2010; Cabeza et al. 2011; Delin 2015). Therefore, we hypothesised that the P recycling potential is clearly over-estimated when it is calculated by traditional PFA without considering the quality of P in secondary resources.

In order to achieve P recycling in practice, it is essential to know the relative agronomic efficiency (RAE) of secondary P products compared with mineral fertiliser. In mineral fertiliser, P is commonly present as simple, water-soluble compounds (e.g. monocalcium phosphate). In secondary products too, P is mainly present as inorganic P. However, inorganic P species in secondary products include a large variety of compounds with accompanying elements such as Ca, Fe and Al that are characterised by variable solubility in soil (Hedley and McLaughlin 2005). Phosphorus fertilisation effects of secondary products can be considerably affected by the pH in the target soil. The solubility of Ca phosphates decreases with increasing soil pH, whereas the solubility of Fe-/Al-bound P decreases with decreasing soil pH (Lindsay 1979). Growth experiments are the most reliable method to determine the RAE of secondary P products, but are too time-consuming to be used as the standard procedure. Therefore simple laboratory methods are required that can be used to predict the RAE of secondary P products when fertilisation plans are established, taking into consideration the pH in the target soil.

Determining inorganic P species in secondary P products is one option to explain the P fertilisation effects of secondary products. Different non-destructive speciation methods have already been used to identify inorganic P species in secondary products, such as X-ray powder diffraction (XRD) and solid-state magic angle spinning nuclear magnetic resonance spectroscopy targeting P (^{31}P MAS-NMR) (e.g. Hinedi et al. 1989; Frossard et al. 1994a, 1997, 2002; Hunger et al. 2004, 2008; Güngör et al. 2007; Nanzer et al. 2014). Sequential chemical fractionation methods were originally developed to study intrinsic inorganic P pools of different solubility in soils, but have also been applied to a variety of secondary products (e.g. Sharpley and Moyer 2000; Ylivainio et al. 2008; García-Albacate et al. 2012; Nanzer et al. 2014). However, so far only a few studies have addressed the relationship between inorganic P species in secondary products and their P fertilisation effects, including the dependence on soil pH (Ylivainio et al. 2008; Nanzer et al. 2014).

Applying chemical standard extraction methods to secondary P products is another option to predict P fertilisation effects and is an operational and straightforward approach. In Norway, it is mandatory to indicate the availability of P in secondary products by stating the fraction of P that is extractable in 0.1 M ammonium lactate and 0.4 M acetic acid adjusted to pH

3.75 (P-AL) according to Egnér et al. (1960) (Norwegian Ministry of Agriculture and Food 2003). This is also the standard soil test used in agriculture. However, the relationship between the fraction of AL-extractable P in secondary P products and their RAE is in fact unknown. A previous greenhouse study has shown poor relationships between AL-extractable P in meat bone meal and wood ash and P uptake in spring cereals (Brod et al. 2014). In other studies, a range of extraction methods, developed for estimation of either plant-available P in soils or fertilisation effects of mineral fertiliser, have been compared with the fertilisation effects of secondary P products, but the results are inconclusive (e.g. Alt et al. 1994; Velthof et al. 1998; Kratz et al. 2010). We hypothesised that distinguishing readily available and late-season fertilisation effects and considering pH in the target soil improves the ability of chemical extraction methods to predict the P fertilisation effects of secondary products.

Secondary P products are complex materials. Therefore, in addition to being affected by the solubility of intrinsic P species, P uptake by plants following secondary P product application might be influenced by the effects on physicochemical or microbial soil processes. Many secondary P products contain organic matter, meaning that organic carbon (C) is applied to the soil when they are used as fertilisers. Organic C application can trigger microbial processes which may cause P immobilisation, P solubilisation or desorption of soil P (Øgaard 1996; Jakobsen et al. 2005; Oberson and Joner 2005). Other secondary P products contain inorganic C, e.g. in the form of calcium carbonate ($CaCO_3$), which neutralises pH in acid soils with associated effects on P availability. The solubility of P in secondary products and mineral fertiliser can also differently affect physicochemical soil processes depending on equilibrium processes in the target soil, since increased phosphate concentration in the soil solution can result in reduced phosphate release from the soil (Achat et al. 2014a). Using radioisotopes of P in growth and incubation experiments provides the possibility to differentiate between plant uptake of P deriving from fertiliser and soil and to study physicochemical or microbial P processes in soil/plant systems. So far, the P fertilisation effects of secondary products have mainly been studied by the difference method (e.g. Erich 1991; Jeng et al. 2006; Ylivainio et al. 2008; Kratz et al. 2010; Cabeza et al. 2011; Delin 2015). The effects of secondary P products on physicochemical or microbial soil processes and their importance for plant P uptake are therefore still poorly understood.

The main objectives of this thesis were:

1. To determine the secondary resources representing the largest amounts of total P in Norway (**Paper I**).
2. To estimate the Norwegian P recycling potential considering plant-availability of P in secondary resources (**Paper II**).
3. To explain the P fertilisation effects of secondary products by inorganic P species present at two distinct soil pH levels (**Paper III**).
4. To determine the standard extraction method which best predicts the P fertilisation effects of secondary products at two distinct soil pH levels (**Paper IV**).
5. To explore the effects of secondary products on physicochemical and microbial soil processes and their importance for P uptake in plants at two distinct soil pH levels (**Paper V**).

2 MATERIALS AND METHODS

2.1 Phosphorus flow analysis

To determine the most important Norwegian secondary P resources in terms of total P, a systems flow analysis (see e.g. Brunner and Rechberger 2004) was conducted on the Norwegian P system (**Paper I**). The system was defined as the economic zone of Norway, including coastal areas where aquaculture production occurs and the marine waters where Norwegian fisheries operate. In our definition, the Norwegian P system consisted of the key processes plant production, animal husbandry, aquaculture and fisheries, food processing and human consumption, and waste management. In an attempt to equal out annual variations, we averaged data from the years 2009, 2010 and 2011 when quantifying P in all associated flows. Data were primarily collected from government statistics, reports, company publications, expert interviews and scientific publications. For details of the method used, see **Paper I**.

Furthermore, we estimated the Norwegian P recycling potential considering plant-availability of P in secondary resources, in order to investigate whether P recycling could meet the demand for P fertiliser in Norway (**Paper II**). To this end, we integrated the results of the PFA (**Paper I**) with the relative agronomic efficiency (RAE, see section 2.3.3 of this thesis) of the most important Norwegian secondary P resources. The RAE is a relative measure of the fertilisation effects of secondary resources and was chosen over an absolute parameter (e.g. apparent phosphorus use efficiency, PUE) because it allowed us to estimate the ability of secondary resources to replace mineral P fertiliser. The RAE of secondary P resources was determined by growth experiments (**Paper IV**, **Paper V**) and using data taken from the literature. We considered the treatment technologies applied to secondary P resources in 2009-2011. If insufficient information was available, we considered the most likely pathways for recycling of secondary P resources in Norway or made assumptions based on available data. For details of the method, see **Paper II**.

2.2 Secondary P products included in the experimental work

For the experimental work (**Papers III-V**), a wide range of secondary P products representing the most important secondary P resources in Norway was selected. Secondary

P products included in the studies are described in Table 1, while Table 2 provides an overview of selected properties. For details of methods used for their determination, see **Papers III** and **Paper V**. The chemical characteristics of the secondary P products were studied using untreated, dried (55°C) and sieved (≤ 2 mm) or milled samples. Sewage sludge was excluded from the experimental work because of its entirely different intrinsic chemical characteristics compared with other secondary P resources. Options for recycling of P with Norwegian sewage sludge are covered in other studies, e.g. Øgaard and Brod (forthcoming).

Table 1. Description of secondary P products used in the experimental work

No.	Secondary P product	Description
1	Wood ash	Bottom ash from a grate-fired boiler system at the Moelven Østerdalsbruket AS mill. Parent material was timber unsuitable for industrial use.
2	Cereal ash	Bottom ash from a grate-fired boiler in the Eidsiva Bioenergi district heating system in Kongsvinger. Parent material was timber unsuitable for industrial use and cereal residues from the local mill.
3	Meat bone meal	Commercial product originating from a slaughterhouse in Hamar and sold by Norsk Protein AS. Slaughterhouse waste of category III according to EC (2002) was stabilised and sanitised at 133°C and 3.0 bar for 20 min.
4	Fish sludge	Collected from the on-land salmon Åsen settefisk hatchery. Fish are bred in closed cages until they are approximately 1 year old. Effluent containing faeces and feed residues (mainly fish meal and soya) was mechanically filtered before the material was treated on-site in a reactor developed by the company Global Enviro.
5	Catering waste	Source-separated catering waste from Rica Sunnfjord Hotel, treated in a reactor developed by the company Global Enviro after removal of grease and water by steam and pressure.
6	Liquid digestate	Untreated digestate based on anaerobic treatment of source-separated household waste collected at the Mjøsanlegget biogas plant.
7	Solid digestate	Solid phase after centrifugation of liquid digestate based on anaerobic treatment of source-separated household waste collected at the Mjøsanlegget biogas plant.
8	Dairy manure	Dried slurry (faeces and urine) of dairy cows collected from cattle houses at the Norwegian University of Life Sciences.
9	Chicken manure	Stabilised, sanitised and pelletised chicken manure produced by Norsk Naturgjødsel.

Table 2. Selected properties of secondary P products. Co = organic C, Po = organic P, Nmin = mineral N (NO_3^- and NH_4^+), n.d. = not detectable

		Wood ash	Cereal ash	Meat bone meal	Fish sludge	Catering waste	Liquid digestate	Solid digestate	Dairy manure	Chicken manure
pH		13	9.6	6.2	5.4	5.8	7.0	7.7	7.0	6.7
Specific surface area	m^2 g^{-1}	0.6	4.5	0.1	0.1	0.1	0.1	2.8	0.8	0.4
Dry matter	g 100g^{-1}	99.6	97.6	96.2	95.0	94.5	2.4	25.0	5.8	93.3
Organic matter	g 100g^{-1} DM	n.d.	17.0	66.6	87.6	81.1	64.6	66.2	81.6	81.7
C	g kg^{-1} DM	19	111	368	503	449	412	400	470	427
Co	% of total C	5.5	124	72	75	82	69	81	84	71
P	g kg^{-1} DM	17	51	54	21	10	12	15	6	10
Po	% of total P	n.d.	n.d.	2	15	7	26	22	24	54
N	^{a}g kg^{-1} DM, ^{b}g L^{-1}	n.d.	4^a	86^a	71^a	50^a	2.3^b	56^a	3.1^b	45^a
Nmin	^{a}g kg^{-1} DM, ^{b}g L^{-1}			5.0^a	2.6^a	5.1^a	1.7^b	7.2^a	1.3^b	0.041^a
K	g kg^{-1} DM	56	93	4	3	6	45	6	42	25
S	g kg^{-1} DM	34	2	34	48	35	59	65	42	58
Ca	g kg^{-1} DM	310	27	110	37	59	33	62	11	44
Mg	g kg^{-1} DM	25	26	3	3	2	8	5	6	6
Al	g kg^{-1} DM	19.1	3.8	0.2	0.3	0.1	8.6	13.8	0.4	0.5
Fe	g kg^{-1} DM	7.6	4.3	0.5	0.7	0.4	2.8	5.9	1.3	1.0

2.3 Fertilisation effects

Fertilisation effects of secondary P products were determined by the difference method (**Paper III**, **Paper IV**) and the ^{33}P indirect labelling method (**Paper V**). In both cases, fertilisation rates were calculated based on total P concentration in secondary P products (Table 2). Fertiliser treatments were applied equivalent to approximately 12 mg P kg^{-1} soil (**Paper III**, **Paper IV**) and 30 mg P kg^{-1} soil (**Paper V**). Fertilisation effects of secondary P products were compared with a treatment receiving no P fertilisation (NoP) and increasing rates of water-soluble mineral P fertiliser (MinP, $Ca(H_2PO_4)_2$). All other essential plant nutrients were applied in sufficient amounts. In both experiments, fertilisation effects of secondary P products were studied at two different pH levels in the same soil.

2.3.1 Difference method

For the studies presented in **Paper III** and **Paper IV**, the fertilisation effects of all secondary P products listed in Table 1 were studied by a bioassay using the difference method. The underlying assumption in the difference method is that unfertilised and fertilised plants take up the same amount of P from the soil, i.e. that the amendments do not affect soil P availability. Here, the P uptake by plants fertilised with the secondary P product was compared with the average P uptake by NoP plants. The difference in P uptake between the two treatments is defined as the P fertilisation effect of the secondary P product. We used ryegrass (*Lolium multiflorum* var. Macho) as the experimental crop, which was harvested four times throughout the season, and a nutrient-deficient 8:2 (v/v) blend of sand and sphagnum peat as the experimental soil. The model soil used is the reason why this experiment is referred to as a bioassay in the following text. Liming each pot (6.37 kg soil per 5 L) with two different amounts of $CaCO_3$ resulted in pH 5.5 (pH level 1) and pH 6.9 (pH level 2) in the soil of the NoP treatments after ryegrass harvest 2. There were three replicates per fertiliser treatment. The bioassay was conducted outdoors under a transparent roof, where the plants were protected from precipitation but otherwise exposed to daylight and outdoor climate. For details of the method, see **Paper III** or **Paper IV**.

Apparent phosphorus use efficiency (%) was calculated as:

$$\text{PUE (\%)} = \frac{\text{P uptake (P+)} - \text{P uptake (NoP)}}{\text{P applied}} \times 100 \qquad (1)$$

where:

P uptake (P+) (mg P pot^{-1}) = Amount of P taken up in aboveground biomass by the fertilised plants

P uptake (NoP) (mg P pot^{-1}) = Amount of P taken up in aboveground biomass by the average NoP plants

P applied (mg P pot^{-1}) = Amount of P applied with the fertiliser.

2.3.2 ^{33}P indirect labelling method

For the study presented in **Paper V**, the fertilisation effects of fish sludge, meat bone meal, wood ash and dairy manure were studied in a pot experiment using the ^{33}P indirect labelling method to differentiate between plant uptake of P deriving from the fertiliser and the soil (Morel and Fardeau 1989; Frossard et al. 1996; Frossard et al. 2011). Barley (*Hordeum vulgare* var. Heder) was chosen as the experimental crop because of its sensitivity to P availability, particularly during establishment (Kristoffersen et al. 2005), and because it is the most important cereal crop in Norway due to its short growing period. The experimental soil originated from plots of a long-term field experiment in Norway that had received 0 kg P yr^{-1} and 0 or 5 kg K yr^{-1} since 1966. The soil is classified as an Albeluvisol according to the World Reference Base for Soil Resources (NIBIO 2015). Plant-available P in the soil was estimated to be low (4.4 mg P-AL 100g^{-1}) based on the Norwegian standard soil test and Norwegian fertiliser recommendations. Before the experiment, the soil was divided into two equal portions, one of which was left unlimed and the other was limed with $CaCO_3$. After incubation for 2.5 months, pH was 5.3 and 6.2 in the unlimed and the limed soil, respectively. There were four replicates of each fertiliser treatment. Before application of the fertiliser, the pool of plant-available P in the soil was labelled with carrier-free ^{33}P-orthophosphate at a rate of 1.1 MBq kg^{-1} soil per pot (1 kg soil). Therefore, fertiliser recovery (P+) (%) in aboveground biomass of barley after application of secondary P products could be calculated by comparing P derived from the fertiliser (Pdf fertiliser, mg P kg^{-1} soil) with P applied (mg P kg^{-1} soil):

$$\text{Fertiliser recovery (P +)(\%)} = \frac{\text{Pdf fertiliser}}{\text{P applied}} \times 100 \qquad (2)$$

where Pdf fertiliser (mg P kg^{-1} soil) was calculated as:

$$\text{Pdf fertiliser} = \text{P uptake (P +)} - \text{Pdfsoil (P +)} - \text{Pdfseed (P+)}$$

where Pdf soil (P+) is the amount of P derived from the soil (mg P kg^{-1} soil) in aboveground biomass of the fertilised plant, which was calculated as:

$$\text{Pdf soil (P+)} = \frac{\text{SA (P+)}}{\text{SA (NoP)}} \times [\text{P uptake(P+)} - \text{Pdf seed (P+)}] \qquad (3)$$

where:

SA (P+) (Bq mg^{-1} P) = Specific activity in aboveground biomass of fertilised plants with P uptake corrected for Pdf seed

SA (NoP) (Bq mg^{-1} P) = Average specific activity in aboveground biomass of plants receiving no P fertiliser with P uptake corrected for Pdf seed

Pdf seed (P+) (mg P kg^{-1} soil) = P derived from the seed in aboveground biomass of fertilised plants, which was calculated from an additional seed P experiment as described in **Paper V**.

2.3.3 Relative agronomic efficiency (RAE)

In **Paper II**, **Paper IV** and **Paper V**, RAE was used as a parameter representing the ability of secondary P products to replace water-soluble MinP. Figure 1a provides a schematic illustration of how RAE was defined: MinP was used as a benchmark by assuming that all MinP is available for plants (RAE defined as 100%). The RAE of secondary P products represents the fraction that is readily available to plants and is defined as the relative P fertilisation effect compared with MinP. For example, if a farmer aims at applying 10 kg P ha^{-1} and uses a secondary P product with RAE = 60%, in order to reach an effective fertilisation effect of 10 kg P ha^{-1}, they can either apply an additional 4 kg P ha^{-1} in the form of mineral fertiliser or increase the dose of the secondary product to 16.7 kg P ha^{-1}.

When the difference method was applied (**Paper IV**), RAE was calculated as illustrated in Figure 1b according to:

$$\mathrm{RAE} = 100 \times \frac{\mathrm{X_1}}{\mathrm{P\ applied}} \tag{4}$$

$$\mathrm{X_1} = \frac{(\mathrm{Y_1} - \mathrm{b})}{\mathrm{a}} \tag{5}$$

where:

Y_1 = P uptake in in aboveground biomass obtained after application of the secondary P product

X_1 = Amount of MinP to which P uptake in aboveground biomass after fertilisation with secondary P product is equivalent

a and b = slope and intercept obtained from linear regression with Y = P uptake in aboveground biomass as an effect of X = increasing application rate of MinP.

When the indirect labelling method was used (**Paper V**), RAE was calculated as:

$$\mathrm{RAE} = \frac{\mathrm{Fertiliser\ recovery\ (P+)}}{\mathrm{Fertiliser\ recovery\ (MinP)}} \times 100 \tag{6}$$

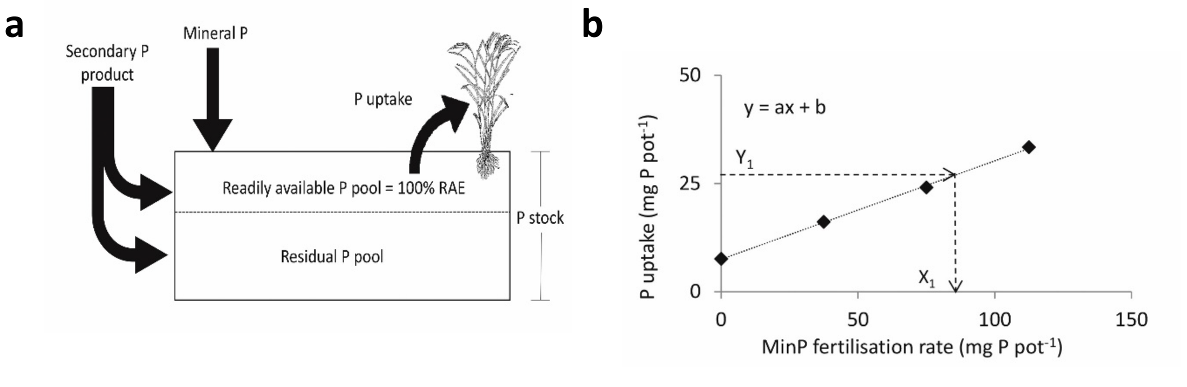

Figure 1. A) Conceptual drawing of relative agronomic efficiency (RAE). B) Calculation of RAE by the difference method with Y_1 = P uptake in aboveground biomass obtained after application of secondary P product and X_1 = Amount of MinP to which P uptake after secondary product application is equivalent, where a and b are determined from linear regression with Y = P uptake as an effect of X = increasing application rate of MinP.

2.4 Characterisation of inorganic P in secondary products

A range of laboratory methods was applied to the secondary P products studied, in order to characterise the inorganic P they contained and to explain observed fertilisation effects.

2.4.1 Chemical extractions

With the aim of determining inorganic P (Pi) fractions of different solubility in the secondary products, the sequential chemical fractionation scheme according to Hedley et al. (1982) was applied (**Paper III**). This sequential chemical fractionation is operationally defined to extract:

- Readily available Pi by H_2O
- Labile Pi by 0.5 M $NaHCO_3$
- Pi adsorbed to Fe-/Al-(hydr)oxides or Fe-/Al-phosphates by 0.1 M NaOH
- Stable Ca phosphates by 1 M HCl
- Total residual P, here determined after digestion in concentrated HNO_3 in an ultraclave.

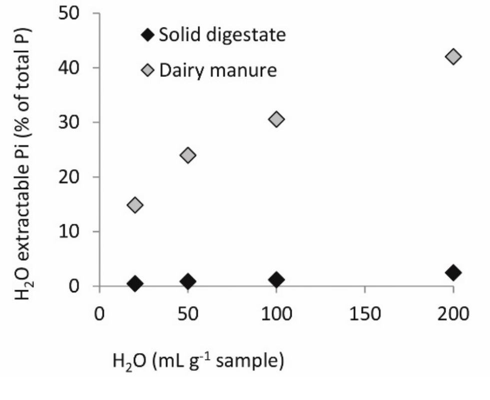

Figure 2. Fraction of inorganic P (Pi) in dairy manure and solid digestate extracted by H_2O, expressed as % of total P with increasing ratio of H_2O (mL g^{-1} sample).

The sequential chemical fractionation according to Hedley et al. (1982) was originally developed for agricultural soils with a total P concentration of 0.2-5 g P kg^{-1} and with an average of 0.6 g P kg^{-1} (Lindsay 1979). The secondary P products studied in this thesis contained 6-54 g P kg^{-1} (Table 2). The pH in some secondary P products was also considerably higher than expected for agricultural soils. Therefore, a modified version of the fractionation scheme (Sharpley and Moyer 2000) with an increased sample:solution ratio was applied to duplicate samples to avoid the extraction capacity being unintentionally reached as a result of equilibrium reactions or significant effects of secondary P products on pH in the extraction solution (**Paper IV**). To determine the appropriate sample:solution ratio, we studied the effect of increasing sample:solution ratios of H_2O on extractable Pi (Figure 2). Finally, we chose a sample:solution ratio of 1:200, as this was the value at which the response of extractable Pi in secondary P products with large fractions of readily available Pi (e.g. dairy manure) started to flatten out, while extractable Pi in secondary P products with small fractions of readily available P (e.g. solid digestate) could still be detected.

For the study presented in **Paper IV**, eight single extractions were also applied to duplicates of each secondary P product. All extraction methods were originally developed for estimation of either plant-available P in soil or P fertilisation effects of mineral fertiliser. The extraction methods were conducted as originally described and compared in terms of their ability to predict readily available P and late-season P fertilisation effects in a bioassay at two soil pH levels (see section 2.3.1). For a summary of the extraction methods, see **Paper IV**.

In the studies presented in **Paper III** and **Paper IV**, Pi in extracts was assumed to be equal to orthophosphate and was analysed by the molybdenum blue method according to Murphy and Riley (1962). In addition, total P concentration in extracts was determined by analysis using inductively coupled plasma-optical emission spectroscopy (ICP-OES). Organic P in the extracts was calculated as the difference between total P and Pi.

2.4.2 Non-destructive speciation methods

With the aim of determining the inorganic P species present in secondary P products, we applied two non-destructive speciation methods. The methods are only briefly explained below. For full details of the methods and data analyses, see **Paper III**.

X-ray powder diffraction (XRD) combined with Rietveld refinements allowed us to quantify detectable Pi in P-bearing phases in the crystalline matter of secondary P products. When X-rays interact with crystalline matter, diffraction patterns which are unique to the crystal lattice are produced. In mixtures, it is possible to separate out the contributions from the crystalline phases present and determine the quantity of each using the Rietveld method (Rietveld 1969). Amorphous material is usually considered part of the background in this method, but spiking samples with e.g. Si allows the amorphous content to be quantified. Quantification of detectable Pi in P-bearing phases contained in the crystalline matter of secondary P products was hence calculated in two steps, on triplicate samples:

- Estimation of the crystalline content in secondary P products
- Quantification of Pi in P-bearing phases in secondary P products (% of total P).

Solid-state ^{31}P MAS-NMR was applied to all secondary P products. The principle of the method is simply that a nucleus confined in an external magnetic field can adsorb energy within a radiofrequency range which depends on the structural environment of that nucleus (Pierzynski et al. 2005). We applied both single-pulse ^{31}P polarisation, which targets all P atoms, and ^{1}H-^{31}P cross-polarisation, which enhances the ^{31}P signal intensity of those P nuclei that have direct or close contact with nearby protons. The peaks obtained were compared with literature data for identification of phosphate species.

2.5 Physicochemical and microbial soil processes

We also conducted soil-fertiliser incubations in order to explore the effects of fish sludge, meat bone meal, wood ash and dairy manure on physicochemical and microbial soil P processes and their importance for total P uptake in barley (**Paper V**). The same soil and fertiliser treatments and rates were used as in the pot experiment employing the ^{33}P indirect labelling method (see section 2.3.2). In addition to soil pH (7, 21 and 42 days after fertiliser application), the following parameters were determined in all fertilised and incubated soil samples (for details of the methods, see **Paper V**):

- P concentration in the soil solution (C_P) and isotopically exchangeable Pi (E_{1min}, in mg P kg^{-1}; Fardeau et al. 1996) as described by Frossard et al. (1994b), 21 days after fertiliser application. E_{1min} is considered the most important source of P for plants (Frossard et al. 1994b) and comprises Pi in the soil solution and Pi adsorbed to soil particles that are exchangeable within the first minute of isotopic exchange kinetics experiments.

- Resin-extractable P (Resin P), in which the pool of plant-available P had been indirectly labelled with carrier-free ^{33}P-orthophosphate at a rate of 5.2 MBq kg^{-1} soil. The indirect labelling allowed us to estimate the fraction of Resin P deriving from the fertiliser (Pdff Resin P %) according to:

$$\text{Pdff Resin P} = \left(1 - \frac{\text{SA (P+)}}{\text{SA (NoP)}}\right) \times 100 \qquad (7)$$

where SA (P+) (Bq mg^{-1} P) is the specific activity in the soil amended with fertiliser and SA (NoP) (Bq mg^{-1} P) is the specific activity in the soil receiving no P fertiliser.

Resin P and Pdff Resin P were studied 7 and 21 days after fertiliser application.

- Phosphorus in microbial biomass (Pmic), which was determined as the difference between extracted Pi using the anion exchange resin membranes from fumigated and non-fumigated (Resin P) soil samples. Pmic was studied 7 and 21 days after fertiliser application.

2.6 Statistical analyses

Statistical analyses were performed using JMP Pro 11.1.1 (SAS Institute Inc. 2013).

In **Paper II**, 95% confidence intervals of all RAE values determined were calculated to express their uncertainty.

In **Paper III**, **Paper IV** and **Paper V**, one- and two-way ANOVA models were applied. The data analysed were checked for normal distribution (normal quantile plots) and homogeneity of variance (residual versus fitted plots) and log-transformed where indicated. For pair-wise comparisons, Tukey's HSD test or t-tests were used at significance level $\alpha = 0.05$. Simple linear regressions were also applied, with *, **, *** representing significance at $p<0.05$, 0.01 and 0.001 probability level, respectively. Two-way ANOVA was used to test differences in slopes and intercepts.

3 MAIN RESULTS AND DISCUSSION

3.1 Norwegian P recycling potential in a systems context

Through the PFA conducted in **Paper I** (Figure 3) and integration of the results with data on the plant-availability of P in secondary resources obtained in **Paper II,** it was possible to show that Norway's demand for fertiliser P could theoretically be fully met by secondary P resources (Figure 4). However, technological and economic feasibility and social acceptance were not considered in the analysis. The total P amount generated yearly in secondary P resources was estimated to be 28,000 Mg P, with the plant-available fraction ranging between 12,700-26,300 Mg P yr^{-1}. In comparison, the P fertiliser demand in Norway was estimated to be 5800 Mg P yr^{-1}, when considering soil P levels according to Hanserud et al. (2015). Each year, 8400 Mg P are applied as mineral fertiliser.

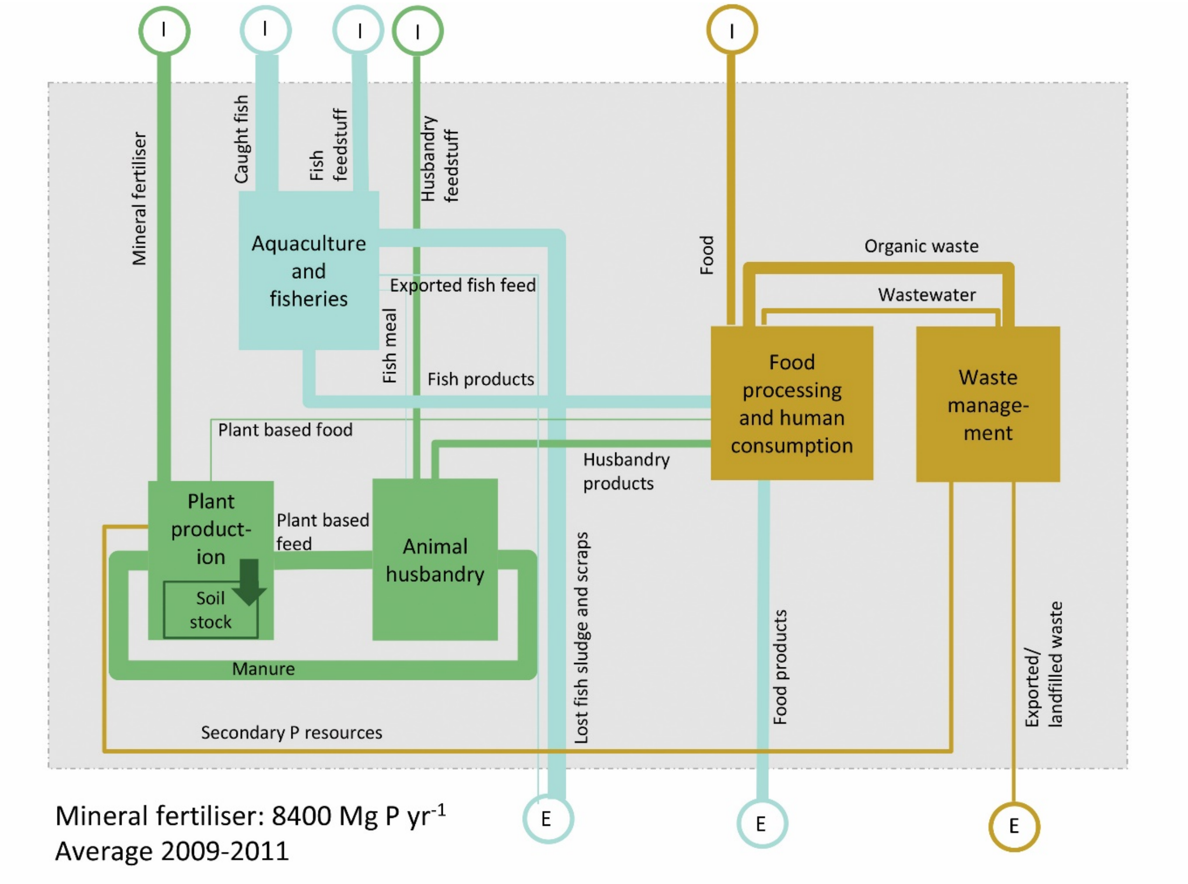

Figure 3. Simplified version of the phosphorus flow analysis (PFA) of the Norwegian food system presented in Paper I, based on averaged 2009-2011 data. The thickness of the flows reflects the amount of total P that they represent and is relative to the amount of P in imported mineral fertiliser (8400 Mg P yr^{-1}). I = P imports to the Norwegian food system, E = P exports from the Norwegian food system.

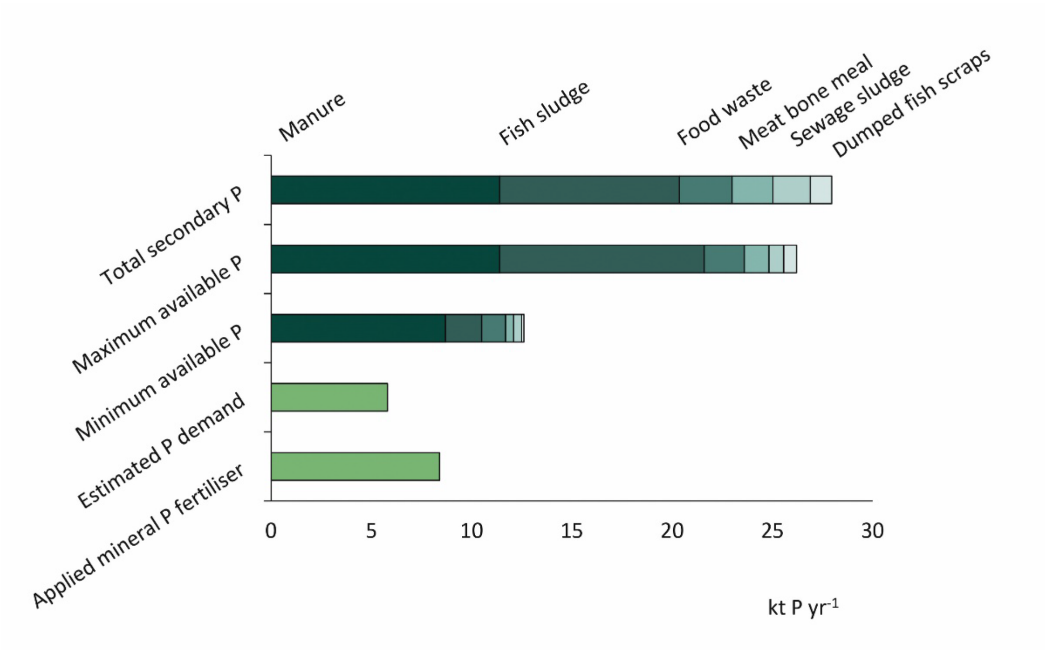

Figure 4. Sum of total P in secondary resources, maximum and minimum P recycling potential of secondary resources depending on plant-availability, estimated P fertilisation demand and mineral P fertiliser applied in 2009-2011 (Paper II).

As Figure 4 indicates, manure alone could fulfil the Norwegian demand for P fertiliser, representing a total P amount of 12,000 Mg yr^{-1} and containing between 8700-11,400 Mg yr^{-1} of plant-available P . However, manure is currently inefficiently utilised. The considerable P over-application in Norway, which results in accumulation of 12,000 Mg P yr^{-1} in the soil (Figure 3), is probably mainly due to the geographical segregation of animal husbandry and arable farming and the resulting uneven distribution of manure within the country (Hanserud et al. 2015). Phosphorus accumulation in soil associated with uneven husbandry distribution is well known also in other industrialised countries such as France (Senthilkumar et al. 2012b) and Denmark (Klinglmair et al. 2015).

In **Paper I** we could further show that, in Norway, agriculture and aquaculture drive P consumption and losses at similar levels (Figure 3). The amount of total P in fish excrement and feed losses from off-shore aquaculture pens (hereafter fish sludge) is 9000 Mg P yr^{-1}, which is of the same order of magnitude as P in animal manure. However, unlike manure, fish sludge is currently not collected or utilised as a secondary P resource, but lost to coastal marine waters. The amount of P in fish sludge is predicted to increase to 45,000 Mg P yr^{-1} by 2050 as a result of the anticipated five-fold increase in Norwegian aquaculture production, assuming that technologies remain constant. With five times more nutrients reaching the Norwegian fjord systems, the risk of eutrophication in coastal marine waters will increase considerably. Furthermore, the increased demand for imported feedstuffs such as soya will increase Norway's indirect dependence on mineral P fertiliser, unless P losses along the production line are recovered and recycled. **Paper II** showed that the amount of plant-available P in fish sludge varies widely (1900-10,300 Mg P yr^{-1}), with the higher end stretching well above 100%, which was mainly due to few observations of RAE of fish sludge included in calculation of the confidence interval. Growth experiments with fish sludge collected from land-based hatcheries (Table 1) showed good P fertilisation effects comparable with those of manure, but somewhat decreasing P fertilisation effects with increasing soil pH (**Paper III**, **Paper IV** and **Paper V**). The N fertilisation effects of fish sludge are also comparable to those of manure (Brod et al. 2012; 2014). The main challenge associated with recycling of P in fish sludge is collection of the sludge from offshore fish pens and production of transportable and safe fertiliser products.

Compared with manure and fish sludge, all other secondary resources represent relatively small amounts of P, the most important being municipal solid waste (2600 Mg P yr^{-1}), meat bone meal (2000 Mg P yr^{-1}), sewage sludge (1900 Mg P yr^{-1}) and fish scraps (1100 Mg P yr^{-1}) (Figure 4). However, when considering feasibility factors such as product quality, spatial distribution and logistics, these secondary P resources may still be important regionally. For example, liquid anaerobic digestate of source-separated municipal solid waste represents a secondary P product with relatively high RAE (**Paper IV**). Furthermore, it has a favourable ratio of plant nutrients relative to crop demand. This means that additional application of plant nutrients is often not required when anaerobic digestate of source-separated municipal solid waste is used as an alternative P fertiliser (Haraldsen et al. 2011). Meat bone meal is another example of a secondary P resource with great potential as an alternative fertiliser because it is collected at central treatment plants and has a low water content. This allows for redistribution of recovered P from meat bone meal if the right technologies are implemented, e.g. chemical extraction of P from bone ash for the production of soluble P fertilisers (Krupa-Żuczek et al. 2008).

3.2 Predicting P fertilisation effects of secondary products

In practice, efficient recycling of P in secondary products can only be achieved if mineral P fertiliser rates are reduced accordingly. As the composition of P species in secondary products and their fertilisation effects are often unknown and vary widely, the aim in **Paper III**, **Paper IV** and **Paper V** was to explain fertilisation effects and to find simple laboratory methods that could predict them and thus enable farmers to adapt their fertilisation plans accordingly.

3.2.1 Fertilisation effects

To achieve this aim, the fertilisation effects of the secondary P products listed in Table 1 were first determined by a bioassay using the difference method.

The fertilisation effects of the secondary P products studied in the bioassay (**Paper III** and **Paper IV)** varied widely, partly depending on soil pH. Differences in fertilisation effects between the treatments were most pronounced at harvest 1 and levelled off thereafter. Dairy manure resulted in higher PUE as the sum of all harvests than MinP at both pH levels (Figure 5), which is in agreement with earlier findings on the P fertilisation effects of cattle manure (e.g. Smith and van Dijk 1987). At pH level 1 (approximately pH 5.5), fish sludge and liquid digestate resulted in equally high PUE as MinP. All the other secondary P products resulted in lower PUE than MinP. At pH level 2 (approximately pH 6.9), all secondary P products except dairy manure resulted in lower PUE than MinP. At pH level 2, meat bone meal, fish sludge and catering waste resulted in significantly lower PUE than at pH level 1.

The fertilisation effects of the studied secondary P products under field conditions remain to be confirmed. Under field conditions, P utilisation from fertiliser is dependent on a range of factors, but is generally lower than in bioassays or pot experiments. One of the reasons is that root density in the field can be expected to be much lower than in pots (Yang et al. 2010). However, we assume that the relative field fertilisation effect of the secondary P products compared with mineral P fertiliser (RAE) are comparable with the experimental values obtained.

Furthermore, long-term P fertilisation effects of secondary P products should be considered during further development of prediction models for RAE. In agriculture, many secondary P products will commonly be applied as N fertiliser, often resulting in considerable over-application of P due to low N:P ratio compared with crop demands (Table 2). Within a crop rotation, secondary P products may still provide sufficient P for the following crops, if additional N is applied. The experiments presented in **Paper III**, **Paper IV** and **Paper V** did not allow the long-term fertilisation effects of secondary P products to be determined.

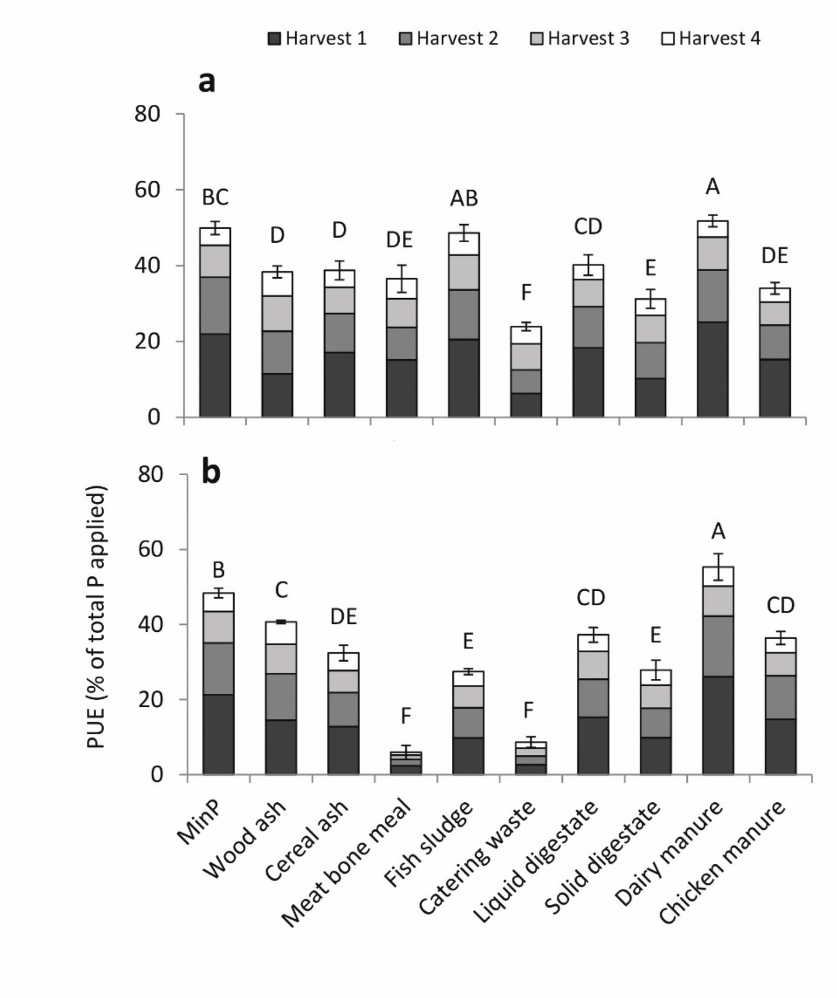

Figure 5. Apparent P use efficiency (PUE, % of total P applied) at: A) pH level 1 (~5.5) and B) pH level 2 (~6.9) at harvests 1, 2, 3 and 4 in the bioassay presented in Paper III and Paper IV. Error bars indicate standard deviation of three replicates for the sum of all harvests. Different letters indicate significant differences between treatments for the sum of all harvests for each pH level (Tukey's HSD).

3.2.2 Inorganic P species affect fertilisation effects depending on soil pH

In **Paper III** we could show that the variation in fertilisation effects for the secondary P products studied could to great extent be explained by the solubility of the inorganic P species they contained, depending on soil pH. Inorganic P species in secondary P products can only be identified by non-destructive speciation methods such as XRD and solid-state ^{31}P MAS-NMR. However, they can also be deduced from sequential chemical fractionations, e.g. according to Hedley et al. (1982). By combining these different methods, in **Paper III** it was possible to demonstrate that the P in the secondary products studied was mainly present as a complex mixture of inorganic P species, primarily Ca phosphates of differing solubility. Organic P constituted only a small fraction of the secondary P products with the exception of chicken manure, in which 53% of total P was present as organic P (Table 2).

Comparing the H_2O-soluble inorganic P fractions in secondary products as studied by the sequential chemical fractionation revealed that significantly more Pi was extracted from dairy manure (42.1%) than from any other secondary product. This explains the equally high or higher PUE of dairy manure compared with MinP at both pH levels studied. Fish sludge contained more H_2O-soluble inorganic P than all secondary P products except dairy manure, explaining the equally high PUE as dairy manure and MinP at pH level 1. The fraction of Pi in secondary products that was soluble in H_2O indicates a product's ability to increase soil solution P concentration and replenish the P concentration after plant P uptake. Phosphorus uptake by plants relies to a great extent on diffusion in addition to mass flow to the roots, especially when root systems are still small. The higher the soil solution P concentration, the more P will reach the roots by these processes. Similarly to the results in this thesis, Achat et al. (2014b) reported a significant relationship between the ability of secondary P products to increase H_2O-soluble Pi in the soil solution (C_P) and their P fertilisation effects on perennial ryegrass (*Lolium perenne*) and red fescue (*Festuca rubra*). In fact, we found a significant positive relationship between PUE as the sum of all harvests at pH level 1 and the H_2O-soluble inorganic P fraction in the secondary P products studied (**Paper III**).

Furthermore, we could show that some secondary P products, e.g. meat bone meal, catering waste and fish sludge, contained considerable fractions of stable Ca phosphates with a molar Ca:P ratio >1, as indicated by all characterisation methods. Using XRD, the stable Ca phosphates in meat bone meal, fish sludge and catering waste were identified as mainly hydroxy- and/or chlor-fluorapatite. The solubility of stable Ca phosphates strongly decreases with increasing soil pH (Lindsay 1979), which explains the lower fertilisation effects of meat bone meal, fish sludge and catering waste at the higher pH level compared with the lower pH level in the bioassay. The HCl-soluble Pi fraction of the sequential chemical fractionation according to Hedley et al. (1982) is operationally defined to reflect the fraction of stable Ca phosphates. In **Paper III** we could therefore show a significant negative relationship between PUE as the sum of all harvests and the HCl-soluble inorganic P fraction of the secondary products at pH level 2.

In the study presented in **Paper III**, the non-destructive speciation methods XRD and solid-state ^{31}P NMR generally confirmed the results of the sequential chemical fractionation, but provided little additional information to explain the observed fertilisation effects.

3.2.3 Physicochemical and microbial soil processes

The fertilisation effects of the secondary P products presented in **Paper III** and **Paper IV** were studied based on the assumption that unfertilised and fertilised plants take up the same amount of P from the soil (see section 2.3.1 Difference method). In the study presented in **Paper V,** we therefore intended to verify this assumption by studying the effects of meat bone meal, fish sludge, wood ash and dairy manure on physicochemical and microbial soil processes after application to an agricultural soil, in order to determine their importance for plant P uptake.

The results of the pot experiment using the ^{33}P indirect labelling method (**Paper V**) were comparable overall with those of the bioassay (**Paper III** and **Paper IV**). In the pot experiment using the ^{33}P indirect labelling method, water-soluble MinP resulted in the highest P uptake on both the unlimed (approximately pH 5.3) and the limed soil (approximately pH 6.2). Fish sludge resulted in equally high P uptake as MinP on the limed soil, while all the other secondary products resulted in lower P uptake on both soils.

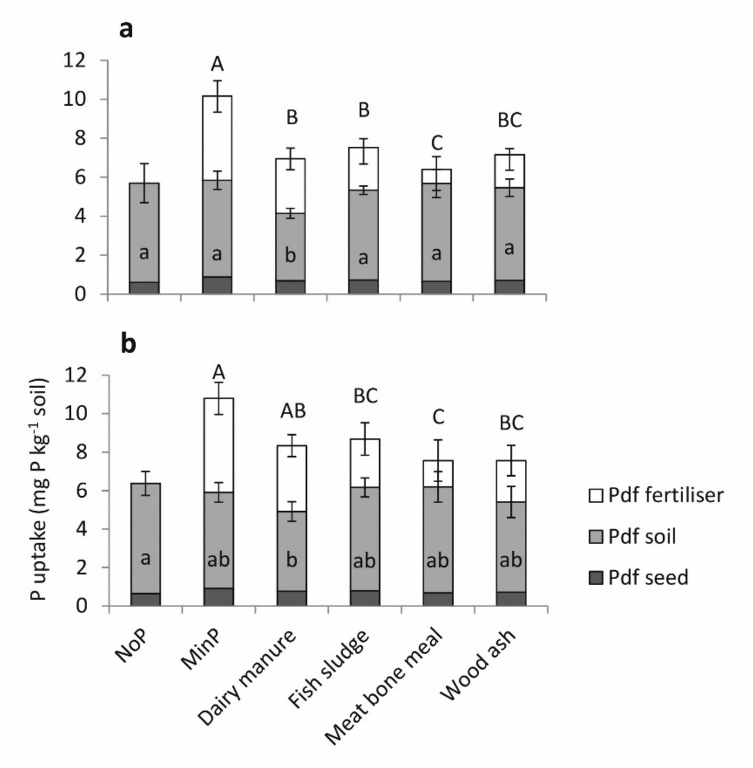

Figure 6. Phosphorus derived from fertiliser (Pdf fertiliser), soil (Pdf soil) and seed (Pdf seed) in barley (mg P kg^{-1} soil) as an effect of the different fertiliser treatments on: A) Unlimed soil and B) limed soil. Error bars represent the standard deviation of four replicates within each treatment. Letters indicate significant differences between treatments according to Tukey's test (one-way ANOVA for each soil); upper case letters refer to Pdf fertiliser and lower case letters to Pdf soil. On the unlimed soil, data on Pdf soil were log-transformed for the statistical analysis. On the limed soil, for Pdf fertiliser of meat bone meal only three observations were considered.

Phosphorus derived from the soil was the most important P source for barley plants with all fertiliser treatments on both soils (Figure 6). There were no differences in Pdf soil between the fertiliser treatments on both soils; only dairy manure resulted in lower Pdf soil than the NoP treatment. This indicates that organic C applied with dairy manure may have resulted in microbial immobilisation of available P. Immobilisation of P following manure application was also indicated in the incubation experiment. Even though organic C was also applied with meat bone meal and fish sludge, microbial P immobilisation seems not to have been a major competitor to barley plants after these treatments. Due to too few products being included in the study presented in **Paper V**, we cannot specify the quantity and quality of organic C in secondary P products at which P uptake by plants might be negatively affected by triggered microbial activity. Effects on physicochemical soil processes seemed not to have influenced P uptake by barley, e.g. the increasing effect of wood ash on soil pH was too small to significantly affect P availability in the soil. Overall, we concluded that physicochemical and microbial soil processes induced by the secondary P products studied were of little overall importance for total plant P uptake. Indeed, in **Paper V** the P uptake by barley was best explained by a linear positive relationship with the H_2O + $NaHCO_3$-soluble Pi fraction or a linear negative relationship with the HCl-soluble inorganic P fraction in secondary products on both the unlimed and the limed soil. Of the parameters studied in the incubation experiment, P uptake by barley was best explained by C_P, the isotopic dilution parameter m and Resin P measured 21 days after fertilisation, all of which represent measures of the solubility of fertiliser P applied to the soil. For the study presented in **Paper V**, an agricultural soil containing little available P and rather low microbial biomass P was used. Therefore the above results remain to be confirmed after application of secondary P products to a wide range of soil types.

3.2.4 Finding the appropriate prediction method

While we found that the fertilisation effects of the secondary P products studied were explained by the inorganic P species they contain (**Paper III** and **Paper V**), sequential chemical fractionations to identify inorganic P pools of different solubility cannot be expected to be applied as a standard procedure. Therefore, an operational approach to predict the RAE of secondary P products with unknown fertilisation effects was sought (**Paper IV**). The appropriate chemical extraction method can be determined by following the decision tree suggested in Figure 7a.

If the P in the secondary product in question has been precipitated with Al and/or Fe, as in the case of chemically precipitated sewage sludge, Øgaard and Brod (forthcoming) suggest predicting the RAE from the concentration of Al and Fe in the product or by extraction of inorganic P with 2% citric acid.

Otherwise, if P in the secondary product has not been precipitated with Al and/or Fe, we found that P is probably mainly present as Ca phosphates of differing solubility (**Paper III**). The solubility of stable Ca phosphates decreases with increasing soil pH (Lindsay 1979). Therefore, we suggest predicting RAE following application of one of two different chemical extraction methods depending on the pH in the target soil. The chemical extraction methods resulting in the best fit of simple linear regression models with X = extractable Pi and Y = RAE studied during the bioassay at two soil pH levels were selected in **Paper IV** after a systematic comparison of eight different extraction methods. Based on studies by

Morel and Fardeau (1990), soil pH 6.5 was set as the critical level at which to switch extraction method.

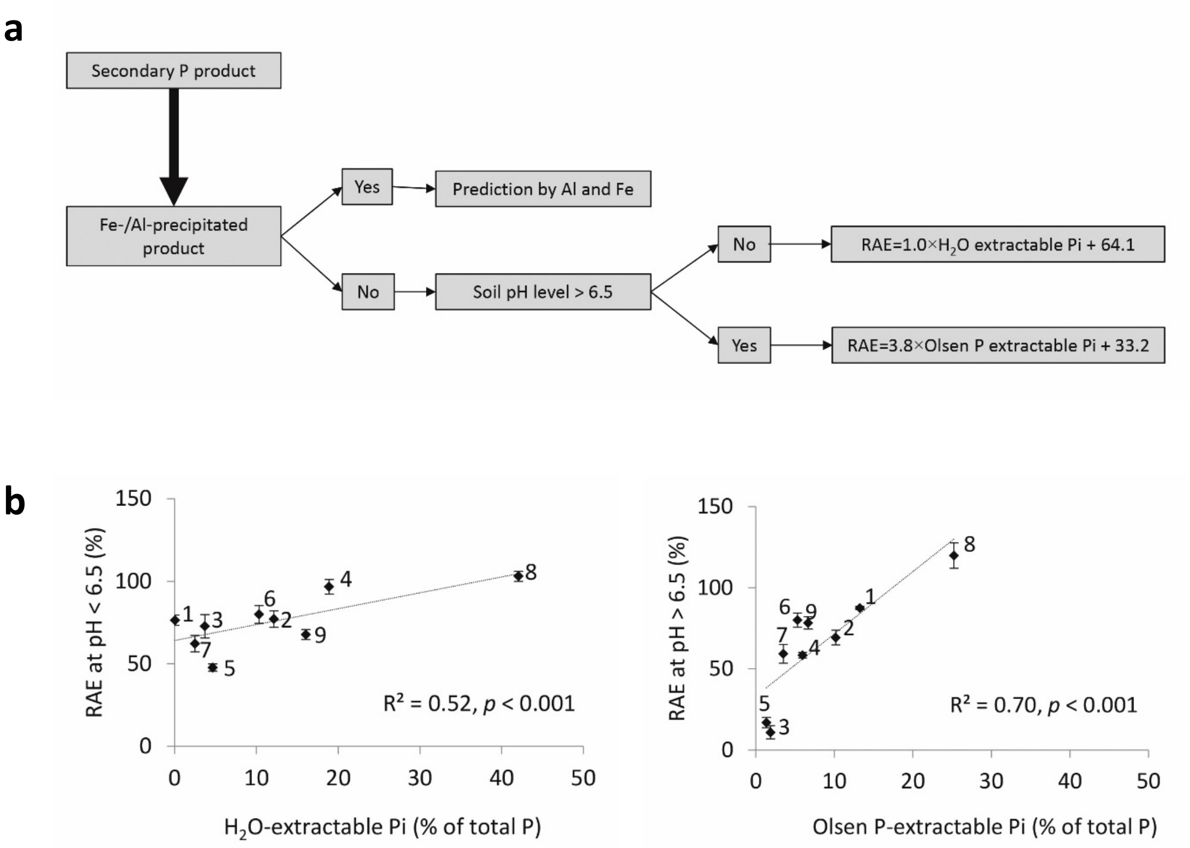

Figure 7. A) Decision tree suggesting an appropriate simple chemical extraction method to determine the relative agronomic efficiency (RAE, %) of secondary P products. B) RAE of the secondary P products studied as an effect of H_2O-extractable Pi and Olsen P-extractable Pi (% of total P) at pH levels 1 and 2 studied in the bioassay with 1=Wood ash, 2=Cereal ash, 3=Meat bone meal, 4=Fish sludge, 5=Catering waste, 6=Liquid digestate, 7=Solid digestate, 8=Dairy manure and 9=Chicken manure. Error bars indicate standard deviation between replicates for the sum of all harvests.

At soil pH <6.5, we propose predicting the RAE of secondary P products by their H_2O-extractable Pi fraction (% of total P), determined at a sample:solution ratio of 1:200, according to:

$$\mathrm{RAE} = 1.0 \times \mathrm{H_2O\ extractable\ Pi} + 64.1 \qquad (8)$$

Figure 7b shows the distribution of secondary P products on the simple linear regression line, which explained 52% of the variation in RAE of the secondary P products studied by their fraction of H_2O-extractable Pi at the lowest soil pH level in the bioassay. Because dairy manure resulted in the clearly largest fraction of H_2O-extractable Pi and RAE among all secondary P products studied, it strongly affected the regression line presented in Figure 7b. Wood ash, on the other hand, contained no H_2O-extractable Pi despite a distinct fertilisation effect at the lowest pH level (Figure 5). This can probably be explained by the highly alkaline wood ash causing a greater increase in pH in the extraction solution compared with the soil pH increase that could be expected after application to soil. The implications of increased pH in the extraction solution for extractability are discussed in detail in **Paper IV**.

In **Paper IV**, we suggest predicting readily available Pi in secondary P products at pH <6.5 by extraction with 0.005 M $CaCl_2$ and simultaneous adsorption to iron-oxide impregnated filter paper (FeOH method). The filter paper acts as a sink, which keeps the P concentration in the extraction solution low and results in continuous release of readily available P from the sample. The fraction of FeOH-extractable Pi resulted in somewhat better prediction of RAE than H_2O-extractable Pi (sample:solution ratio of 1:20). However, use of H_2O as the extraction method is recommended, because it can more easily be conducted as a standard procedure. Essentially, the FeOH method and extraction with H_2O reflect the same principle, indicating a product's ability to increase soil solution P concentration, as explained above. In **Paper IV**, at the lowest pH level late-season fertilisation effects from readily available P were distinguished and it was suggested that these late-season fertilisation effects of secondary P products can be predicted by extraction with neutral ammonium citrate. However, for the sake of simplification, late-season fertilisation effects are omitted from the decision tree presented in Figure 7a. This is also because total P plant uptake as the sum of four harvests was mainly determined by readily available P at harvest 1 studied during the bioassay presented in **Paper III** and **Paper IV** (Figure 5).

At soil pH >6.5, we propose predicting the RAE of secondary P products by their Olsen P-extractable Pi fraction (% of total P) according to:

$$\mathrm{RAE} = 3.8 \times \mathrm{Olsen\ P\ extractable\ Pi} + 33.2 \qquad (9)$$

Olsen P is buffered at pH 8.5, where the solubility of stable Ca phosphates is strongly reduced (Lindsay 1979; Morel and Fardeau 1990). Consequently, in **Paper IV** Olsen P extracted least Pi from those secondary P products containing the largest fractions of stable Ca phosphates (e.g. meat bone meal and catering waste) and was the only extraction method that significantly correlated with RAE representing the readily available P and late-season fertilisation effects at the highest pH level. The fraction of Olsen P-extractable Pi explained 70% of the variation in RAE at the highest soil pH level.

The decision tree in Figure 7a is a first attempt to propose simple laboratory methods that allow consideration of the RAE of secondary P products in fertilisation plans. However, the more data available for a decision tree, the closer the prediction will be to the real fertilisation effects of secondary P products. The equations based on Figure 7b have to be verified with studies on other secondary P products.

4 RECOMMENDATIONS AND CONCLUSIONS

Political interest in achieving a circular economy is increasing in Europe (EC 2015). Also Norway aims at replacing the current oil-based economy with a bioeconomy, which optimally utilises available bioresources. Against this background, better utilisation of secondary P resources will be indispensable in the future. However, P recycling is still too expensive to be an economically viable alternative to mineral fertiliser (Koppelaar and Weikard 2013), despite the increasing price of rock phosphate in past years (Cordell and White 2015). Therefore, P recycling can only be achieved with the implementation of political incentives and a national P management strategy, as already suggested by the Norwegian Environment Agency (2015). Relevant regulations will have to be adapted accordingly. Based on the results presented in **Papers I-V**, the following recommendations can be made:

Restricting P application per soil area. We strongly recommend a further restriction of P application rates per unit soil area. This would result in turn in better utilisation of e.g. P in manure. Manure alone could fulfil the Norwegian demand for P fertiliser, but the current P accumulation of 12,000 Mg P yr^{-1} is probably mainly due to over-application of manure in livestock-dense areas. To better utilise P in manure, it will have to be transported from areas with high livestock density to P-deficient areas. Transporting manure requires treatment to reduce its water content without reducing the high P fertilisation effect. Some work has already been done on technological treatment of manure to improve transportability of P (e.g. Achat et al. 2014a,b; Christel et al. 2014), but suitable treatment technologies have not yet been implemented in Norway. Stricter regulations on P application rates per unit area would most likely encourage the development of transportable manure-based fertiliser products in Norway. According to current regulations, the minimum size of animal farms in Norway is ≥0.4 hectares per livestock unit, which correlates to an average upper limit of 35 kg P ha^{-1} (Norwegian Ministry of Agriculture and Food 2003). However, P uptake in yield is often lower than 35 kg P ha^{-1}, resulting in continuously increasing soil P stocks. In addition, there are currently no obligations to limit over-application of P from both mineral P fertiliser and secondary products. Total P application (mineral fertiliser and secondary P products) per unit soil area should therefore be limited, with stricter adaptation to yield and soil P levels. In Sweden, the application of manure and secondary P products is restricted to

22 kg P ha^{-1} and year as an average over five years (Swedish Board of Agriculture 2012). In the Netherlands, P fertilisation rate is restricted to 44 and 37 kg P ha^{-1} for grassland and arable land, respectively, but also has to be adapted to the soil P status. According to Smit et al. (2015), these regulations have led to a substantial decrease in soil P accumulation, mainly due to a decrease in mineral P fertiliser use and improved utilisation of manure.

Mandatory collection and recycling of P in fish sludge. Aquaculture will be one of the main drivers of the Norwegian bioeconomy in the future and it is likely that its growth will therefore be heavily politically promoted. However, this thesis showed that Norwegian aquaculture production can only grow sustainably if P losses with fish sludge are substantially reduced. Phosphorus losses with fish sludge from offshore aquaculture pens are already of the same order of magnitude as P in mineral fertiliser or manure and are predicted to increase considerably. An essential first step in utilising this P is implementation of political regulations that require collection and recycling of the P in fish sludge. The current P imbalance caused by large feed imports to Norwegian aquaculture could be substantially reduced if the P in fish sludge were to be used for the production of fish feed, either in Norway or abroad. The fish sludge product studied in this thesis had potentially equally high P fertilisation effects as dairy manure. The P fertilisation effects of fish sludge will depend on treatment technologies and the specific secondary P product based on fish sludge. While appropriate technologies exist for fish sludge collection (e.g. multitrophic, floating or land-based closed aquaculture systems) and the production of secondary P products based on fish sludge, they are in their infancy and require further improvement before utilisation of fish sludge as a secondary P resource can be realised (SINTEF Fiskeri og havbruk AS 2006). Stricter political regulation would stimulate technological development to ensure sustainable growth of Norwegian aquaculture. This thesis only examined the fertilisation effects of secondary P products, but it is important to note that harnessed P in fish sludge could also be used for a number of other purposes, e.g. including in fish feed ingredients.

Adapting regulation criteria determining the use of secondary products as fertiliser. Phosphorus recycling with secondary products can only be achieved if mineral P fertiliser rates are reduced accordingly. The P fertilisation effects of secondary products can largely be attributed to the solubility of inorganic P species they contain. Based on the results presented in this thesis, we recommend indicating the plant-availability of P in commercialised secondary P products by parameters that can be determined by simple laboratory methods. The relative agronomic efficiency of secondary P products compared with mineral fertiliser was best explained here by the fractions H_2O-extractable Pi and Olsen P-extractable Pi, depending on soil pH. If the P in the secondary product is precipitated with Al and/or Fe (e.g. chemically precipitated sewage sludge), predicting the relative agronomic efficiency from the concentration of Al and Fe in the product or by extraction of Pi with 2% citric acid has been recommended. The fraction of AL-extractable P in secondary P products must currently be stated when they are sold as alternative fertilisers, although no relationship was found between the fraction of AL-extractable Pi and the P fertilisation effects of the secondary P products studied in this thesis. The Norwegian regulation regulating the use of organic fertiliser products (Norwegian Ministry of Agriculture and Food 2003) is currently under revision. Including the findings in this thesis in the revised version of the regulation would result in immediate political promotion of P recycling in Norway.

Developing secondary P products with high P availability. Even if the fertilisation effects of secondary P products are taken into account in fertilisation planning by the laboratory methods suggested above, their application can still result in considerable accumulation of residual P in the soil. According to the results presented in this thesis, the P fertilisation effects of many secondary products are considerably lower than those of mineral fertiliser, with the P fertilisation effects being variably affected by soil pH. Thus efficient P recycling can only be achieved if the fertilisation effects of commercialised secondary P products are reliable and comparable to those of mineral fertiliser. Furthermore, the NPK ratio in recycling fertilisers has to be in line with crop demands. If one nutrient is limiting, the other nutrients cannot be optimally utilised by the plants. Therefore, development of high-quality residual products is essential for facilitating efficient recycling within food systems, in order to reduce eutrophication risks and the rate of rock phosphate depletion.

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PAPER I

Hamilton HA, Brod E, Hanserud OS, Gracey EO, Vestrum MI, Bøen A, Steinhoff FS, Müller DB, Brattebø H (2015) Investigating cross-sectoral synergies through integrated aquaculture, fisheries and agriculture phosphorus assessments: A case study of Norway. Journal of Industrial Ecology: Doi:10.1111/jiec.12324

Investigating Cross-Sectoral Synergies through Integrated Aquaculture, Fisheries, and Agriculture Phosphorus Assessments

A Case Study of Norway

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:// Supporting information is available on the *JIE* Web site

Summary

Future phosphorus (P) scarcity and eutrophication risks demonstrate the need for systems-wide P assessments. Despite the projected drastic increase in world-wide fish production, P studies have yet to include the aquaculture and fisheries sectors, thus eliminating the possibility of assessing their relative importance and identifying opportunities for recycling. Using Norway as a case, this study presents the results of a current-status integrated fisheries, aquaculture, and agriculture P flow analysis and identifies current sectoral linkages as well as potential cross-sectoral synergies where P use can be optimized. A scenario was developed to shed light on how the projected 2050 fivefold Norwegian aquaculture growth will likely affect P demand and secondary P resources. The results indicate that, contrary to most other countries where agriculture dominates, in Norway, aquaculture and agriculture drive P consumption and losses at similar levels and secondary P recycling, both intra- and cross-sectorally, is far from optimized. The scenario results suggest that the projected aquaculture growth will make the Norwegian aquaculture sector approximately 4 times as P intensive as compared to agriculture, in terms of both imported P and losses. This will create not only future environmental challenges, but also opportunities for cross-sectoral P recycling that could help alleviate the mineral P demands of agriculture. Near-term policy measures should focus on utilizing domestic fish scrap for animal husbandry and/or fish feed production. Long-term efforts should focus on improving technology and environmental systems analysis methods to enable P recovery from aquaculture production and manure distribution in animal husbandry.

Introduction

The linearity of anthropogenic phosphorus (P) flows, from extraction to consumption to waste with limited recycling, makes the current use of P unsustainable. Because P is both a finite resource and a pollutant, the need for improving P management has been deemed as critical (Cordell et al. 2009). In order to do so, a systems-wide understanding is necessary given that P is metabolized across both natural and anthropogenic processes in a variety of sectors. Potentials for reducing P losses and increasing recycling must be identified through a holistic approach, encompassing all processes of the anthropogenic P cycle.

Thus far, data gaps limit our ability to accurately model P flows in the aquaculture, fisheries, and agriculture sectors together on a country scale. The aquaculture and fisheries sectors,

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today, are significant drivers for anthropogenic P use, and this will only increase considering the projected large growth in aquaculture and their dependency on fisheries and agriculture for feed ingredients (Subasinghe 2005; Troell et al. 2009; Abreu et al. 2011). Such increases are likely to shift resource cycles on a global level; however, the implications will be particularly far reaching in countries with large aquaculture sectors. Cordell and colleagues (2013) have noted the particular importance of aquaculture and fisheries and also highlight the problem of data gaps within the aforementioned sectors. Matsubae-Yokoyama and colleagues (2009) have modeled fisheries as a P input to the food system in Japan; however, the implications of the rapidly growing aquaculture sector have not been explored. Because these sectors can be linked, primarily through the production of feed and secondary fertilizers, we use a systems approach and quantify the sources, sinks, and inefficiencies of P metabolism within our case study to identify the most effective strategies for reducing the dependency on finite primary P resources and losses to the environment.

Scope and Research Questions

In order to identify the largest potentials for improving P management, a P balance was conducted on a country that exemplifies the need for integrated fisheries, aquaculture, and agricultural assessments. Norway has large, export-oriented aquaculture and fisheries industries, whereas Norwegian agriculture predominantly serves the national market with a 45% self-sufficiency level in 2010 (NILF 2011). In terms of size, however, Norwegian aquaculture is especially significant. In 2011, Norway was the largest per capita aquaculture producer in the world with 0.23 tonnes of fish and crustaceans per capita and ranked sixth in total production quantities (FAO 2011; SSB 2014). In addition, production in this sector is expected to increase 5 times by 2050, necessitating a corresponding quintupling of fish feed production (DKNVS and NTVA 2012). Because Norwegian fish feed today contains roughly 52% imported agricultural products, it is clear that the agricultural and aquaculture sectors are, to a large degree, interdependent (Pettersen 2013; SSB 2014; EWOS 2010). Sectoral linkages such as this exemplify the need for integrated studies given that P management cannot be optimized if the major drivers are excluded and synergies remain unexplored.

In this article, we aim to, for the first time, integrate the aquaculture, fisheries, and agricultural sectors in a P balance model of Norway in order to address the following questions:

1. What are the current P cycles in aquaculture, fisheries, and agriculture and how are they linked? What is the current state of secondary P recycling (i.e., the use of by-products or waste as secondary P sources)?
2. What are the projected changes and how could this affect P cycles given the current linkages? Specifically, how does this affect waste streams and thus potential future secondary P sources? What challenges could arise?
3. What are the options and opportunities for systems-wide integration of P management?

Methods

To answer the aforementioned questions, a substance flow analysis (SFA) was performed on the Norwegian P system using the free-ware material flow analysis software, STAN, to visualize the results. The system was defined for the economic zone of Norway, including coastal areas where aquaculture production occurs and the marine waters where Norwegian fisheries operate. In an attempt to avoid annual variations, averaged data from 2009 to 2011 were used. The following paragraphs include a further explanation of the key processes and the definitions thereof. For more information regarding the remaining processes, refer to the supporting information available on the Journal's website.

Agricultural and Greening Soil

Agricultural soil refers to permanent grassland that is used for fodder/grazing and arable land that is used for the production of cereals, potatoes, oil seeds, legumes, vegetables, fruits, and fodder. Greening soil includes parks, covering for landfills, gardens, and other areas where mineral and organic fertilizer are utilized, excluding agricultural land. Discharge from soils and the net addition to soil P stocks were included in this process. Forestry soils were omitted because the anthropogenic influence on the P flows in forest systems was determined to be negligible relative to the overall system.

Aquaculture

This process includes all of the fish production taking place within the aquaculture sector, including coastal nets. In Norway, marine aquaculture, or fish farming under controlled conditions inside of cages placed in marine environments, is predominant. Fish farms are typically open-cage systems, where effluent water is continuously exchanged with surrounding waters (Wang et al. 2012; Troell and Norberg 1998). This results in the loss of large amounts of nutrients in a variety of forms. Dissolved inorganic P is released through excretion, particulate organic P is lost through defecation and uneaten feed, and dissolved organic P occurs through the dissolution of particulate P (Wang et al. 2012; Troell and Norberg 1998).

Fisheries and Fish Processing

This process includes all marine fish and shellfish caught by Norwegian vessels and all fish caught by foreign vessels and landed in Norway. Wild fish processing was modeled to include both land-based processing and processing at sea. By-product utilization was included in this analysis including by-products dumped at sea and processed for recycling into new products. Sport fishing was estimated to be negligible.

Fish Feed Production

Fish feed refers to feed for fish farmed in aquaculture for human consumption. Fish feed production includes both domestic and imported raw feed ingredients and prepared imported fish feed. Over time, the composition of fish feed has changed dramatically to favor the use of more plant ingredients owing to the high costs of fish products (Sørensen et al. 2011). In 2013, however, fish feed consisted of 67% plant-based products, including plant protein, rapeseed oil and plant-based binders, and 33% fish-based products, primarily fish oil and fish meal (Ytrestøyl et al. 2014b).

Fertilizer Production

Mineral fertilizer and organic fertilizer production are included in this process. Mineral fertilizer refers to the fertilizer produced from imported phosphates. Organic fertilizer includes domestically produced meat bone meal, sewage sludge, and food waste.

Agriculture

The major animal groups and plant products produced in Norway are included in this process: cereals, potatoes, oil seeds, legumes, vegetables fruits, fodder, dairy and cattle, pigs, sheep and goats, poultry, horses, and fur animals. Imported live animals were estimated to result in small P flows and were therefore not considered. A large amount of sheep, some cattle, horses, and goats graze in forest pastures in the summer. Excreted P in forest pastures was considered to leave the system boundaries, whereas assimilated P in animals during the summer was assumed to be negligible.

Processing, Retail and Consumption

All domestic animal/plant processing and trade is included in this process. Losses during food processing, retail, wholesale, and human consumption are also included. Imported food is assumed to go directly to consumption without an intermediate processing phase.

Data Sources and Quantification

Tables 1and 2 include flow and stock descriptions, equations, and their respective data sources. Data were primarily collected from government statistics, reports, company publications, expert interviews, and scientific publications. Mass balance was utilized in cases of poor data availability. Otherwise, each flow was individually calculated. The net accumulation of P in stocks, including soil stocks, off-site wastewater treatment stocks, and construction and landfill stocks, was calculated by mass balance. Mass balance inconsistencies, designated MBI as seen in figure 1, are marked in red and are given as the total input minus the total output for each process. This was done to allow inconsistencies to be assessed in relation to the size of the relevant process flows.

Data for mineral fertilizer production was obtained from YARA (Nyhus 2013), a Norwegian fertilizer company representing 85% to 90% of the Norwegian market share for mineral fertilizer sales (Mattilsynet 2013). The wastewater calculations were based on a set of assumptions related to the wastewater treatment efficiencies and the part of the P load transported to the wastewater treatment plant. Further information related to this can be found in the Supporting Information on the Web. Additionally, because the process efficiency calculations cannot be calculated through the results presented, detailed explanations and the calculation methods can be found in the Supporting Information on the Web.

Scenario Development

Given the projected growth in aquaculture production, a scenario was developed to shed light on how such production increases could propagate throughout up- and downstream processes. The scenario was based on a fivefold increase in production by 2050, a Norwegian population of 6.6 million, and no growth in the fisheries industry (SSB 2014). Today's technologies were used by scaling up data and holding the transfer coefficients and system structure from the 2009–2011 model constant. Because this estimate does not consider changes within many of the variable drivers, the purpose of this scenario was not to describe the most likely situation in 2050. Rather, the aim was to highlight potential challenges for future P management in Norway. This scenario can be used to inform a more refined analysis needed to direct future policies both within and across the different sectors. The aforementioned mass balance inconsistencies were not visualized for this scenario because the relative error remained the same, given that technologies were held constant.

Results

The P balance, figure 1, indicates the major imports, exports, flows, sinks, and losses of P within the Norwegian system. The results from the 2050 scenario can be seen in figure 2.

Overall, we determined that Norway is a net importer of P (total imported goods – total exported goods), with an average net import of 30,000 tonnes of P per year (P/yr) or 6.2 kilograms of P per capita per year from the recent period 2009–2011. By a substantial margin, the largest P flows were represented by the import of rock phosphates for fertilizer production with the subsequent export of most of the mineral P fertilizers for use in other countries. As shown in figure 1, P flows caused by aquaculture and agriculture drive P consumption and losses at similar levels. Comparing similar flows between the sectors, domestic use of mineral fertilizer based on imported phosphates in soils (8,400 tonnes P/yr) and imported fish feedstuff (9,400 tonnes P/yr) are similar, indicating that both sectors are equally

Table 1 Flow desciptions

Flow origin and destination	*Flow name*	*Flow description*
0,1	Imported rock phosphates	Quantity of P in imported rock phosphate for the production of mineral fertilizer
0,2	Atmospheric deposition	Quantity of P to agricultural land through atmospheric deposition
0,4	Imported husbandry feedstuff	Quantity of P in imported products and phosphates for husbandry feed produced in Norway
0,5	Caught fish	Quantity of P in fish and crustaceans, except mammals, caught by Norwegian vessels
0,6	Imported fish feedstuff	Quantity of P in imported fish feed and feedstuff used for meal and fish feed production. P content based on country of origin
0,8	Imported food	Quantity of P in imported food products, including pet food
1,0	Exported fertilizer	Quantity of P in exported mineral fertilizer
1,2a	Organic fertilizer	Quantify of P in sludge, meat bone meal, and food waste used as fertilizer
1,2b	Mineral fertilizer	Quantity of P in mineral fertilizer applied to agricultural and greening soil
2,0	Runoff	Quantity of P lost from agricultural land to water bodies through the processes of diffusive, point source, and background runoff
2,3	Plant uptake, grazing, and green fodder	Quantity of P taken up by agricultural crops without plant residues, which are assumed to stay on the field. Reference year 1/7–30/6 and the P in green fodder, silage, hay, and pasture. Calculation of P in silage, hay, and pasture is based on: 1 FeM (feed unit) = 1.176 kg DM grass, 2.6 kg P/ton DM grass.
3,0	Manure to forest	Quantity of P excreted in the forest during summer.
3,2a	Manure	Quantity of P excreted by animals in 1 year. Corrected for P excreted by animals grazing in the forest. All manure is assumed to be returned to agricultural land.
3,2b	Seeds and planting potatoes	Quantity of P applied to agricultural land in cereal seeds and planting potatoes. P in grass, vegetable, and fruit seeds is assumed to be negligible. Reference year 1/7–30/6.
3,4	Plant products for husbandry feed	Quantity of P in domestically produced cereals, oil seeds, and legumes for feed
3,8a	Plant products for human consumption	Quantity of P in harvested crops for human consumption. All vegetables, potatoes, and fruits produced are assumed to be used for human consumption.
3,8b	Husbandry products	Quantity of P in milk and eggs produced and living animals leaving the farm to be slaughtered
4,3	Husbandry and fur feed	Quantity of P in husbandry and fur feed
4,8	Pet food	Quantity of P in pet food
5,0	Dumped scrap	Quantity of P in fish scrap that is discarded at sea
5,6	Wild whole fish and scrap	Quantity of P in wild fish and fish scrap from fisheries used for fish feed production
5,8	Processed wild fish, crustaceans, and scrap	Quantity of P in caught fish, fish scraps, and crustaceans
6,0	Exported wild fish feedstuff	Quantity of P in exported fish feed and feedstuff from fisheries fish
6,4	Fish meal and silage	Quantity of P in fish meal and silage used for husbandry feed
6,7	Fish feed	Quantity of P in fish feed used in aquaculture
7,0a	Escaped fish	Quantity of P in escaped and other lost fish from aquaculture
7,0b	Fish excrements and feed losses	Quantity of P in lost fish feed, feces, and excretion from aquaculture including onshore hatcheries
7,8	Farmed fish	Quantity of P in farmed fish from aquaculture, including dead fish
8,0a	Exported fish products	Quantity of P in exported wild fish products, farmed fish products, and crustaceans from Norway
8,0b	Exported fish scrap products	Quantity of P in exported wild fish scrap and farmed fish scrap
8,0c	Exported food	Quantity of P in exported domestically produced meat, milk, eggs, cereals, vegetables, potatoes, and fruits
8,6	Salmon scrap	Quantity of P in salmon scrap used for fish meal and silage for husbandry feed
8,9a	Processing and retail waste	Quantity of P in food processing (animal slaughtering and vegetables), wholesale, and retail waste

(*Continued*)

Table 1 Continued

Flow origin and destination	*Flow name*	*Flow description*
8,9b	Wastewater	Quantity of P from human waste (urine + feces) and municipal wastewater, including wastewater to separate wastewater treatment for <50 person equivalents
8,9c	Municipal solid waste	Quantity of P in food waste generated by households and the service sector
9,0a	Discharge	Quantity of P discharged to water from wastewater treatment
9,0b	Exported meat and bone meal	Quantity of P in exported waste, including meat bone meal, food waste, and fish silage for export
9,1	Waste-based fertilizer	Quantity of P in fertilizer and soil amendment products derived from sludge, meat bone meal, and food waste
9,4	Meat bone meal for husbandry feed	Quantity of P in meat bone meal used for husbandry feed
9,10	Waste for incineration, landfill, and construction	Quantity of P in waste incinerated or directly landfilled

Note: Flow origin and destination refers to the process number in which the flow originates and ends. For example, 6,7 refers to the flow originating from process 6 and ending in process 7.
P = phosphorous; FeM = feed unit for milk production; kg P/tonne = kilograms of phosphorous per tonne; DM = dry matter.

Table 2 Flow origin and destination, flow equations, and sources for both organic matter and P contents (Pc)

Flow origin and destination	*Equation*	*Material quantity sources*	*P contents sources*
0,1	Personal communication with O. Nyhus (2013)	1	
0,2	Agricultural area × rate of atmospheric P deposition per area	2	3
0,4	Imported products × Pc + (Husbandry feed × Pc – raw products used for husbandry feed × Pc)	4	5, 6, 7
0,5	All fish and crustaceans caught by Norwegian vessels × Pc + Fish caught in rivers × Pc	2	8,9
0,6	Imported meal × Pc + Imported fish feed × Pc + Imported fish for fish feed production × Pc + Imported milled fish products for feed × Pc + Imported agriculture products × Pc	2, 10, 11	2, 12, 13
0,8	Sum of imported food products × Pc	2	6, 14, 15, 16
1,0	Personal communication with O. Nyhus (2013)	1, 25	
1,2a	See 9,1	2, 17, 18, 19, 20, 21, 22	23, 24
1,2b	Mineral fertilizer applied to agriculture soil × Pc + Educated estimate	1, 25	
2,0	Agricultural area × rate of diffusive, point source, and background runoff	26	
2,3	Cereal, potato, oil seed, legumes, and vegetable and fruit yields × Pc + Green fodder silage, hay, and pasture × Pc	2, 27, 28	6, 15, 29, 30
3,0	Number of animals in the forest × time in the forest × P excreted per animal	2, 33	33, 34
3,2a	Number of animals × P excreted per animal – number of animals in the forest × time in the forest × P excreted per animal	2, 33	33, 34
3,2b	Cereal, oil seed, legume seeds, and planting potatoes × Pc	27, 31	6
3,4	Cereals, oil seeds, and legumes produced used for husbandry feed production × Pc	4	6
3,8a	Cereals, vegetables, potatoes, and fruit produced used for human consumption × Pc	2, 27	6
3,8b	Cow milk, goat milk, and egg × Pc + (Number of slaughtered animals × slaughter weight/dressing percentage) × Pc + (Number of fur produced × weight fur animals) × Pc	2, 17, 18, 19 28, 32, 35,36	6, 15, 16
4,8	Pet food × Pc	7, 38, 39	50
4,3	Husbandry feed × Pc + fur feed × Pc	4, 7, 38,39	40, 51
5,0	Dumped fish scrap × Pc	2	8, 9
5,6	Fish for fish feed production × Pc + Fish scrap for fish feed production × Pc	2, 41, 42, 43	8, 9, 44

(*Continued*)

Table 2 Continued

Flow origin and destination	*Equation*	*Material quantity sources*	*P contents sources*
5,8	(Landed fish and shellfish for human consumption × Pc – Fish scrap × Pc) + (Fish caught in rivers × Pc)	2, 41, 42, 43	8, 9, 13, 44
6,0	Wild fish meal × Pc + Whole forage fish × Pc + Fish feed × Pc	2	8, 12, 13
6,4	Wild fish meal for husbandry and fur feed × Pc + Wild fish silage for husbandry and fur feed × Pc + Salmon meal for husbandry and fur feed × Pc + Salmon silage for husbandry and fur feed × Pc	2, 4, 41, 42, 43	8, 9, 11, 12, 13,
6,7	Fish feed for aquaculture × Pc	13	13
7,0a	Total amount of escaped fish × average estimated weight × Pc	2, 42, 43	13
7,0b	Total amount of fish feed fed × fish feed loss rate × Pc + Total amount of P in fish feed eaten × ratio lost owing to excretion and feces	2, 41, 42, 43	45, 46, 47, 48
7,8	Total farmed fish produced × Pc + Total quantity of dead fish × Pc	2	13
8,0a	Total exported farmed salmon, herring, pelagic fish, and white fish products × Pc + Total exported crustaceans × Pc	2	9, 13, 44, 47
8,0b	Total exported farmed salmon, herring, white fish, and pelagic fish silage × Pc + Total exported fish scraps × Pc	41, 42, 43	8, 9, 13
8,0c	Amount of exported domestically produced food × Pc	2	6, 14, 15, 16
8,6	Salmon scrap and silage for husbandry × Pc	4, 41, 42, 43	13
8,9a	Quantity of meat bone meal × Pc + Vegetable processing waste × Pc + Wholesale food waste × Pc + Retail food waste × Pc	17, 18, 19, 20,52	6,23
8,9b	Quantity of discharged P to water / (1 – treatment efficiency) + Population connected to separate wastewater treatment × Quantity of P generated per capita – Quantity of P in septic tank content	22	
8,9c	Quantity of organic household waste × Pc + Quantity of organic service waste × Pc	2	24
9,0a	Quantity of discharged P to water	22	
9,0b	Quantity of exported meat bone meal × Pc + Quantity of exported kitchen and canteen waste × Pc + Quantity of exported mixed household waste × Fraction of organic waste in mixed waste × Pc	2, 17, 18, 19, 20, 21, 50	23, 24
9,1	Wastewater – discharge × Fraction of sludge to agriculture, greening, and other use + Quantity of meat bone meal for agriculture and greening × Pc + Quantity of biologically treated municipal waste × Pc	2, 17, 18, 19, 20, 22	23, 24
9,4	Quantity of meat bone meal for husbandry feed × Pc	17, 18, 19, 20	23
9,10	Wastewater – discharge × Fraction of sludge to landfill + Quantity of meat bone meal to incineration × Pc + Quantity of household waste to incineration × Fraction of organic waste in mixed waste × Pc + Quantity of landfilled organic waste × Pc	2, 17, 18, 19, 20, 22	23, 24

Sources: [1](Nyhus 2013); [2](SSB 2014); [3](Oredalen 2000); [4](Norwegian Agriculture Authority 2011); [5](Lentner and Wink 1981); [6](Norwegian Food Composition Database 2014); [7](Mattilsynet 2010aa); [8](Hjerne and Hansson 2002); [9](Czamanski et al. 2011); [10](Norwegian Seafood Federation 2013); [11](Ytrestøyl et al. 2014a); [12](FAO Fisheries Department 1986); [13](Ytrestøyl et al. 2011); [14](USDA and ARS 2013); [15](Antikainen et al. 2005); [16](IFP 2006); [17](Animalia 2010); [18](Animalia 2011); [19](Animalia 2012); [20](Viste 2010); [21](Ahmed 2013); [22](Berge and Mellem 2012); [23](Norsk Protein 2013); [24](Møller et al. 2012); [25](Mattilsynet 2013); [26](Eggestad et al. 2001); [27](Breen 2013); [28](Norwegian Agricultural Economics Research Institute 2011); [29](Bakken et al. 2014); [30](Johansen et al. 2003); [31](Glorvigen 2013); [32](Heimberg 2014); [33](Karlengen et al. 2012); [34](Poulsen 2012); [35](Avdem 2013); [36](Bryhn 2013); [37](IFP 2006); [38](Mattilsynet 2009); [39](Mattilsynet 2011); [40](Ahlstrøm 2013); [41](RUBIN 2009); [42](RUBIN 2010); [43](RUBIN 2011); [44](National Institute of Nutrition and Seafood Research 2007); [45](Lall 1991); [46](Wang et al. 2012); [47](Reid et al. 2009); [48](Bergheim and Braaten 2007); [49](Rørtveit and Nerland 2013); [50](Raadal et al. 2008); [51](Mattilsynet 2010bb); [52](Hanssen and Schakenda 2011)

reliant on imports for production. Additionally, total fish feed consumption (12,000 tonnes P/yr) and husbandry feed, fodder, and grazing in agriculture (17,400 tonnes P/yr) are in the same order of magnitude. In terms of losses and potentially available secondary P sources, fish excrements and feed losses are very comparable to the net accumulation of P in soil stocks for plant production, with 9,000 tonnes P/yr in aquaculture losses versus 12,000 tonnes P/yr net addition to stock in soils (see figure 2).

Although aquaculture and agriculture drive P consumption and losses at comparable levels, we determined that their P efficiencies vary substantially. We estimated that plant production has an efficiency of 70% and animal husbandry has an efficiency of 10%, showing that trophic factors play a major role in the losses of P. In comparison with aquaculture, we estimated a 31% fish production efficiency, making aquaculture more efficient than animal production, but with fish

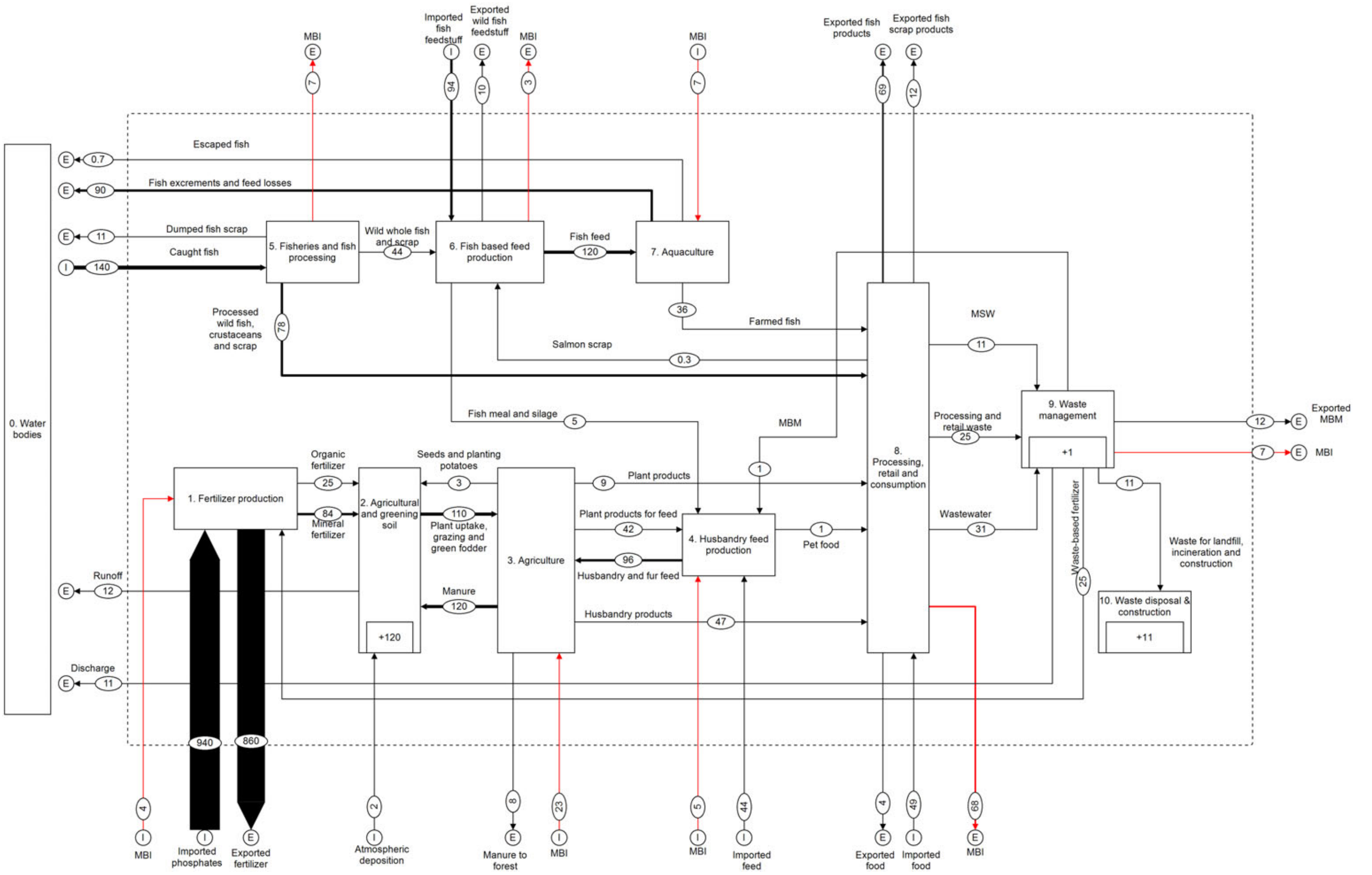

Figure 1 Norwegian phosphorus balance, 100 tonnes P, averaged 2009–2011 data. MBI = mass balance inconsistencies; MBM = meat bone meal; MSW = municipal solid waste; I = import; E = export.

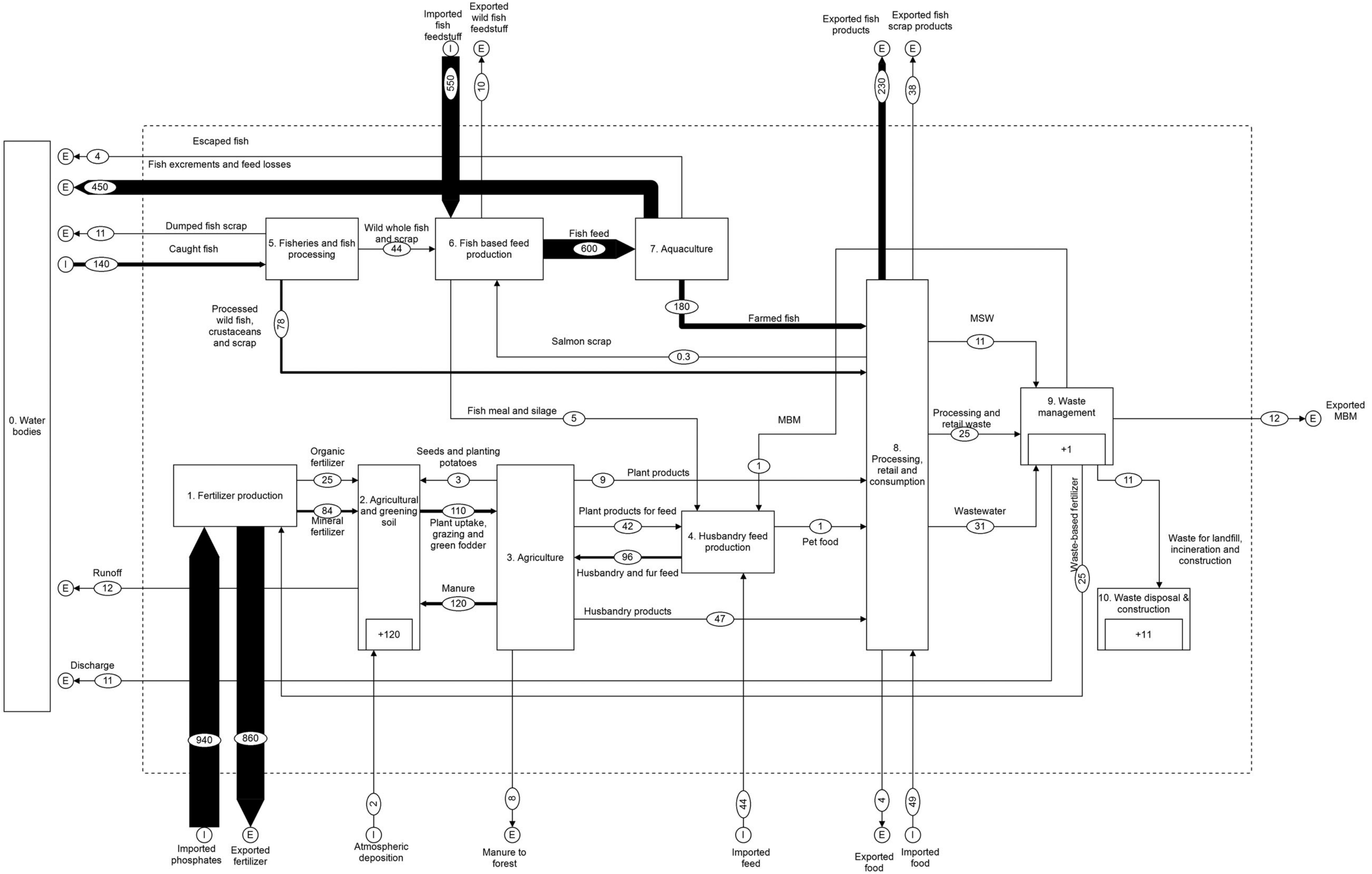

Figure 2 Aquaculture 2050 scenario, 100 tonnes P. MBM = meat bone meal; MSW = municipal solid waste; I = import; E = export.

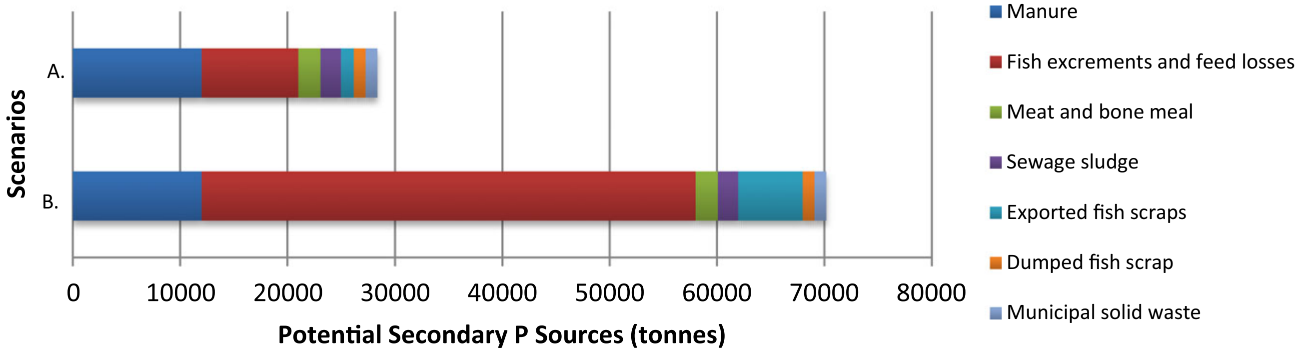

Figure 3 Potential secondary P sources for A. 2009–2011 and B. 2050 scenario in tonnes P.

excrements and feed losses entering water bodies versus P collected in manure.

With respect to fisheries, we determined that the amount of P in landed fish, or fish that were brought onto land from fishery vessels (14,000 tonnes P/yr), is approximately 4 times larger than the P in farmed fish from aquaculture (3,600 tonnes P/yr). We estimated an efficiency of approximately 92% for fisheries. Driving the high efficiency is the lack of feed input and the relatively high utilization of fish scraps from land-based processing. Compared to agriculture products, the P in landed fish far exceeds the P in both plant and husbandry products, with 9,800 tonnes P/yr combined. In terms of existing cross-sectoral synergies between aquaculture, agriculture, and fisheries, the only link found was the use of a small amount of fish meal and silage (500 tonnes P/yr) for the production of husbandry feed.

Waste flows downstream from human consumption were relatively small compared to agriculture, aquaculture, and fisheries. The largest flows include processing and retail waste (2,500 tonnes P/yr) and wastewater (3,100 tonnes P/yr). Waste that was not returned to agricultural and greening soils was accumulated in landfills and construction sites (1,100 tonnes P/yr).

The 2050 scenario reveals the future significance of the aquaculture sector (figure 2). With fish production at 5 million tonnes P/yr and technologies held constant, lost P to marine waters reaches 45,000 tonnes P/yr through fish excrements and feed losses. Compared to the 2009–2011 model, this far exceeds the mineral fertilizer demands of agriculture. Additionally, the aforementioned P losses approach orders of magnitude comparable to imported rock P for fertilizer production, 94,000 versus 45000 tonnes P/yr, respectively. In terms of fish feed needed to sustain the fivefold increase, the 2050 scenario highlights the drastic increase in reliance on imported fish feedstuff, from 9,400 to 55,000 tonnes P/yr. Overall, our results indicate that, with current technologies, such growth could lead to a dramatic increase in P losses to marine waters as well as the dependency on imported P in fish feedstuff. In terms of P recycling, however, the overall amount of potential secondary P sources drastically increases from the base-case scenario to the 2050 scenario (figure 3).

Landed scraps from fisheries and aquaculture are, in general, well utilized either domestically or internationally through the export of fish scraps predominantly for fish feed production (Olafsen et al. 2013). We determined that the only unutilized waste in fisheries, within the system boundaries, is dumped fish scraps, 1,100 tonnes P, mainly from offshore onboard fish processing. This represents a potential secondary source of P if collected and brought to land. In aquaculture, secondary P in fish scraps are also well utilized; however, fish excrements and feed losses represent a substantial amount of unutilized secondary P. Additionally, in order to reduce the dependency on external sources of P, exported fish scraps, 1,200 tonnes P (both wild and farmed), could be considered a source of secondary P if utilized domestically. Compared to agriculture, we determined that 50% of waste flows from agriculture and waste treatment get returned to agricultural land, not including urban greening. This fraction includes all manure produced, 50% of sludge produced, 20% of the meat bone meal produced, and 3% of generated food waste. Further information related to the aforementioned calculations can be found in the Supporting Information on the Web. Treated wastewater effluents were not included as a viable source of secondary P and were thus not included in figure 3. Technological upper limits for P removal during wastewater treatment, a lack of further treatment requirements, and direct dumping make this an unviable source of secondary P.

Discussion

Data Quality

The largest mass balance inconsistencies were found in the process "processing, retail and consumption." Because each flow in this process was separately calculated, errors could be the result of excluded hidden waste flows. Fish domestically consumed, for example, was calculated using data for purchased seafood in Norway. This masks waste flows between the producer and the consumer, and therefore large inconsistencies could be losses from wasted food owing to transportation and expiration. Further inconsistencies could be owing to similar issues related to unaccounted flows for food processing wastes. Poor data availability within food processing and postconsumer wastes weakened the robustness of this portion of the model. Wastewater calculations, for example, were based off of a set of detailed assumptions, found in the Supporting Information on the Web, regarding treatment type and treatment efficiency. Mass balance calculated stocks include a large amount of uncertainty given that errors and missing flows could be masked through the balance. For most flows within aquaculture, fisheries, and agriculture, the data were relatively robust given that data were primarily sourced from governmental reports and national statistics. Fish excretion and feed loss calculations, however, were based on mass balance principles. Though this approach is relatively uncertain, the values found were comparable to an independent study conducted by Wang and colleagues (2012) for 2009. Wang and colleagues found that 9,400 tonnes P/yr was discharged to the environment from Norwegian aquaculture as compared to 9,000 tonnes P/yr found in this study. The lower value could be owing to our use of a higher P content of salmon, meaning that more P was assimilated in the biomass resulting in fewer losses to the environment.

Current Recycling, Inefficiencies, and Potential Improvements

Unlike most other countries where agriculture is clearly the dominating sector, in Norway, the P flows in agriculture, aquaculture, and fisheries are of similar magnitude. Therefore, within these three sectors, there is a substantial opportunity to utilize secondary P sources and reduce the overall domestic demand for mineral P. The following paragraphs discuss the current state of recycling within the different sectors and highlight inefficiencies and areas for substantial improvements.

Aquaculture

In Norway, marine water bodies represent the second largest sink for P. Norwegian aquaculture contributes three P emission streams to the marine environment. Particulate organic P (POP) from fish feces and excess feed represent the largest output of P in volume. Dissolved organic (DOP) and inorganic P (DIP) contribute significantly less P by volume, but are more readily bioavailable than POP. DIP is especially bioavailable and readily taken up by marine phytoplankton in close proximity to the net. Multiple environmental factors determine the dominant phytoplankton species that grow, but studies in Norwegian waters have shown that commercially important kelp and seaweed species achieve higher growth rates close to aquaculture pens (Handå et al. 2013). Multitrophic aquaculture seeks to take advantage of waste nutrient streams by generating valuable biomass from multiple species. Efforts in Norway have focused on kelp/seaweed combined with suspended bivalves to take up DIP/DOP and benthic invertebrates, such as sea urchins, for POP (Bellona 2013).

Currently, for Norwegian sea-based aquaculture, technology limits the ability to utilize this secondary form of P; however, recovery systems are being conducted on a pilot scale. With the development and implementation of technologies such as integrated multitrophic aquaculture, this sector represents a large potential source of secondary P (Wang et al. 2012). Harvested biomass can potentially be used to displace marine ingredients in fish feed, thus directly closing the loop between losses and feed production. However, similar to manure, P recovery from aquaculture is limited by spatial distribution. Fish farms are distributed along the coast of Norway and recovered P must be processed and transported to, for example, fish-feed–producing factories or other areas with P deficits. This could exasperate energy systems and potentially increase costs beyond economic viability.

Land-based aquaculture systems (i.e., controlled on-land recirculating systems used for fish farming) also represent a potential solution for collecting secondary P (Tal et al. 2009). Land-based recirculating tanks result in significantly smaller P losses and allow for easier removal and collection of excreted P, feces, and excess feed. Nonetheless, though land-based systems may be preferable from a P perspective, there are several barriers related to increased costs, energy demand, land demand, and fresh water demand (Aspass et al. 2014). Ocean-based closed containment systems could present a means of reducing pressure on the aforementioned resources while aiding nutrient recovery; however, studies have shown that these technologies are in their infancy and potentially result in animal health issues (Chadwick et al. 2010). The aforementioned barriers must be overcome in order to consider these systems viable solutions.

Fisheries

Secondary P from fisheries is primarily comprised of the scraps and by-products from seafood processing. Seafood processing of fillets from whitefish and herring leave by-products rich in P owing to most of the P being found in the bones. The efficiency of by-product recycling is high in Norway, except for the offshore whitefish fleet, which currently dumps approximately 90% of by-products (Olafsen et al. 2013). Opportunities for improvements in secondary P recycling for fisheries are twofold.

The first way to improve secondary P recycling is to bring all by-products and by-catch to land. The ban on dumping of commercially important species has recently been extended to include all Norwegian by-catch with a few notable exceptions, including fish that are no longer fit for human consumption owing to damage (Gullestad 2015). The ban has been estimated to have reduced dumping of commercially important species to between 2% and 8% of catch (Valdemarsen and Nakken 2002). However, difficulty in enforcement, combined with minimal incentive to land commercially unimportant catch, adds a high degree of uncertainty to dumping estimates. By-products from fish processing can be legally discarded, and this practice is common for large vessels operating far from land. A common platform for the processing of fish by-products and unwanted by-catch onboard fishing vessels is needed to utilize this resource.

The second option to improve secondary P recycling is to process more fish in Norway. The current trend in the Norwegian seafood industry is to outsource fish processing in order to reduce costs (Henriksen 2013). Even exported partially processed whitefish, with the guts and head removed, still contain a large percentage of P in the backbone and pin bones (Opstvedt and Mjelde 1994; Albrektsen et al. 2014). Once the fish leaves Norway, this export becomes a lost source of secondary

P, as seen from a Norwegian resource perspective. Measures to promote a larger degree of processing in Norway have the potential to increase secondary P availability and other important by-product streams.

The Use of Manure and Waste Products in Agriculture

In terms of ability to recycle secondary P, the agricultural and postconsumer waste subsystems have much larger practical capabilities owing to the presence of P in solid and collectable forms, such as manure and sewage sludge. This is in contrast to fish scraps that are dumped offshore or as soluble and particle P lost in fjord systems. Despite the relatively large returns of these products back to agricultural soils, P in manure and sewage sludge are not being recycled efficiently, as shown by the P accumulation in soils. The reason for this, however, is different for each secondary product.

Animal husbandry, and thus manure, is unequally distributed throughout the country, making the distance between the point of manure generation and crop and cereal production needing fertilization potentially vast. The high water content of manure, and thus large weight loads coupled with long transportation distances, puts a strain on costs and energy demand. This limits the ability to transport the secondary P, despite its high plant availability, to agricultural areas with cereal production, for example, in the southeastern part of the country (Knutsen and Magnussen 2011). The spatial distribution of manure issue is not specific to Norway. Whalen and Chang (2001) showed that, in Canada, it is uneconomical to transport manure long distances, and therefore manure is applied mostly to the land surrounding animal husbandry. This combined with the fact that manure is applied based on crop nitrogen requirements resulting in the application of excess P, the accumulation of P in cultivated soils in Canada is the result of long-term manure application. This is likely to be the case in Norway as well.

For postconsumption P flows, such as wastewater and municipal solid waste, the variable plant availability of P introduces barriers. Waste treatment technologies are chosen to meet a variety of criteria, including cost, energy use, and treatment efficiency, and not necessarily P recovery for recycling. In Norway, chemical precipitation is widely used to treat wastewater, despite it drastically decreasing P plant availability (Morse et al. 1998; Vogelsang et al. 2006). This is owing to the need for cost-/space-effective indoor treatment plants that can handle cold winters. In addition, sewage sludge raises concerns surrounding contaminants, such as organic pollutants and pathogens, that could be harmful to human health and affect long-term soil quality. This reduces farmers' willingness to accept them as appropriate substitutes (Refsgaard et al. 2004). The fraction of wastewater sludge that is applied to agricultural soils, however, is used for its soil amendment properties, rather than its P contents, and is thus not primarily used to displace mineral P fertilizer (Refsgaard et al. 2004).

It is likely that P accumulation in agricultural soil is primarily owing to ineffective manure and sludge application: Because of the spatial imbalance between manure generation and crop production areas where mineral P is applied, manure is often applied where P is in excess. Additionally, because the plant availability of P in sludge is poor, it is common practice to apply both mineral fertilizer and sludge to agricultural soils. Therefore, even though the return of secondary agricultural P products to land is relatively high, the reuse or actual plant uptake of secondary P is low and the soil remains a large sink of P.

Options for improving P recycling in agriculture are primarily technology based. Improving manure distribution would require the development and utilization of technologies that can reduce the weight content of manure, thus allowing lower transportation costs. In terms of sewage sludge, despite the challenges related to the Norwegian climate, a shift toward biological wastewater treatment would increase the plant availability of P (Morse et al. 1998). Given that P from the waste sector is easily collectable and regionally concentrated, the distribution of this P source would be manageable. However, this is only viable if barriers related to energy and costs are overcome.

Integrating Aquaculture, Agriculture, and Fisheries for the Utilization of Phosphorous

Scenario: Future Challenges

The future fivefold aquaculture growth, which will result in an inevitable increase in P losses to water bodies, is likely to pose many environmental challenges. With 5 times more P reaching fjord systems, the risk of eutrophication in coastal marine waters drastically increases. The high flux rate of fjord systems currently limits eutrophication concerns, given that the strong currents quickly exchange nutrients with coastal waters (Skogen et al. 2009). However, with drastic increases in P waste flows, concentrations can potentially reach levels that exceed the flux capacity of the fjords. This will have to be considered carefully by public authorities when realizing growth. Additionally, future strains on P resources abroad may cause problems in Norway because of the increased demand for imported feedstuff. Norway does not have feed sources of their own, and feedstuff needs will therefore either have to be sourced from other countries or from the ocean through fisheries. Given that it is highly likely that the imported agricultural feedstuff products are produced using mineral fertilizers, increased fish feed production means that Norway will also indirectly increase its mineral P footprint unless P losses along the production chain are recovered and recycled.

Scenario: Future Opportunities

From a Norwegian standpoint, if P sources are utilized efficiently, the fivefold growth could also represent an opportunity for moving Norwegian agriculture toward becoming import rock P independent. Currently, cross-sectoral utilization of secondary P is hardly explored and is certainly not optimized. The only link between aquaculture, fisheries, and agriculture is the fish waste products that are used as inputs to husbandry and fur animal feed. This represents only a small fraction of the P needed in agricultural feed and approximately one third of the total excess aquaculture fish scrap, with the remainder being exported. With the goal of domestically sourcing P for food production, it is imperative to map and quantify secondary P sources and how they could be optimally utilized within and between sectors.

As discussed, in agriculture, the utilization of returned secondary P is poorly managed and, in order to improve this, related to logistics, energy use and P quality will have to be overcome. Potential improvements that can be realized without substantial technological breakthroughs, however, include optimizing aquaculture and fishery wastes for feed production. Owing to the fact fish feed cannot be sourced from the same species of fish, secondary P from salmon, which comprises over 90% of Norwegian aquaculture, cannot be used to feed salmon (Norwegian Directorate of Fisheries 2014). It can, however, be used for nonsalmon species or as a feed component in animal husbandry. This potential will increase as aquaculture production increases, representing a growing opportunity for displacing imported P in agriculture feed.

Another important step for optimizing fish scrap P recycling is by reducing the amount of dumped fish scrap. Technology and financial incentives currently limit the ability to collect and reuse this waste. However, the prices of by-products are expected to rise with the prices of vital nutrients, such as omega 3-fatty acids, thereby improving the feasibility of utilizing this waste. Marine by-products are especially valuable in Norway because 99.9% of the marine landed catch is nonsalmon species (Norwegian Directorate of Fisheries 2013). This means that the by-products can be used in aquaculture, thus reducing the P burden from agriculture-based P products abroad and the mineral P, which is likely used to produce them.

With secondary P from aquaculture likely reaching orders of magnitude that are comparable to imported rock P for the production of fertilizers, this represents a substantial opportunity to displace not only domestic mineral P needs for fertilizer production, but also global, given that approximately 90% of Norway's produced fertilizers are exported. The P burden would effectively be shifted from mineral P to sea-based P. There are a substantial amount of hurdles to overcome to realize this opportunity. Obstacles related to P quality, salt concentrations, cost, energy needs, and the technologies for recovery will require a substantial amount of research before cross-sectoral synergies can be realized, but if solved, could provide partial solutions to reducing the dual problem of eutrophication and mineral P dependency.

Future Research

In order to identify more in-depth P management solutions, future research is needed. Through this study, it was shown that several methodological adaptations of the model could help to provide a more holistic basis for decision making. In the choice of technological systems, it is clear that energy and other critical materials are closely coupled to P. A good example of this is the increased P demand through the production of bioenergy from crops. To produce this type of energy, biomass inputs require P, indicating that increased bioenergy production would also increase P requirements. Because of these types of relationships, it would be beneficial to study P in combination with other critical materials/nutrients. A multilayer material flow analysis approach could include societally important materials and energy and would preferably lead to overall, not merely P-specific, improvements in resource management.

Further, concerns surrounding technology, plant availability, and practical/economic feasibility for recycling P make the inclusion of a "feasibility assessment" of great value. Such a study would highlight more practical issues regarding recycling given that often quality issues, such as plant availability and/or the presence of heavy metals, play a major role in the ability and willingness to reuse P within agricultural systems. If P is looked at only through an elemental point of view, information regarding the existence of contaminants that reduce the usability of P is masked. Other feasibility elements include the spatial and temporal distribution of P. Conventional SFA methods portray P in an aggregate form and do not include resolution regarding physical location, a factor that plays a key role in the feasibility of reusing P. This would better be examined by spatially explicit SFA modeling.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Table S1. Assumptions utilized for wastewater treatment calculations (Sources: ^{1}(Berge & Mellem, 2012) ^{2}Own assumptions)

Table S2. Calculation for the percentage of meat and bone meal returned to agriculture: DM = dry matter; MBM = meat and bone meal

Table S3. Calculation for the percentage of sludge returned to agriculture

Table S4. Calculation for the percentage of food waste returned to agriculture; MSW =municipal solid waste

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SUPPORTING INFORMATION FOR:

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Summary

This supporting information includes process descriptions and flow calculations, efficiency calculations, and secondary P return calculations.

Process descriptions and flow calculations

Husbandry feed production [process 4]

Husbandry feed production includes all of the animal concentrates and feed that are produced for animal husbandry, fur animals and pets. This includes domestically produced feed based on imported phosphates, domestically produced meat and bone meal and domestic plant products.

Waste management [process 9] and waste disposal and construction [process 10]

Wastewater contains both sanitary and industrial wastewater. Wastewater treatment plants produce sludge used as fertilizer and soil amendment in agriculture and greening and some P is lost to water through discharge. The stock accumulation is due to the retention of P in on-site treatment. Loss of P from sewage pipe leakages was considered partly offset by leakage of water into the same pipes and negligible. Slaughter waste is processed into meat and bone meal (MBM), which is either used as waste-based fertilizer, in pet- and fur feed production, exported, or incinerated. Ashes from incineration of MBM are used in cement production for the construction sector. The Norwegian company Norsk Protein has a market share of about 85% of the slaughter waste processing (Viste, 2010), and values are corrected for the remaining 15%. Mixed household and service waste is incinerated domestically or exported for incineration abroad, while source separated food waste is used as fertilizer after processing in biogas- or composting plants or exported.

Wastewater discharge/net addition to stock calculation

The calculations for wastewater discharge and net addition to stock in process 9 were done using the below assumptions. For some of the treatment solutions the percentage (column 2) coincides with the treatment effect meaning that all P retained in the treatment system is collectable and taken to a wastewater treatment plant. For other solutions, the percentage is less than the treatment effect. This means that some of the P is retained on-site either in soil or adsorbed to another filter medium.

Treatment type	**Treatment effect (%)[1]**	**Part of P load transported to wastewater treatment plant[2]**
Direct discharge	0	0
Septic tank	5	5
Packaged domestic wastewater treatment plant, biological treatment	15	15
Packaged domestic wastewater treatment plant, chemical treatment	90	90
Packaged domestic wastewater treatment plant, biological-chemical treatment	90	90
Subsurface soil infiltration	75	5
Sand filter	15	5
Blackwater holding tank	75	75
Dry toilet	75	0
Total wastewater holding tank	100	100
Constructed wetland	90	0
Blackwater holding tank, grey water granular medium filter	90	75
Dry toilet, grey water granular-medium filter	90	0
Other treatment method	50	0

Table S1. Assumptions utilized for wastewater treatment calculations (Sources: [1] (Berge & Mellem, 2012) [2]Own assumptions)

Efficiency calculations

Plant and animal production

The plant efficiency calculations were done in the following steps:

1. Obtain the total amount of agricultural land used for the production of agricultural production types* in hectares.
2. Multiply this amount by the recommended P fertilizer application rates (Bioforsk, 2012) based on agricultural land types*

$$\left(Amount\ of\ agricultural\ land\ per\ agricultural\ land\ type\ (ha)\right) \times \left(P\ fertilizer\ application\ rate\ per\ agricultural\ land\ type\ \left(\frac{kg}{ha}\right)\right) = x_{a-e}$$

*agricultural production types: a = cereals; b = oil seeds; c = fruits and vegetables; d = potatoes; e= grazing land, cultivated pastures, etc.

3. Derive the P share of each agricultural production type in relation to total agricultural land

$$\frac{X_{a-e}}{\sum_{i=a}^{i=e} x_i} = f_{a-e}$$

4. Multiply the share by separate data for total P in mineral and manure applied to get the amount of P per agricultural land type.

$$a\ \times f_{a-e} = t_{a-e}, where\ a = total\ P\ in\ fertilizer\ applied\ (manure\ and\ mineral)\ and$$

$$t_{a-e} = total\ P\ per\ agricultural\ land\ type\ a\ through\ e$$

5. Using the share of cereals for husbandry feed versus human consumption versus planting seeds as well as knowledge about which products are used for husbandry feed, the amount of P needed for husbandry versus plant production was derived.

$$P_{pl} = \ C_s \times t_a + C_{hc} \times \ t_a + t_c + t_d$$

$$P_h = C_h \times t_a + t_b + t_e,$$

$$where\ C_s = \%\ of\ cereals\ for\ seeds;\ C_h = \%\ of\ cereals\ for\ husbandry\ feed;$$

$$C_{hc} = \%\ of\ cereals\ for\ human\ consumption;$$

$$P_{pl} = P\ in\ manure\ and\ mineral\ fertilzier\ for\ plant\ production;$$

$$P_{h} = P\ in\ manure\ and\ mineral\ fertilizer\ needed\ for\ husbandry$$

6. The efficiency for animal husbandry production was then calculated by summing the P content in the total outputs which included meat, eggs and milk and dividing by the sum of the total inputs: $P_{h,}$and the imported P in feed concentrates.

$$\frac{P_m + P_e + P_{mi}}{P_h + P_{co}} = e_h \times 100 = efficiency\ of\ husbandry\ production,$$

$$where\ P_m = P\ content\ in\ produced\ meat; P_e = \ P\ content\ in\ produced\ eggs;$$

$$P_{mi} = P\ content\ in\ produced\ milk; P_{co} = Imported\ P\ in\ feed\ concentrates$$

7. The efficiency for plant production was calculated by summing total outputs including cereals, oil seeds, potatoes, vegetables and fruits and dividing by the total P requirements for plant production, P_{pl} .

$$\frac{P_c + P_{cs} + P_p + P_f + P_v}{P_{pl}} = e_{pl} \times 100 = efficiency\ of\ plant\ production,$$

$$where\ P_c = P\ content\ in\ cereals\ produced; P_{cs} = P\ content\ in\ oil\ seeds\ produced;$$

$$P_p = P\ content\ in\ potatoes\ produced; P_f = P\ content\ in\ fruit\ produced;$$

$$P_v = P\ content\ in\ vegetables\ produced$$

8. Steps 1-7 were done for the chosen reference years, 2009, 2010 and 2011, and the three values were averaged to obtain the final efficiency.

Aquaculture and fisheries efficiency

1. Aquaculture efficiency was calculated by summing the total outputs and dividing by the total inputs.

$$\frac{P_{ff}}{P_{feed}} = e_a \times 100 = efficiency\ of\ aquaculture\ production, where$$

$$P_{ff} = P\ content\ in\ farmed\ fish\ produced\ and\ P_{feed} = P\ content\ in\ fish\ feed$$

2. Fisheries efficiency was calculated by summing the total utilized outputs and dividing by the total inputs. The calculated efficiency was very high because the only

unutilized wastes inside the system boundaries were dumped fish and scrap. Additional wastes that occur at sea were not considered inside of the system boundaries.

$$\frac{P_{wf} + P_{wfs}}{P_{lf}} = e_f \times 100 = efficiency\ of\ fisheries\ production, where$$

$$P_{wf} = P\ content\ in\ wild\ fish\ to\ fish\ feed\ production\ and\ processing, retail\ and\ consumption;$$

$$P_{wfs} = P\ content\ in\ wild\ fish\ scrap; P_{lf} = P\ content\ in\ landed\ fish$$

Secondary P return calculations

Percentage of meat and bone meal returned to agriculture as fertilizer

Meat and bone meal	**Amount**	**Unit**	**Source**
Average 2009-2011 total amount of MBM returned	8870	tonnes MBM	(Animalia, 2010, 2011, 2012)
Dry matter content	96	% of wet weight	(Norsk Protein, 2013)
P content in dry matter	4.5	% of dry matter	(Norsk Protein, 2013)
Market share of Norsk Protein	85	%	(Viste, 2010)
Average P amount	451	tonnes P	
Total	2173	tonnes P	Flow 8,9a
% of P in MBM returned	**21**	**%**	

Table S2. Calculation for the percentage of meat and bone meal returned to agriculture: DM = dry matter; MBM = meat and bone meal

Percentage of P in sludge returned to agriculture

Assuming that P in sludge is equally distributed, the share of sludge to agriculture was calculated from (Berge & Mellem, 2012).

Year	Calculation	Percentage returned
2009	63866 t DM to ag./113200 t DM in total	56.4 %
2010	57244 t DM to ag./105771 t DM in total	54.1 %
2011	65579 t DM to ag./116336 t DM in total	56.4 %
Average % of sludge P to agriculture		56%

Table S3. Calculation for the percentage of sludge returned to agriculture

Percentage of P in food waste returned to agriculture

Food waste	**Amount**	**Unit**	**Source**
Avg. amount of biologically treated food waste 2009-2011	162000	tonnes	(Statistics Norway, 2014)
DM content of organic household waste	33	% of wet weight	(Møller et al., 2012)
P content in dry matter or organic household waste	0.4	% of dry matter	(Møller et al., 2012)
Fraction of biologically treated waste to agriculture	17	%	(Lystad & Vethe, 2002)
Amount of P in biologically treated food waste to agriculture	36	tonnes P	
Total amount of P in MSW	1142	tonnes P	Flow 8,9a
Average % of P returned to agricultural soils = 3 %			

Table S4. Calculation for the percentage of food waste returned to agriculture; MSW = municipal solid waste

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PAPER II

Hamilton HA, Brod E, Hanserud OS, Müller DB, Brattebø H, Haraldsen TK. Estimating the recycling potential of secondary phosphorus resources by integrating substance flow analysis and plant-availability. Manuscript

Estimating the recycling potential of secondary phosphorus resources by integrating substance flow analysis and plant-availability

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Abstract

The plant-availability of phosphorus (P) plays a central role in determining the recycling potential of secondary resources. Secondary P plant-availability varies significantly, often with large fractions of residual P that, if applied, accumulates in soils thereby increasing the risk of P runoff and eutrophication. Substance flow analyses (SFA), used to identify potentials for improved P management, have, to date, not considered this well-known recycling barrier. We, therefore, argue that P recycling potentials estimated by traditional SFA are overestimated. Using Norway as a case, we present a newly developed methodology for assessing plant-availability within a mass balance framework through the integration of SFA and the concept of relative agronomic efficiency. We found, while the method has uncertainties particularly for long-term estimations, it more realistically estimates secondary P recycling potentials and is adaptable to other countries. For Norway, we found the overall secondary P potential reduced by 6-55% when considering plant-availability. The most important secondary product was manure, which had i) the highest P plant-availability and ii) quantities large enough to meet Norway's entire P fertilization demand, with 10.9 kt plant-available P/yr. Fish sludge was also an important product, with 6.1 kt plant-available P/yr but with uncertain plant-availability data. Food waste and sewage sludge were relatively negligible sources of plant-available P, with 1.6 and 0.5 kt plant-available P/yr, respectively. We argue that secondary P products can meet Norway's P fertilization demand but need to be carefully regulated based on their plant-availability to eliminate the soil accumulation of both available and residual P.

1 Introduction

Phosphorus (P) is essential for food production but also acts as a pollutant if accumulated in sensitive aquatic systems. Currently, agricultural P demands are primarily met through the mining of non-renewable phosphate rock and, overall, P use is inefficient implying minimum recycling, large losses and soil accumulation.[1] Therefore, in order to reduce the rate of rock phosphate depletion and eutrophication risks while meeting the growing demand for food, a high level of effective P recycling is essential.[2]

The research community has recognized the need for using substance flow analysis (SFA) to describe the anthropogenic P cycle and estimate P recycling potentials.[1,3,4] Conducted studies, however, neglect one of the greatest barriers to P recycling: the plant-availability of secondary resources.[3,5–12] Growth experiments have shown that P forms in secondary resources vary considerably, often with large fractions of P bound in complex and slowly soluble compounds that, if applied, can accumulate in non-labile P soil pools.[13] Chemically precipitated sewage sludge from wastewater treatment, for example, has relatively low fertilization effects due to the high percentage of P that is adsorbed to Fe-/Al-hydr(oxides) or present as Fe-/Al-phosphates.[14] Due to this, we hypothesize that secondary P recycling potentials calculated by traditional SFA are over-estimated.

Matsubae-Yokoyama and colleagues[5] have highlighted the need for exploring the quality of untapped secondary P resources. Ringeval and colleagues[15] have recognized that secondary P resources, such as sewage sludge, have both a plant-available and plant-unavailable fraction. Nonetheless, SFA has not been adapted to include these aspects. Such advancements are important in order to obtain more realistic estimates of secondary P recycling potentials and inform strategies for improved P management.

Here, we use the concept of relative agronomic P efficiency (RAE) [also called mineral fertilizer equivalents (MFE)] and propose a new method for assessing plant-availability within a mass balance framework. RAE is a measure of crop response as a factor of P fertilization as it compares to mineral P application. This metric provides the fraction of P within secondary products that is directly available to plants, assuming that water-soluble mineral P fertilizer is 100% plant-available. Growth experiments are considered the most reliable method for quantifying RAE and, therefore, we integrate the results from secondary P product growth experiments with SFA and apply this adapted method to our case study of Norway. This is in order to answer the following research questions:

What are the strengths and weaknesses of the developed plant-availability-explicit SFA approach?

What is the current P recycling potential of Norwegian secondary products based on plant-availability? Can it meet the demand for P fertilization?

What are the policy implications of a plant-availability explicit phosphorus SFA?

2 Methods

Substance flow analysis. We determined the most important potential secondary P resources in Norway from the results of an average 2009-2011 SFA of P.[12] We excluded: i) products that were better utilized at a higher trophic level, e.g. animal husbandry feed and ii) P losses that were a result of technological treatment limitations, such as the P remaining in treated wastewater that was discharged to water bodies. For further information regarding the methods and results from this study, refer to Hamilton and colleagues.[12]

Technology assumptions. We considered the treatment technologies applied to secondary P resources in 2009-2011. Exceptions were i) fish excrements/feed losses generated in marine aquaculture (later referred to as fish sludge), which were not collected in 2009-2011, ii) fish scrap where losses entered directly to water bodies untreated and iii) food waste that was incinerated or exported. For these secondary P resources, we assumed treatment technologies based on available data and the most likely pathways for recycling in Norway. For fish sludge, we obtained an RAE value for only one product treated via reactor composting[13] and, due to data limitations, applied this to all generated fish sludge. Also due to data limitations, we assumed the plant-availability in fish scrap to be similar to that of meat bone meal, as P in fish scrap is also primarily sourced from the bones.[16,17] For food waste treatment, we assumed 50% composting and 50% anaerobic digestion.

Relative Agronomic Efficiency (RAE). In order to quantify the plant-available fraction of P in secondary products, we applied the concept of RAE. We chose RAE as a relative measure for the fertilization effect of secondary products over an absolute metric (e.g. P use efficiency) to estimate the substitutability of mineral fertilizer. Figure 1 provides a schematic illustrating how RAE was defined in this study: mineral fertilizer is used as a benchmark by assuming that all mineral P is available for plants (RAE=100%). RAE represents the plant-available P fraction of returned secondary P products, while the remaining P (referred to as residual P) enters the residual P soil pool. In reality, different biotic and abiotic soil processes transfer P from the plant-available soil pool to the residual P pool and vice versa. These processes are complex and depend on various soil characteristics such as the P status, P adsorption capacity, soil pH, organic P mineralization rates, time and the crop grown (e.g. Pierzynski et al.[18]). However, we assume that the relative fertilization effect of a secondary P product during the first growing season is mainly due to the solubility of the P species it contains and that P uptake after secondary resource and mineral fertilizer application is not affected differently by soil processes.

To consider a wide range of studies for each secondary product, RAE values were sourced from both international and Norwegian pot and field experiments (table 1), where only one-year experiments or the first year of long-term experiments were considered. All obtained values were weighted equally. The RAE values for fish sludge, chemically and chemically-biologically treated sewage sludge were sourced from experiments on only Norwegian products, as described by Brod et al.[13] and Øgaard and Brod[19]. We assume that the obtained RAE values are representative of Norwegian conditions.

We assumed an apparent P use efficiency of 100% of mineral fertilizer. However, organic fertilizers e.g. manure have been shown to be able to, upon application, increase the flow from the residual P stock to the directly available stock relative to mineral fertilizer. This can be explained by low molecular organic acids reducing the phosphate retention of soil particles[20] allowing for RAE above 100%, as the excess P is sourced from the existing soil stock. However, because we cannot quantify the exact amount transformed from the residual stock to the available, we have left this flow unquantified, represented by the dotted arrow in figure 1.

For products with no available RAE values, we used similar products as proxies. For fish scrap, we assumed the RAE for meat bone meal. For horse manure, we assumed an RAE of 80% due to a lack of data. Finally, 100% RAE was assumed for mechanically treated sludge based on Hanserud and colleagues.[21]

To express the uncertainty of RAE values, we calculated 95% confidence intervals of all collected RAE values for their respective secondary P products based on the assumption that the RAE values are normally distributed. For sheep and goat manure, for which we had only one observation, we applied an uncertainty of ±25% to the given value to produce uncertainty ranges. For horse manure, dumped fish scrap and mechanically treated sewage sludge, where there were no observations, we applied an uncertainty of ±25% to their assumed RAE values.

P fertilization demand. The P recycling potential of Norwegian secondary products was compared with the domestic demand for P fertilizer.[22] We corrected the P fertilization demand by accounting for the existing levels of plant-available P in soils based on Hanserud and colleagues.[21] More information regarding this method can be found in the supplementary information.

Scenario. We applied the calculated RAE values with the aforementioned treatment technology and plant-availability assumptions to the 2009-2011 system. The system structure was held constant and, thus, no changes were made regarding fertilizer application or the return of secondary P products as compared to Hamilton and colleagues.[12] However, we included a fertilizer market that includes all primary and secondary P resources, in order to enable the visualization of the theoretical recycling potential and their subsequent uses. The presented RAE corrected system, using average RAE values, does not allow for mass balance consistency because the RAE values only consider a fraction of the P contained in the flows. We, therefore, included the total P content in parenthesis for flows of returned secondary P products in order to enable mass balance consistency for the plant production process.

3 Results

A comparison between the total P and RAE corrected P balances is shown in figure 2. We found that the overall plant-available recycling potential of Norwegian secondary P products ranged from 12.7 to 26.3 kt plant-available P/year, which was a reduction of between 6 and 55% as compared to total P. In terms of both total P amounts and plant-available P, manure was the most important P resource with between 8.7 and 11.4 kt plant-available P/yr, which is large enough to meet the entire P fertilization demand of Norway. Fish sludge was the second most important P resource, with between 1.9 and 10.3 kt plant-available P/yr. Food waste amounted to between 1.2 and 2.0 kt plant-available P/yr, followed by meat bone meal, sewage sludge and dumped fish scrap, with 0.4-1.2, 0.4-0.7 and 0.2-0.6 kt plant-available P/yr, respectively. We estimated that in 2009-2011 between 9.2 and 12.4 kt/yr of plant-available P was returned to soils, however, due to various factors discussed in Hamilton and colleagues[12], was likely to have accumulated in the soil stock. Between 1.8-0.3 and 0.1-0.6 kt plant-available P/yr was lost to water bodies from aquaculture in the form of fish sludge and fish scraps, respectively. Finally, it was estimated that between 0.5 and 1.3 kt plant-available P/yr was incinerated, landfilled or exported.

Applying the RAE values to the different secondary P resources had a varied effect. For manure, between 76 and 100% of the total P was available for plants while, for fish sludge, between 21-115% of the total P was plant-available. Food waste had a relatively high plant-available fraction of 41-75%, however, applying RAE values to the remaining products drastically reduced their P recycling potential due to their low plant-available fractions. Fish scrap, meat bone meal and sewage sludge had plant-available fractions of 10-60%, 19-60% and 22-40%, respectively.

With an estimated P fertilization demand of 5.8 kt plant-available P/yr (figure 3), overall, we determined that Norwegian secondary P products could potentially meet the P demand even when considering the minimum recycling potential. Therefore, in theory, the Norwegian secondary P recycling potential based on plant-availability is large enough to displace mineral P fertilizer use.

When applying secondary products, however, it is also important to consider the residual P contained within these products, as accumulated soil P increases the risk of runoff and eutrophication. We estimated that during the 2009-2011 period, between 11.8 and 15.0 kt plant-available P/yr was over applied to agricultural soils and included with these products was an additional 2.8 kt of residual P/yr. This contributed to the large net accumulation of stock (12 kt P/yr), owing to the over-application of both plant-available and residual secondary P. In terms of individual products, we found that chemically and chemical-biologically treated sewage sludge had particularly high fractions of residual P of 63-80%. Meat bone meal and fish scrap also contained high amounts of residual P with between 40-81% and compost contained residual P amounts between 35 and 61%.

4 Discussion

Our newly adapted method provides a first approximation for including plant-availability in SFA P studies and, through this, we have shown that traditional SFA overestimates the recycling potential of secondary P resources. The inclusion of plant-availability within SFA is an important advancement for P assessments, as it provides a more realistic estimation of the overall secondary P recycling potential and, furthermore, highlights the most promising secondary products in terms of amounts and mineral P substitutability. Because traditional SFA quantifies total P, there is a risk of focusing on secondary products that are significant in terms of total P amounts but not from a plant-availability perspective. In addition, a lack of consideration for plant-availability increases the risk of applying P products that have high fractions of residual P and, thus, result in soil P accumulation and an increased risk for eutrophication. A focus on reducing the risk for environmental degradation coupled with finding viable substitutes for mineral P is central for long-term P management planning.

4.1 Method

4.1.1 Limitations of plant-availability estimates

Integrating plant-availability estimates with SFA, however, has several limitations, which we discuss in the following paragraphs. Additional limitations regarding SFA methodology are discussed in Hamilton and colleagues.[12]

Soil pH. While the calculated values for plant-available P in secondary resources are applicable on average, their applicability for local soil conditions can be limited. This is primarily due to the RAE of many secondary P resources being dependent on the pH of the target soil. Several secondary P resources contain P that is present as different Ca-phosphates[13] and the solubility of Ca-phosphates is known to decrease with increasing soil pH.[37] This explains the wide confidence interval for the RAE of meat bone meal (table 1), in which P is mainly present as apatite.[13] The P fertilization effect for fish sludge is also dependent on soil pH, owing to the wide range of observed RAE values. Therefore, the RAE values presented serve only as indicative values.

Time aspect. Studies suggest that the long-term P fertilization of secondary resources is higher than the short-term fertilization effects. Bøen and Haraldsen[38], for example, found that the P fertilization effects of meat bone meal and biosolids were higher than the unfertilized control the third year after application to a silty loam, while there were no differences between the fertilizer treatments and the unfertilized control the second year after application. Therefore, simplifying the soil mechanisms to consider 1 year likely underestimates the real recycling potential of secondary resources over the long-term. Conversely, we can assume that secondary P resources with RAE of over 100% will result in reduced P fertilization effects with decreasing soil P status. Therefore, over longer time-frames, the suggested RAE values may not be useful for long-term strategies.

Technology. Because waste treatment technologies dictate the RAE of secondary P products, the technology assumptions had a heavy influence on the results of this study. In the future, new treatment technologies can be developed or existing technologies can improve. An example of this is sewage sludge from wastewater treatment. Currently, the widespread use of chemical precipitation in Norwegian wastewater treatment plants removes 90 to 95% of the P contents in wastewater but leaves it in a mostly unavailable form.[14,19,39] However, a range of studies examine the development of alternative solutions for technically advanced P recovery from sewage sludge.[40] Nanzer et al.[41], for example, found that P fertilizer from sewage sludge ash prepared with $MgCl_2$ as chemical reactant during thermal treatment had RAE of 71 and 88% after application to an acidic and a neutral soil. This is clearly higher than current RAE of sewage sludge treated chemically or chemical-biologically (20-29%) shown in table 1.

Statistical treatment. The statistical treatment of the RAE values contributed to the large RAE uncertainty ranges, as shown by the presented confidence intervals (table 1). This is important to keep in mind when interpreting the results, especially concerning fish sludge. The RAE range for fish sludge, for example, was large (21-115%), with the higher end stretching well above 100%. This was primarily due to only 3 RAE observations being obtained. While fish sludge RAE values of over 100% are theoretically possible[20], it is unlikely that they would be to the extent presented here.

Data availability. The validity of the horse manure, fish scrap and mechanically treated sewage sludge assumptions on P plant-availability is unknown due to a lack of experimental studies on these particular secondary P products. However, the P amounts in these products were insignificant relative to the overall system and, therefore, the aforementioned assumptions are unlikely to have significantly influenced the results.

4.1.2 Applicability of the method

As SFA studies on P are widespread, this approach can easily be adapted to other countries or regions by supplementing or replacing the above RAE values with region and product specific values. Because the uncertainties associated with this method will decrease with improved data availability, more research oriented towards developing and testing the plant-availability of secondary P products is needed in order to apply this on a large scale.

It is worth noting, however, that this study is fertilizer centered, as it only focuses on the use of secondary products as P fertilizer. There are other uses for these products, such as ingredients for animal or fish feed, that could be more beneficial from a resource perspective due to P uptake by animals being at a higher trophic level as compared to plants. These possibilities were not evaluated due to the vast number of additional food and safety barriers associated with using waste products as feed.[16] In the future, however, these potentials should be explored in order to fully optimize the use of secondary P products.

4.2 Policy implications

Regulatory frameworks that govern secondary resources are arguably the largest determining factor for how efficiently they are used.[42] Therefore, it is essential that crucial information, such as P plant-availability, becomes incorporated into policy-making. A lack of information can lead to a disregard for the P fertilization effects of secondary resources and, thus, result in the over-application of both available and residual P with subsequent soil P accumulation. The following paragraphs detail how future policy can incorporate our findings to improve the utilization of P.

4.2.1 Restricting P application per soil area.

Based on the results presented here, we argue for the further restriction of P application to agriculture soils on a per hectare basis. This is particularly important for manure management due to i) its high P plant availability and, thus, large P resource potential and ii) the common practice of over-applying manure resulting in regional P accumulation and related pollution risks.

We have shown that, in Norway, manure, alone, has a large enough potential, both in terms of amounts and plant-availability, to meet the entire P fertilization demand and this potential is not specific to Norway. Van Dijk and colleagues[43] have shown the importance of manure as a P source in the European Union member states, with the amount of P in manure representing about 76% of the total P in agricultural crops produced. However, despite manure's large potential, in Norway, as in many industrialized countries, it is not efficiently used as a secondary P resource, with the primary barrier being the spatial distribution between areas with P surpluses (areas with intensive animal husbandry) and areas with P deficits (crop production areas).[21,44,45] Large transportation distances combined with manure's high water content and, thus, high weight loads make the distribution of manure uneconomical. This leads to the spreading of manure locally, resulting in P over-application, soil accumulation and subsequent eutrophication risks. Large discrepancies in regional P balances due to a lack of manure management are an international challenge, particularly for countries with intensive animal husbandry, including Norway[12,21], the United States[46], the United Kingdom[42], Northern Ireland[42] and Canada[47], to name a few.

For this to be overcome, one key solution to improve the cost-effectiveness of transporting manure is to implement new technologies related to reducing manure's water content or extracting P (as well as other essential nutrients), as for example suggested by Achat and colleagues[48]. We argue, however, that in order to incentivize this technological development, it is essential to implement stricter regulations for manure use, as the current practices will not change within the regulatory frameworks with insufficient manure spreading rules based on P contents and its plant-availability. This is especially the case when the low cost of mineral fertilizer provides little economic incentive for efficiently managing manure.

In Norway, current regulations do not stipulate the utilization of manure P despite the manure spreading area, in fact, being based on the manure's P content: In order to manage livestock density, the minimum farm size has to be ≥ 0.4 hectares per livestock unit (a measure of an animal's P excretion), which correlates to an upper limit of 35 kg P/ha.[49,50] There is a requirement for farmers to make a fertilization plan for their agricultural areas based on the expected crop yield and the residual soil P levels in order to receive production subsidies. However, this plan is not further controlled to ensure that it is being followed accordingly.[39] In addition, there are currently no obligations to limit the over-application of P from both mineral P fertilizer and secondary sources.[50] Therefore, we suggest a stricter limitation of total P application (mineral fertilizer and secondary P resources) per soil area with an adaptation to soil P levels, which will consequently stimulate the development of transportable manure-based fertilizer products. For example, Sweden, as compared to Norway, is more tightly regulated with regards to manure spreading (limited to 22 kg P/ha/yr) and subsidies are used to cover the costs of conserving and managing nutrients.[42,51] According to Kleinman and colleagues[42], this has led to, from a pollution standpoint, positive results with lowered amounts of P losses from agricultural areas. The authors did not, however, comment on the improvements from a resource perspective. In the Netherlands, regulations restrict P fertilization rates based on the soil P status, up to a maximum of 44 and 37 kg P/ha for grassland and arable land, respectively. These regulations have led to a substantial decrease in soil P accumulation, at the national level from 31 Mkg P in 2005 to 11.7 Mkg in

2011.[52] According to Smit and colleagues[52], this was met with a decrease in mineral fertilizer use due to the resulting improved utilization of manure.

Meat bone meal is another example of a secondary P resource that is applied to agricultural soil with low P utilization. In Europe, the use of meat bone meal as a fertilizer was banned in 2002 due to the risk of bovine spongiform encephalopathy (BSE) or “mad cow disease”. However, the European Union (EU) lifted these restrictions in 2006 and meat bone meal can now be used as a fertilizer in all EU countries, unless it is suspected to be infected or based on specified risk material.[33] Nonetheless, according to van Dijk and colleagues, the meat bone meal P potential is hardly utilized, as most meat bone meal is incinerated - amounting to 20% of the total P losses from all sectors in the EU-27 combined [244 kt P/yr]. In Norway, however, the meat bone meal that is applied to agriculture soils is done so inefficiently. The N:P ratio in meat bone meal (1.5-2[53]) is low compared to the ratio required by agricultural crops (N:P 7.5-8[54]), resulting in the considerable over-application of P since meat bone meal is usually applied as N fertilizer. Thus, meat bone meal would only be efficiently utilized as an alternative P fertilizer if it was applied based on the crops’ P needs and if N was applied in addition for example as mineral fertilizer. However, the application of meat bone meal based on plant-available P would still result in the additional application of the accompanied residual P, unless appropriate technologies are implemented as e.g. chemical extraction of P from bone ash for the production of soluble P fertilizers[55].

4.2.2 Mandatory collection and recycling of P fish sludge

Our results highlight that, in Norway, fish sludge represents a large fraction of the P recycling potential (figure 3) and, therefore, improvements in this sector are crucial for sustainable P management. Additionally, the importance of P management within aquaculture is everincreasing due to the sector's rapid growth and anticipated quintupling in salmon production by 2050.[56] This expected expansion will heavily depend on imported plant-based fish feed ingredients that have high P fertilizer requirements abroad and, thus, potential resource limitations. Therefore, such growth will likely shift P cycles also on a global scale.[12]

Overall, the P from aquaculture waste is lost directly to water bodies. Therefore, we argue that an essential first step for utilizing this P is through the implementation of political regulations that mandate the collection and recycling of P in fish sludge from Norwegian offshore and onshore aquaculture pens. The political regulation of aquaculture will stimulate the industry to develop, mature and implement technologies for recovering P successfully and to develop transportable fish sludge-based fertilizer products that can be used in Norway and abroad. Today, there are only few technologies in place to recover P from aquaculture. However, ongoing pilot projects for integrated multi-trophic aquaculture, that harness excess nutrients through the growth of macroalgae in the proximity of fish farms[57], might provide a large-scale solution for recovering P. Additional potential future technologies include (semi-) closed land-based systems, where the fish sludge can be collected in combination with anaerobic digestion to produce biogas.[58,59] It is important to note that harnessed aquaculture P can also be used for a number of purposes including fish feed ingredients, biofuels feedstock and pharmaceuticals.

4.2.3 Adapting regulation criteria determining the use of secondary resources as fertilizer

According to the current Norwegian regulations, the use of secondary P resources as fertilizer is determined by specific baseline criteria regarding their heavy metal content, sanitation, stabilization and contamination risk.[50] Currently, regulations do not consider nutrient concentrations in secondary resources as compared to the concentration of heavy metals and dry matter. As a result, current regulations restrict the use of nutrient-rich products with low contents of organic carbon. Food waste-based anaerobic digestate, for example, represents a secondary P product that has a relatively high P plant-availability and, in addition, a favorable ratio of nutrients (P:nitrogen (N): potassium (K)) for plant uptake.[60] While we found that there are comparatively insignificant levels of P in food waste-based anaerobic digestate at a national level, it could represent an immediate replacement for mineral fertilizer, if accordingly promoted by regulations on the use secondary resources as fertilizer.

Additionally, current regulations that restrict the use of secondary resources based only on heavy metal concentrations can result in the considerable over-application of nutrients. For example, about 55% of Norwegian sewage sludge is currently returned to agricultural land, mainly due to its positive effects as soil conditioner and as liming material.[61] With this current practice, P tends to be heavily over-applied as compared to the plants needs[14], while the P contents of sewage sludge, both residual and plant-available, are usually not accounted for in fertilization plans. However, even if policies accounted for the plant-available P fraction, the application of sewage sludge as a fertilizer would still result in a large accumulation of residual P with associated environmental risks, unless new technologies for sewage sludge treatment are applied.

Supporting Information.

Description of the phosphorus fertilization demand methodology as well as its associated limitations. This material is available free of charge via the Internet at http://pubs.acs.org.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Abbreviations

MFE, Mineral Fertilizer Equivalents; P, phosphorus; SFA, Substance Flow Analysis; RAE, Relative Agronomic Efficiency

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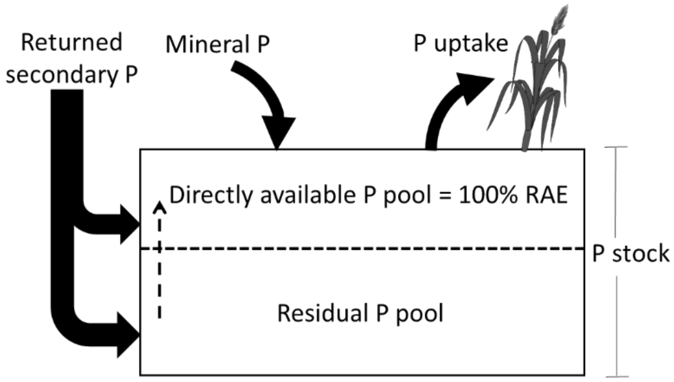
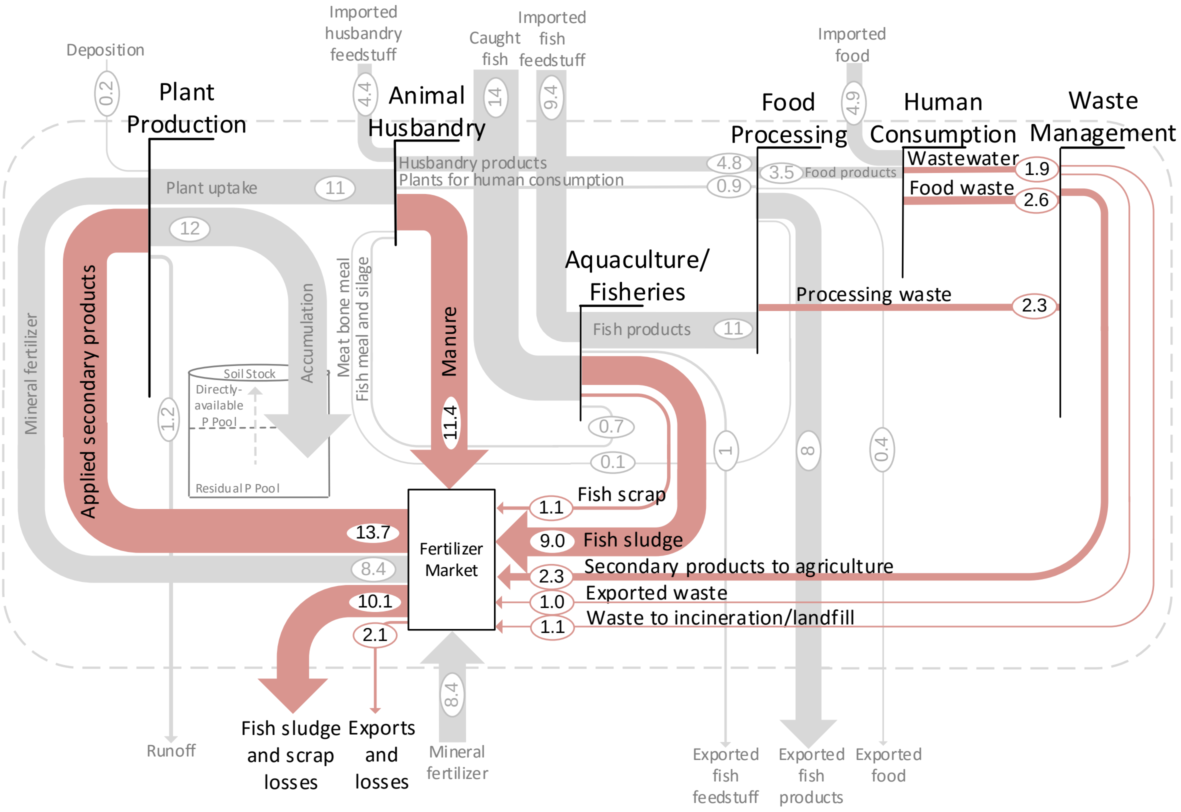


Figure 1. Conceptual drawing of relative agronomic efficiency (RAE)



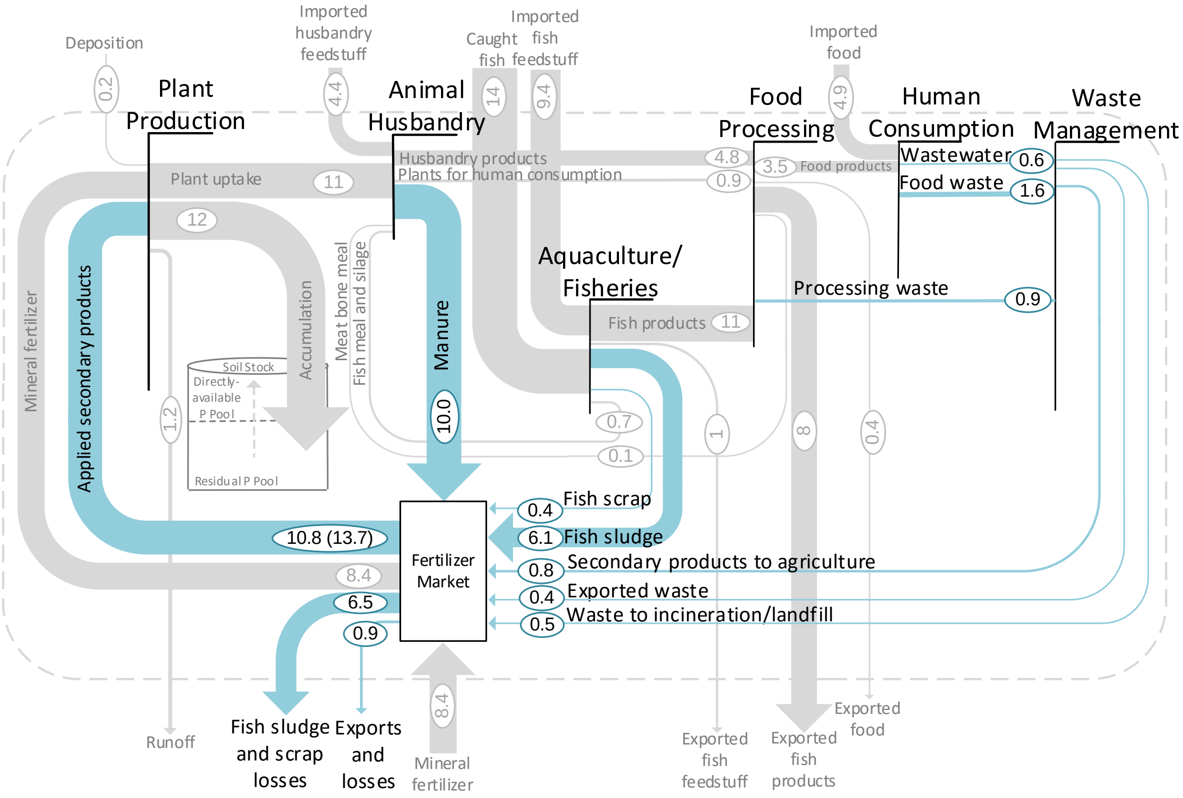


Figure 2. Norwegian phosphorus balance (top), kt P/yr, averaged 2009-2011, red flows highlighting the secondary P resources considered; Norwegian phosphorus balance corrected for RAE (bottom), average RAE presented, kt plant-available P/yr, averaged 2009-2011, blue flows highlighting the products that RAE were applied to.

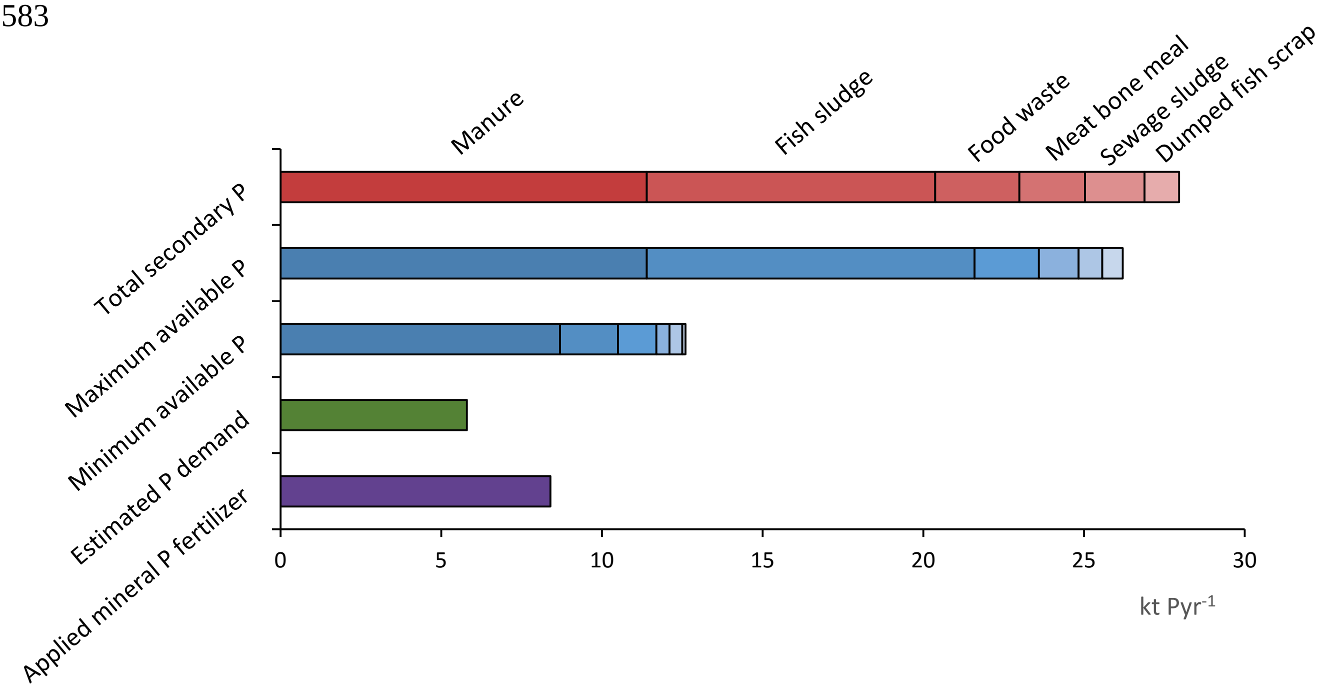


Figure 3. Total secondary P, maximum and minimum P recycling potential of secondary products, estimated P fertilization demand and mineral P fertilizer applied in 2009-2011.

Table 1. Secondary products, their respective RAE ranges and sources; RAE=relative agronomic efficiency; n= number of observations

Secondary P resource	Category/treatment	RAE (%)	n	Reference
Manure	Cattle manure	[82, 112]	15	[23–30]
	Sheep and goat manure	[75, 125]	1	[31]
	Pig manure	[77,123]	6	[27,28,32]
	Poultry manure	[63, 73]	4	[27,29,30]
	Fox and mink manure	[29, 94]	2	[29,33]
	Horse manure [a]	[55, 105]		Own assumption
Fish sludge	Reactor-composted	[21, 115]	3	[30,34]
Food waste	Compost	[39, 65]	10	[30,35]
	Digestate	[55,86]	4	[30]
Meat bone meal	Treated with heat and pressure	[19, 60]	5	[29,30,33,36]
Sewage sludge	Chemical or chemical-biological treatment	[20,37]	9	[19]
	Biological treatment	[75,125]	1	[14]
	Mechanical treatment	[75,125]		Own assumption
Dumped fish scrap [b]		[19,60]		Own assumption

[a] Assumed to be equal to cattle manure, [b] assumed to be equal to meat bone meal

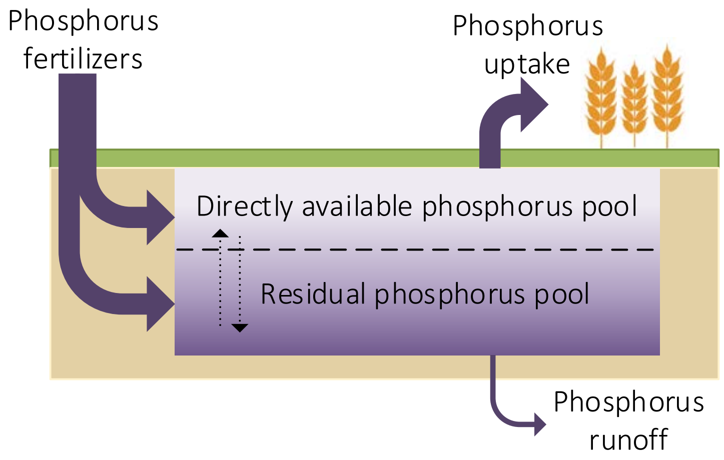


Figure. Graphical abstract

Supplementary material

Estimating the recycling potential of secondary phosphorus resources by integrating substance flow analysis and plant-availability

Helen Ann Hamilton; Eva Brod; Ola Hanserud; Daniel B. Müller; Helge Brattebø; Trond K. Haraldsen

Page numbers: 2

1 P fertilization demand

1.1 Method

The accumulated plant-available P stock resulting from yearly fertilizer over-application is used as a P source by plants in addition to P applied with fertilizer the same year. Here, we define the P demand to be equal to the plant uptake minus a correction for the existing soil P levels. Therefore, the P fertilizer applied should be reduced to below the amount of P removed by plants, according to fertilization recommendations.[1] This is with goal of, over time, reaching a soil P level that is considered optimal in Norwegian fertilizer planning to both optimize yield and reduce the risk of surface runoff. While Norwegian plant-available soil P values, estimated as ammonium lactate-extractable P according to Egner et al.[2], vary geographically both above (this is primarily the case) and below the optimal soil P concentration, we have applied a weighted national average correction factor of a reduction of 48.1% to the P fertilization demand, defined to be equal to P uptake.[3] It is important to note, however, that if this recommendation is followed, the aforementioned correction factor would decrease over time and is, therefore, not constant.

1.2 Limitations of the method

Limitations associated with the P fertilization demand estimation relate to time aspects and the soil P status. This method accounts for long-term soil interactions, as the measured concentration of plant-available soil P, P-AL, is positively related to the total soil P stock[4] and is slow responding to annual changes in P balances within the soil.[5] Overall, the representativeness was considered satisfactory, based on the number of samples to area ratio. Additional uncertainty relates to the use of mean values for the different P-AL classes, which assumes that measurements are well distributed within each class. More information regarding this method can be found in Hanserud and colleagues.[3]

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PAPER III

Brod E, Øgaard AF, Hansen E, Wragg D, Haraldsen TK, Krogstad T (2015) Waste products as alternative phosphorus fertilisers part I: Inorganic P species affect fertilisation effects depending on soil pH. Nutrient Cycling in Agroecosystems 103: 167–185

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ORIGINAL ARTICLE

Waste products as alternative phosphorus fertilisers part I: inorganic P species affect fertilisation effects depending on soil pH

Eva Brod · Anne Falk Øgaard · Eddy Hansen · David Wragg · Trond Knapp Haraldsen · Tore Krogstad

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Abstract One of the bottlenecks to efficient phosphorus (P) recycling is limited understanding of the relationships between inorganic P species in waste products and their P fertilisation effects. In this study, we characterised inorganic P species in seven waste products (two biomass ashes, meat bone meal, fish sludge, catering waste and two food waste-based digestate products) and two manure products (dairy and chicken manure) by: (1) Sequential chemical fractionation, (2) X-ray powder diffraction and (3) solid-state ^{31}P MAS-NMR spectroscopy. We then used the characterisation data to explain the results of a bioassay studying the fertilisation effects of waste and manure products after application to a nutrient-deficient model soil that was limed to two pH levels (approximately pH 5.5 and 6.9 at pH level 1 and 2), with ryegrass (*Lolium multiflorum*) as the experimental crop. The P in waste products was mainly present as a complex mixture of inorganic P species, predominantly Ca phosphates with differing solubility. Fertilisation effects were largely explained by sequential fractionation data, with a positive relationship between apparent P use efficiency and the H_2O-soluble inorganic P fraction at pH level 1 ($R^2 = 0.52$) and a negative relationship between apparent P use efficiency and the HCl-soluble inorganic P fraction at pH level 2 ($R^2 = 0.66$). X-ray powder diffraction and solid-state ^{31}P MAS-NMR spectroscopy confirmed the sequential fractionation data, but provided little additional information.

Keywords Speciation · Sequential fractionation · XRD · 31-P solid state NMR · Meat bone meal · Wood ash · Digestate

Introduction

It is widely acknowledged that rock phosphate is a limited resource, but in practice mineral phosphorus (P) fertiliser is still seldom replaced by P-rich waste products. One of the reasons is that the fertilisation effects of mineral P fertilisers are well studied and reliable, whereas those of P-rich waste products are strongly variable (e.g. Kratz et al. 2010; Cabeza et al. 2011). Therefore, it is crucial to understand how the

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chemical properties of waste products influence their P fertilisation effects, in order to optimise P recycling and avoid accumulation of unavailable P in soils. Also in waste products P is mainly present as inorganic P (Hedley and McLaughlin 2005), and these inorganic P species include a large variety of compounds with accompanying elements such as Ca, Fe and Al that are characterised by variable solubility in soil. The solubility of Ca phosphates decreases with increasing soil pH, whereas the solubility of Fe-/Al-bound P decreases with decreasing soil pH (Lindsay 1979). Characterisation of inorganic P species can therefore be used to explain the P fertilisation effects of waste products depending on soil pH. Different methods can be used for the identification of inorganic P species, but all of these methods have advantages and disadvantages.

Different sequential chemical fractionation methods have been applied to a variety of waste products to study inorganic P pools of different solubility (e.g. Sharpley and Moyer 2000; Ylivainio et al. 2008; García-Albacete et al. 2012; Nanzer et al. 2014). In brief, labile P, Fe-/Al-bound P and stable Ca phosphates are distinguished when P is sequentially extracted from samples using solvents with different pH and increasing extraction capacity. However, chemical fractionation schemes fail to identify specific P species (Pierzynski et al. 2005).

X-ray powder diffraction (XRD) can be applied to waste products for the detection of crystalline phases containing inorganic P. It has been used for the identification of different Fe-/Al-bound P, Ca phosphates such as whitlockite and apatite in sludge and sludge ashes (Frossard et al. 1997; Kim et al. 2013; Nanzer et al. 2014) and struvite in dairy manure (Güngör et al. 2007; Hunger et al. 2008). When X-rays interact with crystalline matter, diffraction patterns are produced, which are unique to the crystal lattice. In mixtures, it is possible to separate out the contributions from the crystalline phases present and determine the quantity of each. The most common method currently used for the quantification is the Rietveld method (Rietveld 1969). Amorphous material is usually considered part of the background in this method. However, using the spiking method described below, it is also possible to quantify the amorphous content (Scarlett and Madsen 2008).

Solid-state ^{31}P MAS-NMR has also been successfully applied to waste and manure products or their extraction residues and soils for the identification of different Ca phosphates and Al-bound P (Hinedi et al. 1989; Frossard et al. 1994, 2002; Hunger et al. 2004, 2008; Nanzer et al. 2014). The principle of the method is simply that a nucleus confined in an external magnetic field can adsorb energy within a radiofrequency range which depends on the structural environment of the nucleus in question (Pierzynski et al. 2005). Single-pulse ^{31}P polarisation targets all P atoms, whereas ^{1}H–^{31}P cross-polarisation enhances the ^{31}P signal intensity of those P nuclei that have direct or close contact with nearby protons. However, paramagnetic metal cations in samples can result in severe line broadening and loss of sensitivity. Therefore, phosphate adsorbed to Fe- and Mn-phases may become invisible in solid-state ^{31}P MAS-NMR (Toor et al. 2006).

Numerous publications describe characterisation of inorganic P species in different waste products by means of various methods (see above). Many studies have also been published on the P fertilisation effects of different waste products (e.g. Cabeza et al. 2011). However, only a few studies address the relationship between characterisation of inorganic P species in waste products and their P fertilisation effects (Ylivainio et al. 2008; Nanzer et al. 2014).

In the present study, we characterised inorganic P species in a wide range of Norwegian P-rich waste and manure products using a combination of different characterisation approaches: (1) Sequential chemical fractionation, (2) X-ray powder diffraction and (3) solid-state ^{31}P MAS-NMR spectroscopy. We then used the data on inorganic P species to explain the P fertilisation effects of the different waste and manure products at two distinct soil pH levels. We determined these fertilisation effects using a bioassay with ryegrass (*Lolium multiflorum*) and a nutrient-deficient sand-peat mixture as model soil, which was limed to two pH levels.

Materials and methods

Waste and manure products

Waste and manure products are described in Table 1, while Table 2 provides an overview of selected chemical properties, determined as described below.

Table 1 Description of waste and manure products

No.	Waste/manure product	Description
1	Wood ash	Bottom ash from a grate-fired boiler system at the Moelven Østerdalsbruket AS mill, Norway. Parent material is timber unsuitable for industrial use
2	Cereal ash	Bottom ash from a grate-fired boiler system in the Eidsiva Bioenergi district heating system in Kongsvinger, Norway. Parent material is timber unsuitable for industrial use and cereal residues from the local mill
3	Meat bone meal	Stabilised and sanitised slaughterhouse waste from the slaughterhouse in Hamar, Norway
4	Fish sludge	Faeces and feed residues from the Åsen settefisk salmon hatchery, Norway, treated in a reactor developed by the company Global Enviro after separation of water by mechanical filtering
5	Catering waste	Source-separated catering waste from Rica Sunnfjord Hotel, Norway, treated in a reactor developed by the company Global Enviro after separation of grease and water by steam and pressure
6	Liquid digestate	Untreated digestate based on anaerobic treatment of source-separated household waste collected at the Mjøsanlegget biogas plant, Norway
7	Solid digestate	Solid phase after centrifugation of liquid digestate based on anaerobic treatment of source-separated household waste collected at the Mjøsanlegget biogas plant, Norway
8	Dairy manure	Slurry (faeces and urine) of dairy cows collected at the Norwegian University of Life Sciences, Norway
9	Chicken manure	Stabilised, sanitised and pelletised chicken manure produced by Norsk Naturgjødsel, Norway

Table 2 Selected general chemical properties of waste and manure products

	Wood ash	Cereal ash	Meat bone meal	Fish sludge	Catering waste	Liquid digestate	Solid digestate	Dairy manure	Chicken manure
pH	13	9.6	6.2	5.4	5.8	7.0	7.7	7.0	6.7
SSA (m^2 g^{-1})	0.6	4.5	0.1	0.1	0.1	0.1	2.8	0.8	0.4
DM (g 100 g^{-1})	99.6	97.6	96.2	95.0	94.5	2.4	25.0	5.8	93.3
OM (g 100 g^{-1} DM)	0.0	17.0	66.6	87.6	81.1	64.6	66.2	81.6	81.7
P (g kg^{-1} DM)	17	51	54	21	10	12	15	6	10
Pi (% of total P)	103	100	98	86	93	74	78	76	47
C (g kg^{-1} DM)	19	111	368	503	449	412	400	470	427
N ([a]g kg^{-1} DM, [b]g L^{-1})	0[a]	4[a]	86[a]	71[a]	50[a]	2.3[b]	56[a]	3.1[b]	45[a]
Nmin ([a]g kg^{-1} DM, [b]g L^{-1})	n.d.	n.d.	5.0[a]	2.6[a]	5.1[a]	1.7[b]	7.2[a]	1.3[b]	0.041[a]
K (g kg^{-1} DM)	56	93	4	3	6	45	6	42	25
S (g kg^{-1} DM)	34	2	34	48	35	59	65	42	58
Ca (g kg^{-1} DM)	310	27	110	37	59	33	62	11	44
Mg (g kg^{-1} DM)	25	26	3	3	2	8	5	6	6
Al (g kg^{-1} DM)	19.1	3.8	0.2	0.3	0.1	8.6	13.8	0.4	0.5
Fe (g kg^{-1} DM)	7.6	4.3	0.5	0.7	0.4	2.8	5.9	1.3	1.0

SSA specific surface area, *DM* dry matter, *OM* organic matter, *Pi* inorganic P, *Nmin* mineral N (NO_3^- and NH_4^+)

Dry matter (DM) content was determined after drying at 105 °C and organic matter (OM) content was determined after incineration at 550 °C. Before further analyses, waste and manure products were dried at 55 °C and sieved at mesh width $\leq$2 mm or milled. The pH was determined on sieved samples in deionised H_2O in a solid:solution ratio 1:2.5 (v/v), while the pH in dairy manure and liquid digestate was determined in fresh samples. Specific surface area was determined on sieved samples by Brunauer–Emmett–Teller gas adsorption analysis, using N_2 as the adsorbent (Brunauer et al. 1938). Total C was analysed on milled samples using a Leco TruSpec CHN analyser. Total N content was determined by the modified

Kjeldahl method (EN 13654-1 2001) and NO_2-/NO_3-N and NH_4-N were determined after extraction with 2 M KCl (Henriksen and Selmer-Olsen 1970; Selmer-Olsen 1971), using a Konelab Aqua 60 analyser. To determine total P, duplicate milled samples were incinerated at 550 °C and digested in 6 M H_2SO_4 (Møberg and Petersen 1982) and then colorimetric analysis of ortho-P was performed by the molybdenum blue method according to Murphy and Riley (1962). Inorganic P was determined in an unincinerated sample as described for total P, and organic P was calculated as the difference between total and inorganic P. Total K, Mg, Ca, S and heavy metal contents were determined by ICP–OES after digestion with concentrated nitric acid in an ultraclave (EN ISO 11885 2009). Based on their heavy metal content, under Norwegian regulations (Norwegian Ministry of Agriculture and Food 2003) all waste products included in the study could be applied as fertiliser to agricultural land.

Sequential chemical extraction

Inorganic and organic P fractions of different solubility in waste and manure products were analysed in duplicate using the sequential fractionation scheme according to Hedley et al. (1982) modified by Sharpley and Moyer (2000). 1 g of dried, milled sample was extracted in 200 mL deionised H_2O for 1 h and then extracted respectively in 200 mL 0.5 M $NaHCO_3$ (labile P), 0.1 M NaOH (P adsorbed to Fe-/Al-hydroxides/oxides or Fe-/Al-phosphates, hereafter referred to as Fe-/Al-bound P) and 1 M HCl (stable Ca phosphates), each for 16 h. After centrifugation at 1160 relative centrifugal force (rcf) for 20 min, ortho-P was analysed in all extracts as described above. Total P concentration was determined by analysing the extracts on ICP–OES. Organic P in the extracts was calculated as the difference between total and inorganic P. Because of uncertainties, in some cases organic P was estimated to be <0. Although organic P content could be assumed to be zero in these cases, for reasons of transparency the results are expressed without data modification. Residual P in the samples was analysed by ICP–OES after digestion of the dried residual sample in concentrated HNO_3 in an ultraclave. Phosphorus recovery was calculated as the sum of all fractions.

X-ray powder diffraction combined with Rietveld refinements

XRD patterns of waste and manure products were determined with Cu Kα_1 radiation over a 2Θ range of 10°–70° and a step size of 0.02°, using a Bruker D8 Advance diffractometer with Ge (111) monochromator and LynxEye detector. The count time was 0.5 s per step. Phase identification was conducted with the Bruker EVA software using the PDF2 database (ICDD 2013). Rietveld quantitative phase analysis (QPA) was performed with the programme Topas 4.2 (Coelho 2006) based on structural models from the PDF2 database. A detailed description of QPA and Rietveld plots for each of the waste and manure products can be found in the supplementary material. Analyses were conducted on triplicate dried, milled samples spiked with <5 % silicon (Si).

Crystalline content in samples (CC, wt%) was estimated as:

$$\mathrm{CC\ (wt\%)} = 100 \times \frac{[\mathrm{CC_{Si}} - \mathrm{Si_{weighed}}]}{[\mathrm{CC_{si}} - \mathrm{Si_{weighed}}] + [\mathrm{AC_{Si}}]} \tag{1}$$

where $\mathrm{CC_{Si}}$ = crystalline content in the spiked sample (wt%) was calculated as:

$$\mathrm{CC_{Si}\ (wt\%)} = 100 \times \left(\frac{\mathrm{Si_{weighed}}}{\mathrm{Si_{measured}}}\right) \tag{2}$$

with $\mathrm{Si_{weighed}}$ (wt%) = weighed internal standard, $\mathrm{Si_{measured}}$ (%) = internal standard measured by Rietveld refinement and $\mathrm{AC_{Si}}$ = amorphous content in the spiked sample (wt%), calculated as:

$$\mathrm{AC_{Si}\ (wt\%)} = 100 - \mathrm{CC_{Si}} \tag{3}$$

Phosphorus in P bearing phases detected by XRD was expressed as a percentage of total P and calculated in two steps. First, the share of the P bearing phase in the sample (expressed as wt%) was corrected for the Si spike:

$$\mathrm{P\ phase\ (wt\%)} = \frac{\mathrm{P\ phase\ _{Si}}}{(100 - \mathrm{Si_{measured}})} \times \mathrm{CC\ (wt\%)} \tag{4}$$

where P phase (wt%) = P bearing phase in unspiked sample and P phase $_{\mathrm{Si}}$ (wt%) = detected P bearing phase measured by Rietveld refinement in spiked sample.

Then, P contained in the P bearing phase was expressed as a percentage of total P in the sample:

$$\text{P in P phase (\% of total P)} = \frac{\text{P phase} \times \text{Molar ratio}}{\text{Total P}} \quad (5)$$

where Molar ratio = fraction of P contained in P bearing phase and total P as described above (Table 2).

Solid-state ^{31}P MAS-NMR

Solid-state ^{31}P MAS-NMR spectra of dried, milled waste and manure products were recorded on a Bruker Avance III spectrometer at 11.7 T, corresponding to a resonance frequency of 200 MHz and with a Magic Angle Spinning (MAS) rate of 15 kHz. ^{31}P single-pulse spectra were acquired using a ^{31}P $\pi/2$-radiofrequency pulse of 4.5 µs duration and a recycle delay of 30 s between successive transients of scans. The total number of scans varied between 64 and 2000, depending on the sample. ^{1}H–^{31}P cross-polarisation spectra (CP-MAS) were acquired using the same parameter setting as in single-pulse experiments, except that a contact time of 2 ms for magnetization transfer from protons to P was applied. The spectra were processed using Topspin 3.2 with Lorentzian line broadening of 50 Hz before Fourier transformation to slightly enhance the signal-to-noise ratio and subsequently phase-corrected in order to perform reliable spectral fitting. Chemical shifts δ (ppm) of ^{31}P were externally referenced to 85 % H_3PO_4 with $\delta = 0$ ppm. The spectra were model-fitted using Excel and the derived chemical shifts/intensities were accurate to within ±0.1 ppm/±5 %. Spectra were deconvoluted according to the procedure outlined by Hunger et al. (2004) which implies fitting to the sum of a minimum number of Gaussian/Lorentzian line shape functions using a non-linear least squares technique to obtain a statistically reasonable representation of the observed spectrum. The peaks obtained were compared with the literature for identification of phosphate species.

Bioassay experiment

A bioassay was conducted with 5-L pots filled with a nutrient-deficient 8:2 (v/v) blend of sand and sphagnum peat (6.37 kg pot^{-1}), selected chemical properties of which are presented in Table 3. This model soil was chosen to avoid P fertilisation effects of fertiliser treatments being masked by soil P. It was limed to pH level 1 and 2 (approximately pH 5.5 and 6.9) by mixing 1.5 and 7.5 g $CaCO_3$ pot^{-1}, respectively, into the soil volume.

Waste product fertilisation rates were calculated based on total P content. Our intention was to apply 75 mg P pot^{-1}, equivalent to 30 kg P ha^{-1} (assuming 20 cm topsoil depth), but actual fertilisation rates deviated slightly from this (Table 8). The fertilisation effects of waste products were compared with those of dairy and chicken manure, for which fertilisation rates were calculated as for waste products, with a treatment receiving no P fertilisation (NoP) and a mineral control treatment (MinP) that received $Ca(H_2PO_4)_2$ at a rate of 75 mg P pot^{-1}. To study the response of ryegrass to P fertilisation on the model soil, $Ca(H_2PO_4)_2$ was also applied at a rate of 37.5 and 112.5 mg P pot^{-1}, equivalent to 15 and 45 kg P ha^{-1}, respectively. All other nutrients were applied in sufficient amounts. Mineral N in waste products was assumed to become plant-available during the growing season. Organic N was assumed to be immediately available at a rate of 30 % for dairy manure, chicken manure, solid and liquid digestate, 80 % for meat bone meal and fish sludge and 50 % for catering waste (Jeng et al. 2004; Bioforsk 2014; Brod et al. 2014). The remaining N to give a total of 500 mg N pot^{-1} was applied as $Ca(NO_3)_2$. In addition, 500 mg K pot^{-1} was applied as K_2SO_4 and 62.5 mg Mg pot^{-1} was applied as $MgSO_4$ together with Fe, Mn, Cu, Mo, B and Zn. After each harvest, 250 mg N and 250 mg K pot^{-1} were applied as $Ca(NO_3)_2$ and K_2SO_4, respectively. Due to a calculation error, the solid digestate treatments initially received too little mineral N fertiliser and an extra 350 mg N were applied 12 days before harvest 1. There were three replicates per treatment at each pH level studied.

Waste and manure products, lime and additional mineral fertiliser were applied 5 days before sowing in May 2013. Waste products were applied after drying (55 °C) and sieving (≤2 mm). Only liquid and solid digestate and dairy manure were applied without previous drying. Ryegrass (*L. multiflorum* var. Macho) was sown at a rate of 0.5 g seeds pot^{-1}. The pots were placed side by side on a table outdoors under a transparent glass roof, where the plants were protected

Table 3 Chemical properties of the model soil used in the bioassay

pH	P-AL mg P 100 g^{-1}	Olsen P mg P kg^{-1}	K-AL mg P 100 g^{-1}	Mg–AL mg P 100 g^{-1}	Ca-AL mg P 100 g^{-1}
5.2–5.3	1.1–1.2	4.5–4.8	1.0–1.1	1.8–1.9	11

AL = extraction with 0.1 M ammonium lactate and 0.4 M acetic acid adjusted to pH 3.75 (Egnér et al. 1960) and analysis on ICP–OES, Olsen P = extraction with 0.5 M $NaHCO_3$ adjusted to pH 8.5 (Olsen et al. 1954) and colorimetric analysis (Murphy and Riley 1962)

from precipitation but otherwise exposed to daylight and outdoor climate. Due to wind protection provided by the north and east side of the roof, the mean temperature was somewhat higher than the mean outdoor temperature for the area, which was lowest in September (11.1 °C) and highest in July (17.5 °C) (Meteorologisk Institutt 2013). The pots were watered based on weight to 60 % of water holding capacity every day, with differences in biomass production between treatments assumed to be negligible in relation to total pot weight. Pot positions were randomised twice a week. 6 and 11 days after harvest 3, all plants were successfully treated with Thiacloprid (Bayer) against aphids (*Acyrthosiphon pisum*). Aboveground biomass was harvested at a height of 5 cm above the soil surface four times during the season, 6 weeks after sowing and then every 4 weeks. Harvested biomass was dried at 55 °C before weighing (g DM pot^{-1}) and milling. Total N concentration in the material was determined by the Dumas method (EN 13654-2 2001). Concentrations of all other nutrients in plant biomass were determined by the same method as described for the waste products. Plant P uptake (mg pot^{-1}) was computed by multiplying concentration by aboveground yield for each replicate.

The apparent phosphorus use efficiency (PUE, %) was calculated based on the difference method for each harvest and their sum as:

$$\text{PUE}\ (\%) = \frac{\text{P uptake}(\text{P}+) - \text{P uptake}(\text{P}-)}{\text{P applied}} \times 100 \tag{6}$$

where P uptake (P+) (mg P pot^{-1}) = amount of P taken up in aboveground biomass by fertilised plants, P uptake (P−) (mg P pot^{-1}) = amount of P taken up in aboveground biomass by the average of NoP plants and P applied (mg P pot^{-1}) = amount of P applied with the fertiliser (mg P pot^{-1}).

Data analysis

One-way analysis of variance (ANOVA) was used to study the effect of the waste and manure products on extracted inorganic P, organic P, residual P and P recovery during the sequential fractionation and the effect of fertiliser treatments on soil pH, biomass production and PUE. Biomass production and PUE were also analysed by two-way ANOVA to study the effect of the factors fertiliser, soil pH level and their interaction. To perform multiple comparisons, Tukey's honestly significant difference (HSD) test ($\alpha = 0.05$) was used. Simple linear regression analysis was used to study the relationship between PUE and extracted P.

Results

Total and inorganic P in waste and manure products

The waste products contained 10–54 g P kg^{-1} DM, with the lowest content in catering waste and highest content in meat bone meal (Table 2). Manure products contained less total P, with 6 and 10 g P kg^{-1} DM in dairy and chicken manure, respectively. The P in waste products was mainly present as inorganic P, with organic P content varying between −3 and 26 % of total P. Dairy manure and chicken manure contained 24 and 53 % organic P, respectively.

Sequential chemical extraction

Extraction by H_2O removed more inorganic P from dairy manure than from all waste products (Table 4). Among the waste products, the largest H_2O-soluble inorganic P fraction was found in fish sludge, whereas

Table 4 Inorganic and organic P (Pi and Po) and residual P extracted by sequential fractionation of waste and manure products expressed as % of total P

Waste/manure product	H_2O		$NaHCO_3$		NaOH		HCl		Residual	Recovery
	Pi	Po	Pi	Po	Pi	Po	Pi	Po	Total P	Total P
Wood ash	0.0i	0.1d	42.9a	1.2bc	0.2f	0.1e	62.5c	2.5cd	16.1	125.7a
Cereal ash	12.2d	3.7b	38.2b	14.8a	3.2e	3.7d	22.7e	1.2d	5.5	105.1bc
Meat bone meal	3.7g	0.7cd	5.4e	0.0bc	3.2e	−0.2ef	88.1a	5.0bcd	11.4	117.2ab
Fish sludge	18.9b	4.7a	19.1d	−0.1bc	12.1c	0.9e	37.4d	7.0b	7.4	107.4abc
Catering waste	4.7f	1.5c	6.4e	−0.7c	6.8d	−1.5f	76.6b	5.5bcd	9.2	108.4abc
Liquid digestate	10.3e	4.9a	21.3d	1.8bc	31.4a	12.0b	18.5ef	5.5bc	13.8	119.2ab
Solid digestate	2.5h	1.0c	19.5d	−1.1c	23.0b	6.7c	33.7d	7.0b	5.5	97.6c
Dairy manure	42.1a	5.4a	32.5c	−4.0c	3.6e	3.9d	1.7g	1.8cd	4.4	91.4c
Chicken manure	16.1c	4.7a	7.6e	5.1b	4.4e	24.9a	14.7f	16.1a	4.4	97.9c
SEM	0.2	0.1	0.4	1.0	0.3	0.3	1.0	0.8	2.2	3.3
HSD	0.9	0.8	2.5	5.7	1.5	1.5	5.5	4.3	12.4	18.5

Recovery is the sum of all fractions and expressed as % of total P. SEM is the pooled standard error of the mean, HSD is the honestly significant difference with $p < 0.05$ between waste and manure products according Tukey's test after one-way ANOVA and different letters indicate significant differences between waste and manure products

no P was extracted by H_2O in wood ash. The largest $NaHCO_3$-soluble inorganic P fraction was found in wood ash, while the smallest fraction was found in chicken manure, catering waste and meat bone meal. Comparing labile inorganic P (the sum of H_2O- and $NaHCO_3$-soluble inorganic P fractions), more was extracted from dairy manure (74.6 %) than from any of the waste products. Among the waste products, the largest fraction of labile inorganic P was found in cereal ash (50.4 %). The smallest fraction of labile inorganic P was found in catering waste and meat bone meal (9.1–11.1 %). The largest fraction of NaOH-soluble inorganic P was found in liquid digestate, followed by solid digestate and fish sludge. In all other materials, the amount of inorganic P that was soluble in NaOH was <7 %. The largest HCl-soluble inorganic P fraction was found in meat bone meal, followed by catering waste and wood ash.

Extraction of organic P was generally small due to the low content of organic P in the materials. The largest fractions of organic P were extracted by NaOH, e.g. 24.9 % of total P in chicken manure, whereas the NaOH-soluble organic P fraction was <11.9 % of total P for the waste products. Organic P was also extracted by HCl, with the largest fraction in chicken manure. Even though cereal ash only contained inorganic P (see Table 2), 23 % of the P in cereal ash was defined as organic P according to the sequential fractionation procedure because it was not detected by the colorimetric molybdenum blue method.

Residual P was between 4.4 and 16.1 %, with the largest amount in wood ash. The total recovery by sequential fractionation, including residual P, was 91.4–125.7 % of total P.

X-ray powder diffraction combined with Rietveld refinements

The crystalline content in the waste products was estimated to be highest in wood ash and cereal ash, followed by meat bone meal (Table 5). For all other waste and manure products, the crystalline content was estimated to be <6 %. The true accuracy of Rietveld QPA is estimated to be ±3–6 % (Scarlett et al. 2002).

The sum of P in phases detected by XRD and assessed by Rietveld refinement was between 7 and 71 % of total P, with the largest fraction in wood ash. No crystalline P bearing phases were detected in liquid digestate and the manure products. When P bearing phases were detected, the sum of P present (% of total P) was always higher than the estimated crystalline content in the sample (wt%), meaning that more P was present in crystalline than in amorphous structures.

The P bearing phases present in each sample are summarised in Table 5. In wood ash, calcium

Table 5 Crystalline content (wt%) and crystalline P phases (% of total P) in waste and manure products assessed by Rietveld refinement of XRD data obtained on Si-spiked samples

Waste/manure product	Crystalline content	Gof[a]	Rwp[b]	P phases detected by XRD			
	wt%			Σ P phases % of total P	P phase Name	Formula	% of total P
Wood ash	48.1 ± 3.5	2.4	9.6	71.4 ± 10.7	Calcium phosphate silicate	$(Ca_2(SiO_4))_6(Ca_3(PO_4)_2)$	41.6 ± 0.9
					Sodium calcium magnesium phosphate	$Na_{18}Ca_{13}Mg_5(PO_4)_{18}$	28.1 ± 9.6
					Hydroxyapatite	$Ca_5(PO_4)_3(OH)$	1.8 ± 0.5
Cereal ash	26.8 ± 2.4	1.7	6.3	33.2 ± 2.0	Calcium dipotassium diphosphate	$CaK_2(P_2O_7)$	15.3 ± 1.4
					Struvite-K	$KMg(PO_4)$	8.9 ± 1.7
					β-Lomonosovite	$Na_4(H,Na)_6Ti_4(Si_2O_7)_2(PO_4)_2O_4$	5.2 ± 1.8
					Whitlockite	$Ca_{18}Mg_2H_2\ (PO_4)_{14}$	3.8 ± 0.3
Meat bone meal	15.9 ± 1.7	1.7	6.8	52.6 ± 8.0	Chlor-fluorapatite	$Ca_5(PO_4)_3F_{0.29}Cl_{0.47}(OH)_{0.24}$	29.2 ± 4.5
					Hydroxyapatite	$Ca_5(PO_4)_3(OH)$	23.4 ± 4.3
Fish sludge	4.0 ± 0.3	1.7	5.2	27.0 ± 2.5	Chlor-fluorapatite	$Ca_5(PO_4)_3F_{0.29}Cl_{0.47}(OH)_{0.24}$	27.0 ± 2.5
Catering waste	3.3 ± 0.2	1.5	5.1	24.5 ± 4.2	Chlor-fluorapatite	$Ca_5(PO_4)_3F_{0.29}Cl_{0.47}(OH)_{0.24}$	24.5 ± 4.2
Liquid digestate	6.9 ± 0.3	1.5	5.3	No P phases detected			
Solid digestate	6.2 ± 0.3	1.5	5.1	7.3 ± 1.3	Hydroxyapatite	$Ca_5(PO_4)_3(OH)$	7.3 ± 1.3
Dairy manure	0.8 ± 0.2	1.4	4.4	No P phases detected			
Chicken manure	2.6 ± 0.2	1.7	5.6	No P phases detected			

The reported errors are standard deviations calculated for three replicates per sample

[a] Gof = goodness of fit $(Rwp\ Rexp^{-1})^{-2}$, [b] RwP = residual factor for the weighted Rietveld profile and Rexp = expected residual

phosphate silicate was assessed to be the most abundant crystalline P phase, in addition to sodium calcium magnesium phosphate and hydroxyapatite. Cereal ash mainly contained calcium dipotassium diphosphate, as well as struvite-K, β-lomonosovite and whitlockite. Otherwise, only apatite (as hydroxy- or chlor-fluorapatite) was detected, with the largest fraction in meat bone meal, followed by fish sludge, catering waste and solid digestate.

The calcium bearing phase calcite ($CaCO_3$) was also identified in some of the samples and quantified as described. The highest abundance of $CaCO_3$ was found in wood ash (8.1 ± 0.2 wt%), followed by solid digestate (2.6 ± 0.1 wt%), chicken waste (2.3 ± 0.0 wt%), catering waste (2.0 ± 0.2 wt%), cereal ash (1.8 ± 0.2 wt%), liquid digestate (1.4 ± 0.1 wt%) and fish sludge (0.2 ± 0.1 wt%). It should be noted that most of these phase fractions are very small and may be of limited significance.

Solid-state ^{31}P MAS-NMR

All waste and manure products revealed isotropic chemical shifts in the range $\delta = 2.1$–3.8 ppm (Fig. 1; Table 6). Chemical shifts in this range have been assigned to stable Ca phosphates with a molar Ca:P ratio >1, hereafter referred to as stable Ca phosphates (Rothwell et al. 1980; Aue et al. 1984; Hinedi et al. 1989; Frossard et al. 2002; Nanzer et al. 2014): Hydroxyapatite ($\delta = 2.2$–2.8 ppm), chlor-fluorapatite ($\delta = 2.0$–2.8 ppm), carbonate apatite ($\delta = 2.8$–3.0 ppm), octocalcium phosphate ($\delta = 2.8$–3.4 ppm) or amorphous Ca phosphate ($\delta = 3.0$ ppm). Therefore, the results suggest that stable Ca phosphates were present in varying abundance in all waste and manure products. More specific assignment of Ca phosphates was difficult, however, as their chemical shifts overlap.

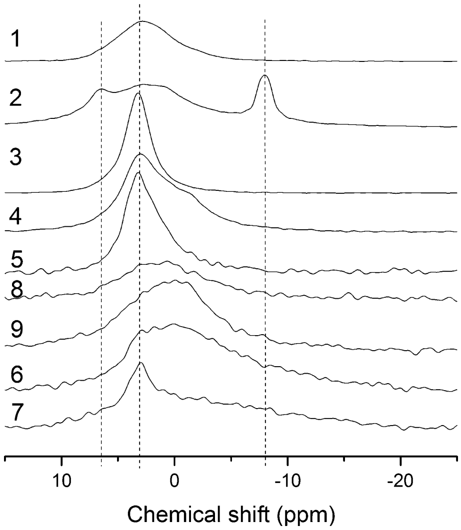

Fig. 1 Single-pulse ^{31}P MAS-NMR spectra of waste and manure products, where *1* wood ash, *2* cereal ash, *3* meat bone meal, *4* fish sludge, *5* catering waste, *8* dairy manure, *9* chicken manure, *6* liquid digestate and *7* solid digestate

The spectrum of cereal ash was clearly different from that of all other products. In addition to the peak indicating stable Ca phosphates ($\delta = 2.2$ ppm), cereal ash also resulted in a chemical shift at $\delta = 6.7$ ppm, which was assigned to struvite (Hunger et al. 2008). This peak was selectively enhanced by CP-MAS NMR (results not shown), as also observed by Hunger et al. (2008). Moreover, cereal ash revealed a chemical shift at $\delta = -8$ ppm, which was assigned to pyrophosphate (Hinedi et al. 1989). The peak disappeared during CP-MAS NMR (results not shown), confirming the presence of condensed phosphates as pyrophosphates due to the absence of protons in the close vicinity of P. The peak at $\delta = 1.5$ ppm was tentatively assigned to brushite according to Rothwell et al. (1980) and Aue et al. (1984). However, the relatively large peak width (1412 Hz) reflected the uncertainty of this assignment.

Wood ash, fish sludge, catering waste, liquid and solid digestate, dairy manure and chicken manure all showed chemical shifts between $\delta = -2.1$ and 1.1 ppm, with rather broad peak widths between 954 and 2200 Hz. These could either represent various unspecified forms of organic P (Frossard et al. 2002; Hunger et al. 2004) or different weakly bound inorganic phosphates (Rothwell et al. 1980; Aue et al. 1984; Hunger et al. 2008).

Liquid and solid digestate also produced broad, asymmetrical resonance bands above $\delta = 0$ ppm. The spectra of liquid and solid digestate were generally characterised by more severe line broadening than those of the other waste products, which might indicate interaction between P and Fe and the presence of Fe-hydroxides/oxides or Fe-phosphates. Furthermore, according to Hunger et al. (2008) phosphate adsorbed to aluminium hydroxide cannot be excluded.

Table 6 Chemical shifts (ppm) with assignments, width (Hz) and relative area (%) derived from deconvolution of the single-pulse ^{31}P MAS-NMR spectra

Waste/manure product	Chemical shift/assignment ppm	Width Hz	Relative area %	Spinning side bands
Wood ash	2.8[a]	490	66	Significant
	2.6[a]	868	21	
	−2.1[b]	964	13	
Cereal ash	6.7[c]	466	11	Significant
	2.2[a]	542	29	
	1.5[d]	1412	42	
	−8.0[e]	302	18	
Meat bone meal	3.8[a]	506	16	Observable
	3.1[a]	496	84	
Fish sludge	3.1[a]	674	43	Observable
	2.1[a]	500	19	
	−1.0[b]	1232	38	
Catering waste	3.4[a]	452	38	Weak
	2.4[a]	366	43	
	−1.7[b]	1228	19	
Liquid digestate	3.0[a]	256	6	Weak
	−0.4[b]	1478	49	
	−5.7[b]	3122	45	
Solid digestate	3.1[a]	424	17	Weak
	1.1[b]	2200	37	
	−4.5[b]	1584	46	
Dairy manure	3.1[a]	846	23	Weak
	0.0[b]	1542	77	
Chicken manure	2.6[a]	1432	40	Weak
	−0.7[b]	1368	60	

[a] Stable Ca phosphates with a molar Ca:P ratio >1 (Rothwell et al. 1980; Aue et al. 1984; Hinedi et al. 1989, Frossard et al. 2002)

[b] Unresolved and unspecified organic/inorganic phosphates (Toor et al. 2006)

[c] Struvite (Hunger et al. 2008)

[d] Brushite (Aue et al. 1984)

[e] Pyrophosphates (Hinedi et al. 1989)

Effect of waste and manure products on soil pH

Liming the model soil resulted in pH 5.5 (hereafter referred to as pH level 1) and 6.9 (hereafter referred to as pH level 2) at harvest 2 in the pots that received NoP (Table 7). Soil pH increased throughout the experiment to pH 6.0 and 7.2, respectively, in these pots, probably as an effect of fertilisation with $Ca(NO_3)_2$ and associated OH^- release by plants during NO_3^- uptake in the poorly buffered model soil. Soil pH in the model soil was also significantly affected by fertiliser treatments. At pH level 1 and harvest 2, wood ash and catering waste resulted in significantly increased soil pH compared with NoP. At harvest 4, wood ash resulted in significantly higher soil pH than NoP. At pH level 2 and harvest 2, wood ash resulted in equally high soil pH as NoP and catering waste, but in significantly higher soil pH than the other fertiliser treatments. At harvest 4, fish sludge, liquid and solid digestate and dairy manure resulted in lower soil pH than NoP.

Table 7 pH in model soil after harvests 2 and 4 of the bioassay at soil pH level 1 and 2

Waste/manure product	Harvest 2	Harvest 4
pH level 1		
NoP	5.5cd	6.0b
MinP	5.3d	6.1b
Wood ash	6.2a	6.7a
Cereal ash	5.7bc	6.2b
Meat bone meal	5.5cd	6.2b
Fish sludge	5.3d	6.2b
Catering waste	5.8b	6.3b
Liquid digestate	5.7bc	6.0b
Solid digestate	5.7bc	6.4ab
Dairy manure	5.7bc	6.1b
Chicken manure	5.6bc	6.2b
SEM	0.1	0.1
HSD	0.3	0.4
pH level 2		
NoP	6.9ab	7.2ab
MinP	6.8b	7.0abc
Wood ash	7.1a	7.2a
Cereal ash	6.7b	7.0bc
Meat bone meal	6.8b	7.2ab
Fish sludge	6.7b	6.9c
Catering waste	6.8ab	7.2ab
Liquid digestate	6.6b	6.8c
Solid digestate	6.7b	6.9c
Dairy manure	6.8b	6.9c
Chicken manure	6.8b	7.0abc
SEM	0.1	0.2
HSD	0.3	0.0

SEM is the pooled standard error of the mean, HSD is the honestly significant difference with $p < 0.05$ between treatments according Tukey's test after one-way ANOVA and different letters indicate significant differences between treatments. Statistical analysis of the data was performed on pH values instead of H^+ concentration

Fertilisation effects of waste and manure products

The clear response of ryegrass to P application on the model soil was shown by a linear increase in P uptake in aboveground biomass as a function of increasing mineral P fertiliser application rates (0, 37.5, 75 and 112.5 mg P pot^{-1}) at both pH levels tested, as described in Part II of this study (Brod et al. 2015). After application of waste and manure products, P uptake was always in the linear range of the P response curve (results not shown), even though P application rates deviated slightly from the intended values (Table 8).

At pH level 1, biomass production (sum of all harvests) was equally high after application of MinP and all waste products except catering waste and solid digestate (Table 8). Differences in biomass production between fertiliser treatments were most pronounced at harvest 1 and levelled off thereafter. By calculating PUE, deviations in P fertilisation rates were corrected for. PUE as the sum of all harvests was highest for dairy manure and fish sludge (Fig. 2). PUE for dairy manure was higher than for MinP. Fish sludge and liquid digestate resulted in equally high PUE to MinP, while the other waste products resulted in lower PUE than MinP. Liquid digestate, cereal ash, wood ash, meat bone meal and chicken manure resulted in PUE at the same level, followed by solid digestate. Catering waste resulted in the lowest PUE among all fertiliser treatments. Biomass production increased from harvest 1 to 2 in pots supplied with waste products and decreased thereafter, whereas biomass production was equally high at harvest 1 and 2 in pots supplied with MinP and manure products and decreased thereafter (results not shown). PUE generally tended to be highest at harvest 1 and to decrease over time. Only wood ash, catering waste and solid digestate resulted in no significant difference between PUE at harvests 1 and 2 (results not shown).

At pH level 2, biomass production as the sum of all harvests and PUE were both generally lower than at pH level 1 (Table 8; Fig. 2). Meat bone meal, fish sludge and catering waste resulted in significantly lower total biomass production and PUE than at pH level 1 and NoP resulted in significantly lower biomass production. At pH level 2, biomass production as the sum of all harvests was significantly lower after application of meat bone meal, fish sludge, catering waste and solid digestate than after application of MinP. PUE as the sum of all harvests was significantly highest for dairy manure, followed by MinP. All waste products resulted in lower PUE than MinP. Wood ash, liquid digestate and chicken manure were the waste products with the highest PUE, followed by cereal ash, solid digestate and fish sludge. At pH level 2, biomass production increased from harvest 1 to 2 for all treatments and decreased thereafter (results not shown). PUE tended to be

Table 8 Fertilisation rate (mg P pot^{-1}) and biomass production (g DM pot^{-1}) for harvests 1 and 2 and the sum of all harvests at soil pH level 1 and 2

Waste/manure product	Fertilisation rate mg P pot^{-1}	Harvest 1 g DM pot^{-1}	Harvest 2 g DM pot^{-1}	Σ Harvest 1–4 g DM pot^{-1}
pH level 1				
NoP	0	10.1d	10.4d	27.3d
MinP	75	14.2a	14.5abc	44.9a
Wood ash	75	12.0bc	15.4a	45.5a
Cereal ash	77	12.5abc	14.8ab	42.6abc
Meat bone meal	78	12.9abc	14.7ab	43.7abc
Fish sludge	79	13.0abc	15.0a	45.5a
Catering waste	79	11.1cd	13.3c	40.0c
Liquid digestate	79	11.9cd	15.2a	43.2abc
Solid digestate	80	8.1e	15.9a	40.2bc
Dairy manure	76	13.8ab	13.5bc	43.6abc
Chicken manure	88	14.0a	14.6abc	44.2ab
SEM		0.4	0.3	0.8
HSD		1.9	1.4	4.1
pH level 2				
NoP	0	6.1c	9.2c	21.3e
MinP	75	12.6a	15.2a	44.8ab
Wood ash	75	11.5ab	16.4a	45.2a
Cereal ash	77	11.3ab	14.7a	41.3abc
Meat bone meal	78	7.1c	10.3bc	25.2de
Fish sludge	79	10.1b	14.9a	39.9bc
Catering waste	79	6.7c	11.6b	28.5d
Liquid digestate	79	10.6b	16.1a	43.5abc
Solid digestate	80	7.3c	16.3a	39.0c
Dairy manure	76	12.6a	15.7a	44.9ab
Chicken manure	88	12.2a	15.9a	43.7abc
SEM		0.3	0.4	1.0
HSD		1.5	1.9	5.1
2-Way ANOVA, source of variation				
Fertiliser		***	***	***
pH level		***	n.s.	***
Fertiliser × pH level		***	***	***

SEM is the pooled standard error of the mean, HSD is the honestly significant difference with $p < 0.05$ between treatments according Tukey's test after one-way ANOVA and different letters indicate significant differences between treatments

n.s. not significant

*,**,*** Represent significance at $p < 0.05$, 0.01 and 0.001 probability level, respectively

highest at harvest 1 and to decrease over time. However, meat bone meal, fish sludge, catering waste and solid digestate resulted in no significant difference between PUE at harvest 1 and 2 (results not shown).

The observed fertilisation effects were ascribed to P supplied by the treatments rather than N, because the N:P ratio in plant biomass (g kg^{-1}) was ≥9 (Liebisch et al. 2013, results not shown). Estimation of the N fertilisation effects of the waste products was hence successful. Only dairy manure resulted in N:P <9 (7.8) at pH level 1 and harvest 1, probably because of the high availability of P in dairy manure and subsequent N limitation. However, the N:P ratio in plants fertilised with dairy manure increased again at the following harvests. Also growth limitation by other nutrients was excluded based on normal concentrations in aboveground biomass at harvests 1–4 (Bergmann 1993, results not shown).

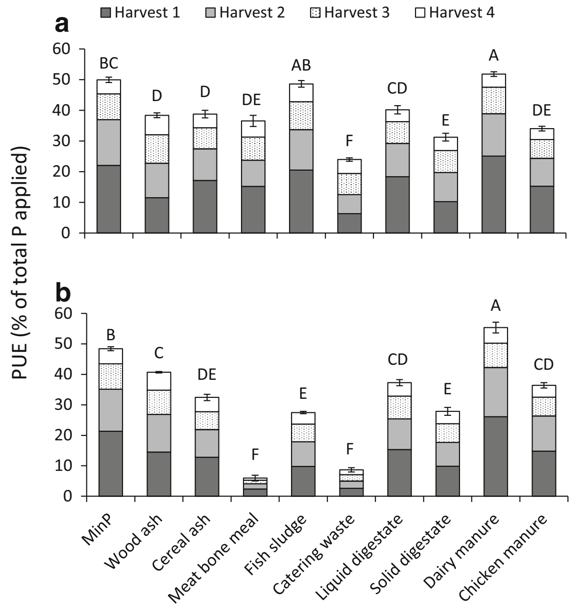

Fig. 2 Apparent P use efficiency (PUE, % of total P applied) at **a** pH level 1 and **b** pH level 2 at harvests 1, 2, 3 and 4. *Error bars* indicate standard deviation between replicates for the sum of all harvests. *Different letters* indicate significant differences between treatments for the sum of all harvests for each pH level (Tukey's HSD)

Discussion

Complex mixtures of inorganic P species affected P fertilisation effects of waste products depending on soil pH level

By combining different approaches, we were able to demonstrate that the P-rich waste products studied were characterised by a complex mixture of inorganic P species, mainly Ca phosphates of differing solubility. Different inorganic P species resulted in turn in variable performance of P-rich waste products as alternative P fertilisers, an effect that was also dependent on soil pH. The fertilisation effects of waste and manure products were largely explained by the sequential fractionation data, as illustrated by the positive relationship between PUE as the sum of all harvests and the H_2O-soluble inorganic P fraction at pH level 1 and the negative relationship between PUE as the sum of all harvests and the HCl-soluble inorganic P fraction at pH level 2 (Fig. 3). The other characterisation methods supported the findings of the sequential fractionation, as discussed below.

Meat bone meal and catering waste

Meat bone meal and catering waste were characterised by poor P fertilisation effects at pH level 2, as indicated by both biomass production and PUE. PUE was in the same order of magnitude as the labile fraction identified by sequential fractionation. The majority of inorganic P was present as stable Ca phosphates that were only soluble in HCl. This is in agreement with Ylivainio et al. (2008), who found that 92 % of the P in meat bone meal was in the HCl fraction. XRD and ^{31}P MAS-NMR also suggested the presence of crystalline and stable Ca phosphates, such as hydroxyapatite and chlor-fluorapatite. The largest fraction of P in animals is present in bones, where it forms hydroxyapatite (Roufosse et al. 1984). We assume that the P in catering waste is also largely present in bone material, as leftovers of bone steaks

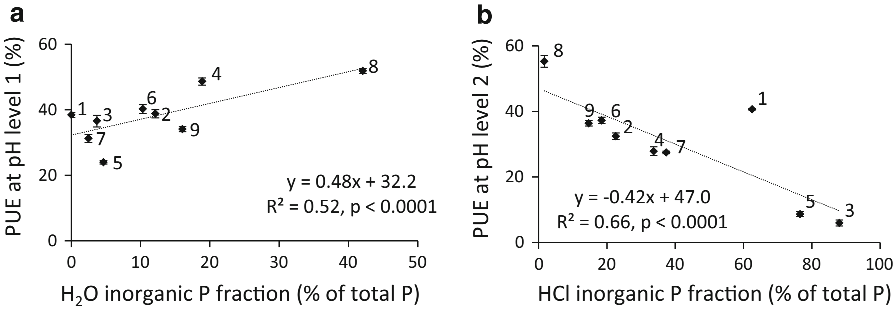

Fig. 3 Apparent P use efficiency (PUE, %) expressed as sum of all harvests and as effect of **a** H_2O-soluble inorganic P fraction and **b** HCl-soluble inorganic P fraction, where *1* wood ash, *2* cereal ash, *3* meat bone meal, *4* fish sludge, *5* catering waste, *6* liquid digestate, *7* solid digestate, *8* dairy manure and *9* chicken manure. *Error bars* indicate standard deviation between replicates for the sum of all harvests

etc. The solubility of stable Ca phosphates strongly decreases with increasing soil pH (Lindsay 1979), which explains the poor fertilisation effects of meat bone meal and catering waste at pH level 2.

Also at pH level 1, PUE was significantly lower after application of meat bone meal and catering waste than after application of MinP. However, PUE was significantly higher for meat bone meal than for catering waste, which resulted in the lowest PUE of all fertiliser treatments. One possible reason for this difference might be the increasing effect of catering waste on soil pH, although differences in soil pH following application of meat bone meal and catering waste were not significant. According to XRD, catering waste contained some crystalline $CaCO_3$, which increases soil pH according to $CaCO_3 + H^+ \rightarrow Ca^{2+} + CO_2 + OH^-$. No $CaCO_3$ was detected in meat bone meal. Therefore, increased soil pH might have resulted in less P being dissolved from stable Ca phosphates in catering waste. Dissolution of $CaCO_3$ would also have increased the Ca concentration in the soil solution, which in turn would have decreased the solubility of Ca phosphates. Another possible reason for reduced growth after application of catering waste may be phytotoxins contained in the reactor-composted food waste, as reported for unstable compost by Keeling et al. (1994). Poor effects of catering waste when used as N fertiliser and clear residual effects in the year after application have previously been explained by recalcitrant N (Brod et al. 2014), but it is possible that catering waste showed reduced effects as both alternative N and P fertiliser because it had not yet stabilised.

Each year, Norway produces slaughter waste corresponding to 2200 Mg P (Hamilton et al. 2015). Slaughter waste is also of great importance internationally, with European animal and vegetal waste accounting for 290 Gg P (Ott and Rechberger 2012). However, this study demonstrated that the recycling potential of slaughter waste cannot be fully exploited unless it is treated, e.g. with chemical extraction of P from bone ash for the production of soluble P fertilisers (Krupa-Żuczek et al. 2008).

Fish sludge

Fish sludge was the only waste product that resulted in equally high PUE as dairy manure and mineral fertiliser at pH level 1. This was also reflected by fish sludge being the waste product with the largest fraction of H_2O-soluble inorganic P. When root systems are still small, P uptake by plants is mainly determined by diffusion in addition to mass flow to the roots. It is likely that H_2O extraction reflects the ability of a fertiliser product to increase soil solution P concentration (Kratz et al. 2010). Once the root systems of plants fertilised with fish sludge were well developed, the root-induced decrease in pH in the rhizosphere (Hinsinger 2001) could also have helped dissolve stable Ca phosphates. Similarly, Krogstad et al. (2005) reported increased plant availability of P in sewage sludge after combined application with water-soluble inorganic P and explained the effect by early developed roots utilising sewage sludge P more efficiently.

Fish sludge showed significantly reduced fertilisation effects at pH level 2 compared with pH level 1, as shown by significantly lower biomass production at harvest 1 and PUE than after MinP application at pH level 2. Our XRD analysis detected chlor-fluorapatite in fish sludge in the same order of magnitude as for catering waste, and a large fraction of P in fish sludge was assigned to stable Ca phosphates by ^{31}P MAS-NMR. Fish sludge still resulted in higher PUE than meat bone meal and catering waste, which can be explained by both the sequential fractionation and ^{31}P MAS-NMR. The fraction of labile P in fish sludge was significantly larger and the HCl-soluble inorganic P fraction was significantly smaller than in meat bone meal and catering waste, indicating that fish sludge contained less stable Ca phosphates. Moreover, its stable Ca phosphates might have been less well ordered than in meat bone meal and catering waste, as indicated by broader peak widths in ^{31}P MAS-NMR (Frossard et al. 2002).

In Norway, the amount of P lost each year to the sea through fish sludge (9000 Mg P) is greater than the amount of P applied to land as mineral fertiliser (8500 Mg P, Hamilton et al. 2015). Our results show that fish sludge has great potential as an alternative P fertiliser when applied to acidic soils. However, technological development is required before fish sludge can substitute mineral fertiliser on a large scale, which requires collection from open cages, dewatering, desalinisation and the development of safe fertiliser products.

Wood ash and cereal ash

It is difficult to draw general conclusions about the P fertilisation effect of biomass ash because we identified large differences in inorganic P species in wood and cereal ash, which can explain their variable performance as P fertilisers over time and at different pH levels.

None of the P in wood ash was soluble in H_2O, which can explain its delayed P fertiliser effect during the start of the bioassay compared with cereal ash and the other waste products tested here. However, this initially delayed P fertilisation effect was compensated for later in the season, despite the increasing effect on soil pH, and PUE of wood ash as the sum of all harvests was equally high at both pH levels studied. This was unexpected, because most P was present as stable Ca phosphates according to both sequential fractionation (>60 % of total P) and ^{31}P MAS-NMR (>80 % of total P). We assume that these stable Ca phosphates were the calcium phosphate silicate or sodium calcium magnesium phosphate identified by XRD, in addition to hydroxyapatite (>70 % of total P). The P fertilisation effect of wood ash was therefore underestimated by the HCl-soluble inorganic P fraction compared with the HCl inorganic P fraction in the other waste products (Fig. 3). Even though most P in wood ash seemed to be present as stable Ca phosphates, more than 40 % of total P was extracted by $NaHCO_3$, an extraction solution that is buffered at $pH > 8$ and is therefore expected to dissolve only labile Ca phosphates and labile Fe-/Al-bound P (Kuo 1994). The relatively high Fe and Al concentrations in wood ash (Table 2) might indicate the presence of $NaHCO_3$-soluble Fe-/Al-bound P. The solubility of Fe-/Al-bound P increases with increasing soil pH and its presence could therefore explain the equally good performance of wood ash as P fertiliser at both pH levels.

Cereal ash resulted in lower total PUE than fish sludge at pH level 1 and wood ash at pH level 2, which were the waste products with the highest PUE at these pH levels. This was unexpected because cereal ash contained larger amounts of labile P than wood ash and in fact the largest labile P fraction of all waste products (>60 % of total P). Further characterisation by XRD and ^{31}P MAS-NMR also led us to expect higher fertilisation effects of cereal ash than observed. Struvite-K was detected by XRD and confirmed by ^{31}P MAS-NMR and can be expected to have as good P fertilisation effects as struvite (e.g. Cabeza et al. 2011), also because of a somewhat higher solubility product compared with struvite (Taylor et al. 1963). Pyrophosphate was detected by ^{31}P MAS-NMR and further specified as $CaK_2(P_2O_7)$ by XRD, which was suggested to be a promising fertiliser by Brown et al. (1964). Lindsay (1979) also suggests high solubility of pyrophosphates in soils. The reason why P fertilisation effects of cereal ash were lower than expected was not identified.

The presence of pyrophosphates in cereal ash might explain the incorrect measurement of organic P in cereal ash during sequential fractionation, which resulted from the difference between total P and inorganic P. Total P was measured by ICP–OES, while inorganic P was measured as ortho-P by the colorimetric molybdenum blue method. Pyrophosphate-P was hence not detected as inorganic P and was therefore defined as organic P as an artefact of the method. Another possible explanation for the lack of agreement between the results of ICP–OES and the molybdenum blue method could be inorganic P bound to colloids remaining in the centrifuged supernatant, which was not filtered despite the suggestion by Hedley et al. (1982). Inorganic P bound to colloids may not have been detected by the molybdenum blue method, but would have been detected by ICP–OES.

The relatively large crystalline content in wood and cereal ash as quantified by XRD may explain the pronounced spinning side bands in the ^{31}P MAS-NMR spectra of both materials (Table 6), but did not have a reducing effect on the P fertilisation effect of ash compared with the other waste products. This observation contradicts findings by Nanzer et al. (2014), who describe a significant negative relationship between crystalline phases in different sewage sludge ashes and their PUE. Furthermore, in comparison with Nanzer et al. (2014), we did not find any relationship between crystalline content and specific surface area of waste products (Table 2) that could be used to explain P fertilisation effects.

Liquid and solid digestate

Our results indicate that both P fertilisation effects and inorganic P species can vary considerably between waste products with the same origin as a result of different treatment processes, despite similar total P concentrations. During the treatment process and centrifugation of liquid digestate, mineral N and K followed the liquid phase, whereas P and organic matter followed the solid phase and ended up in solid digestate. There was also relatively more Ca, Fe and Al compared with P in solid than in liquid digestate (Table 2).

Liquid digestate resulted in higher PUE than solid digestate at both pH levels, which was equivalent to a larger fraction of labile P in liquid compared with solid digestate according to the sequential fractionation. In addition, significantly more P was present as stable Ca phosphates in the solid than in the liquid digestate according to all characterisation methods. Solid digestate resulted in higher soil pH than liquid digestate at pH level 1 and harvest 4, a finding which can have resulted from $CaCO_3$ being detected by XRD and again in reduced solubility of stable Ca phosphates as described above. However, we cannot exclude the possibility that reduced P fertilisation effects of solid compared with liquid digestate can also be attributable to unintended low mineral N fertilisation during the set-up of the experiment and the P in solid digestate therefore not being fully exploited at harvest 1. Still, we assume that ryegrass compensated for this discrepancy later in the season, when N was in sufficient supply.

Liquid and solid digestate were in addition to fish sludge the only waste products that contained noteworthy amounts of NaOH-soluble P, which is defined as Fe-/Al-bound P. As Fe-/Al-bound P were not detected by XRD, we can conclude that these compounds had an amorphous structure. The ^{31}P MAS-NMR results suggested that the presence of condensed, crystalline Al phosphates can be excluded, as they would have resulted in chemical shifts in the area $\delta = -5$ to -30 ppm (Bleam et al. 1989). More specific assignment of Fe-/Al-bound P in liquid and solid digestate and further discussion of possible implications for P plant-availability are therefore not possible.

Untreated liquid digestate based on source-separated household waste is commonly characterised by N:P:K ratios similar to those of mineral compound fertiliser and good fertilisation effects (Haraldsen et al. 2011). However, to turn untreated liquid digestate into a competitive alternative fertiliser, the water content has to be efficiently decreased without reducing the fertilisation effects, as was the case for solid digestate in the present study.

Dairy and chicken manure

Dairy and chicken manure were included in the present study as secondary P resources with well-studied P fertilisation effects. It is widely accepted that the P fertilisation effects of dairy manure are comparable to those of mineral fertilisers (e.g. Smith and van Dijk 1987), whereas chicken manure has been reported to result in lower P fertilisation effects than mineral fertiliser (Oladeji et al. 2008).

In the present study, dairy manure resulted in equally high or higher PUE than MinP, which is in agreement with the results of the P characterisation studies showing that the majority of total P in dairy manure was soluble in H_2O and $NaHCO_3$. Similar results have been reported by Sharpley and Moyer (2000) and Ylivainio et al. (2008), who found 74 and 91 % of total P in dairy manure, respectively in the same fraction. According to our XRD analyses, all P was present in amorphous structures, which was also supported by the ^{31}P MAS-NMR spectrum. The higher P fertilisation effects of dairy manure compared with MinP can be explained by low molecular organic acids reducing P retention in the highly P-deficient model soil (Øgaard 1996).

Chicken manure resulted in lower PUE than dairy manure and MinP. In comparison with the waste products and dairy manure, the P in chicken manure was mainly present as organic P. The largest fraction of organic P was extracted by NaOH, but some was also extracted by HCl, as reported previously by He et al. (2006), although the original method only assumed inorganic P to be present in the HCl extract (Hedley et al. 1982). Nevertheless, the relationship between the solubility of organic P obtained by sequential fractionation and its P fertilisation effects is poorly understood (Condron et al. 2005), and therefore no conclusions can be drawn about the plant availability of organic P in chicken manure. Reduced P fertilisation effects of chicken manure can partly be explained by the likely presence of stable Ca phosphates, since >30 % of inorganic P in chicken manure was extracted by HCl and a ^{31}P MAS-NMR chemical shift suggested stable Ca phosphates, although the resolution was poor.

Manure is the most important of all the secondary P resources in Norway, with a yearly accumulation of 12,000 Mg P (Hamilton et al. 2015), of which cattle manure represents 6500 Mg P and poultry manure represents 1000 Mg P (Hamilton et al. unpublished data). In Europe, 1600 Gg P are returned to agricultural land with manure each year, while 1080 Gg P are applied with mineral fertiliser (Ott and Rechberger 2012). Despite high P fertilisation effects of dairy manure, efficient P recycling can only be realised if technology is developed to transport manure from areas with concentrated animal husbandry activity and a P surplus to arable areas where P inputs are needed.

Conclusions

The aim of this study was to explain the P fertilisation effects of a range of Norwegian P-rich waste products by characterising the inorganic P species they contain. Inorganic P species in waste products were identified as a complex mixture of mainly Ca phosphates of differing solubility. The apparent phosphorus use efficiency (PUE) of all waste products was lower than that of mineral P fertiliser and dairy manure and was strongly affected by pH in the model soil (approximately pH 5.5 and 6.9 at pH level 1 and 2). The fertilisation effects of waste and manure products were largely explained by sequential fractionation, with a positive relationship between PUE and the H_2O-soluble inorganic P fraction at pH level 1 and a negative relationship between PUE and the HCl-soluble inorganic P fraction at pH level 2. XRD and solid-state ^{31}P MAS-NMR spectroscopy confirmed the findings of the sequential fractionation but provided little additional information.

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Supplementary Material

Waste products as alternative phosphorus fertilisers, Part I: Inorganic P species affect fertilisation effects depending on soil pH

Nutrient Cycling in Agroecosystems

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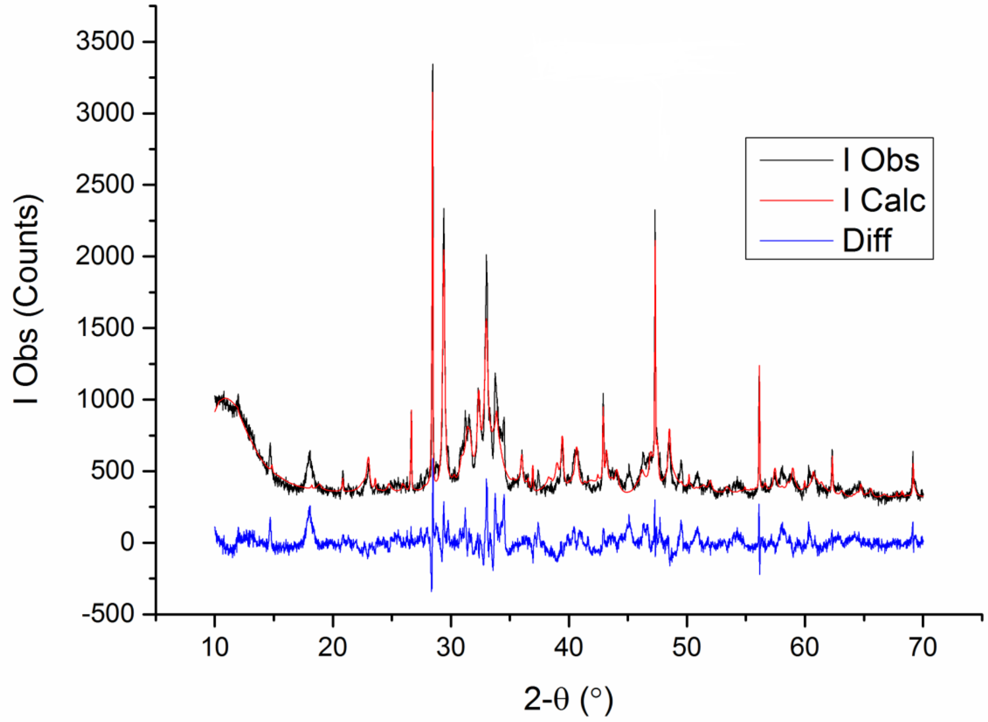
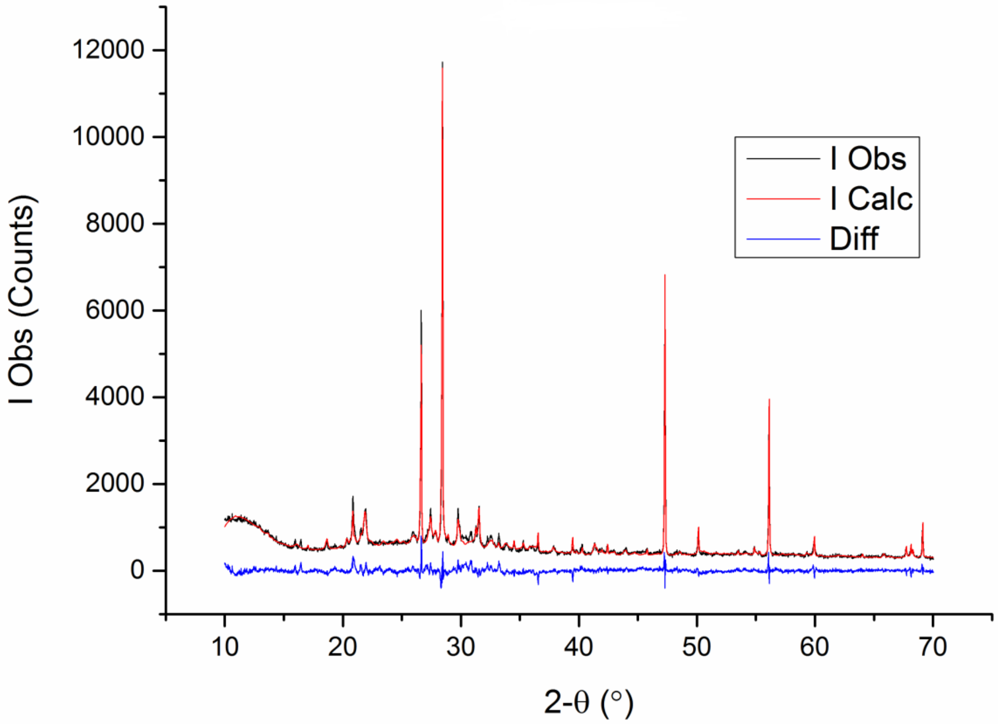
Rietveld quantitative phase analysis (QPA)

Rietveld QPA was carried out using TOPAS version 4.2. The fundamental parameters method was used to generate the peak shapes and for each phase-independent scale, Lorentzian crystallite size broadening and lattice parameters were refined. Atomic positions were not refined. A seven-term Chebyshev polynomial background function was used to fit the main background shape, with additional broad peaks at fixed positons to fit the large amorphous background contributions in some samples. The diffractometer zero point was also refined.

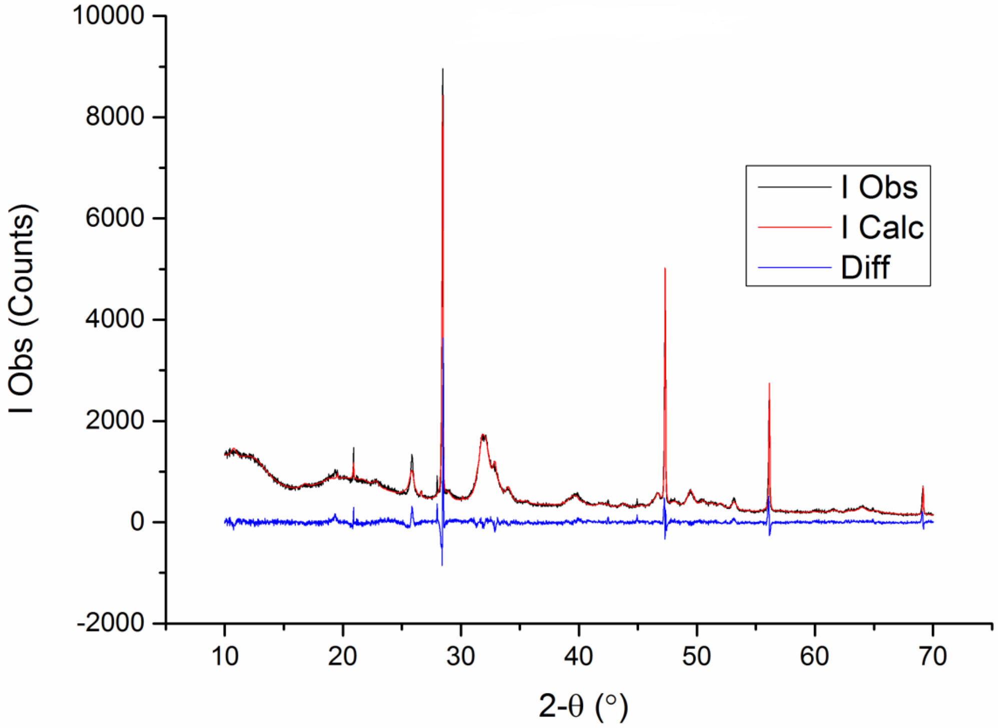
For wood ash, catering waste, liquid digestate and chicken manure, it was not possible to identify all the crystalline phases present, with several non-fitted peaks at lower angles being observed. Changing the 2-θ range to omit these peaks had little effect on the QPA results. Independent peaks were used to fit contributions from unknown phases in the liquid digestate and chicken manure samples, improving the overall pattern fit. The effect on the QPA of including these unidentified peaks or ignoring them was negligible.



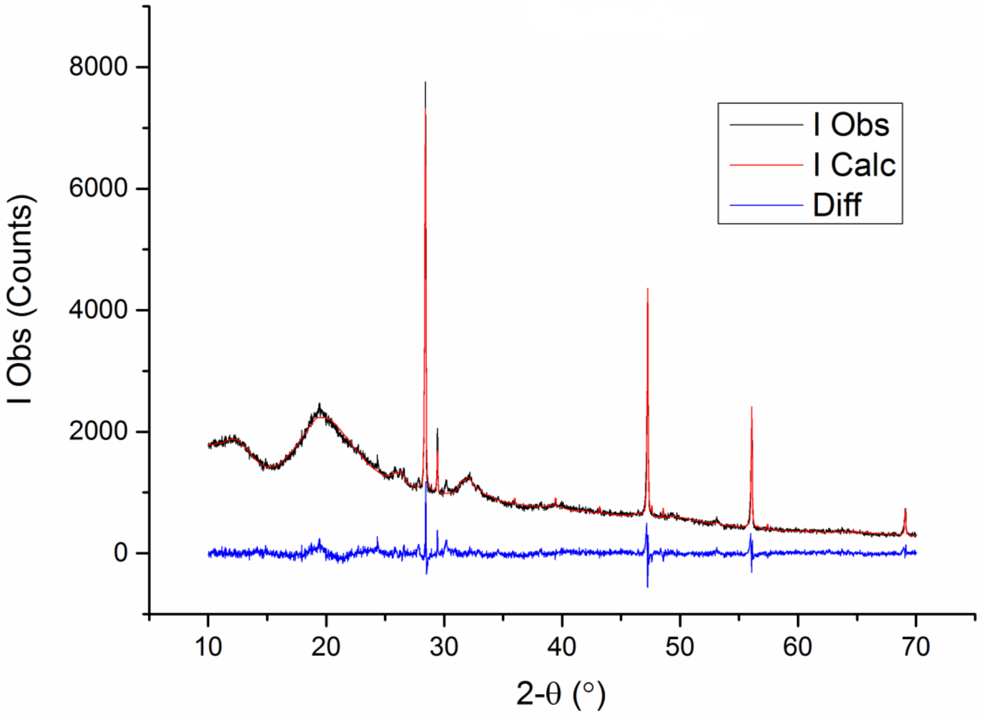
Supplemental Figure S 1. Rietveld plot for wood ash, replicate 1. Note the peak from an unidentified phase at ~18°. I Obs = Intensity observed, I Calc = Intensity calculated, Diff = Difference between I Obs and I Calc.



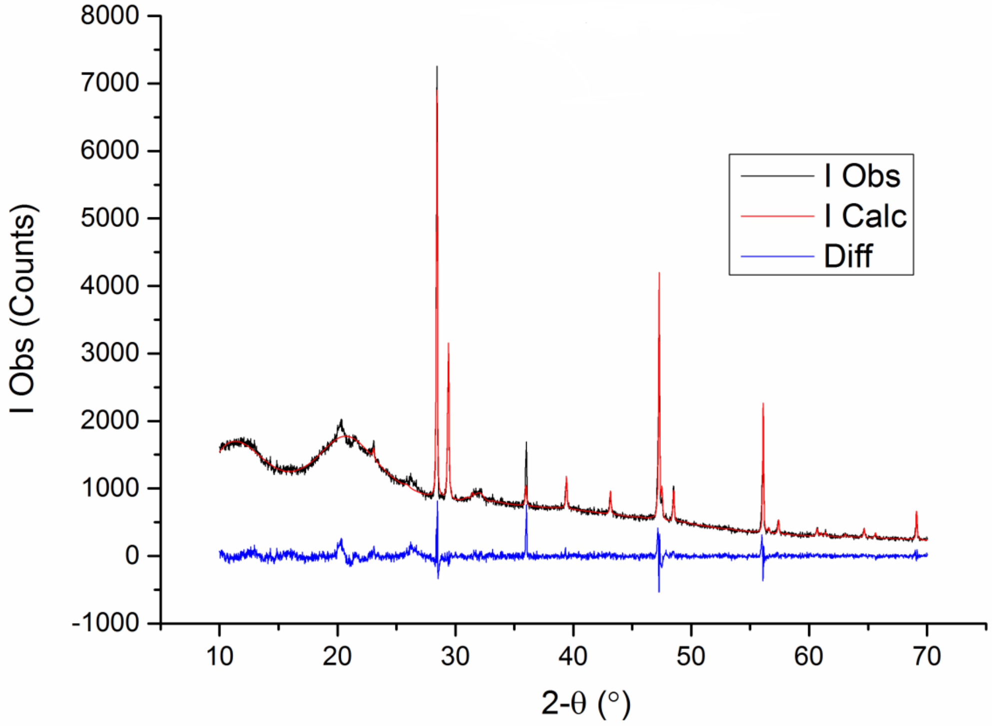
Supplemental Figure S 2. Rietveld plot for cereal ash, replicate 1. I Obs = Intensity observed, I Calc = Intensity calculated, Diff = Difference between I Obs and I Calc.



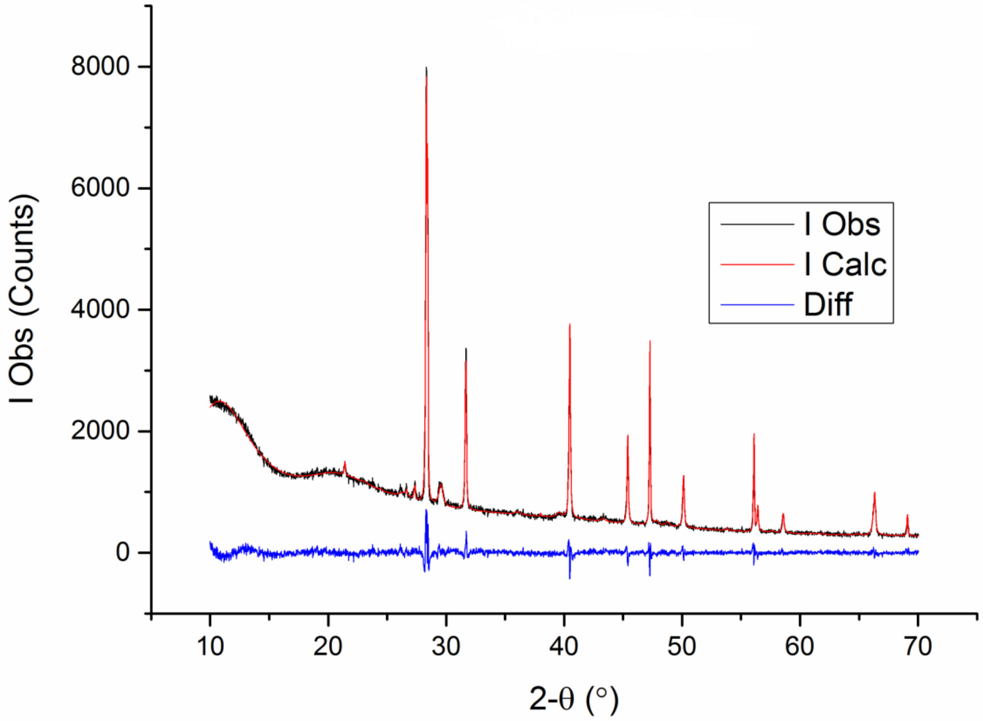
Supplemental Figure S 3. Rietveld plot for meat bone meal, replicate 1. I Obs = Intensity observed, I Calc = Intensity calculated, Diff = Difference between I Obs and I Calc.



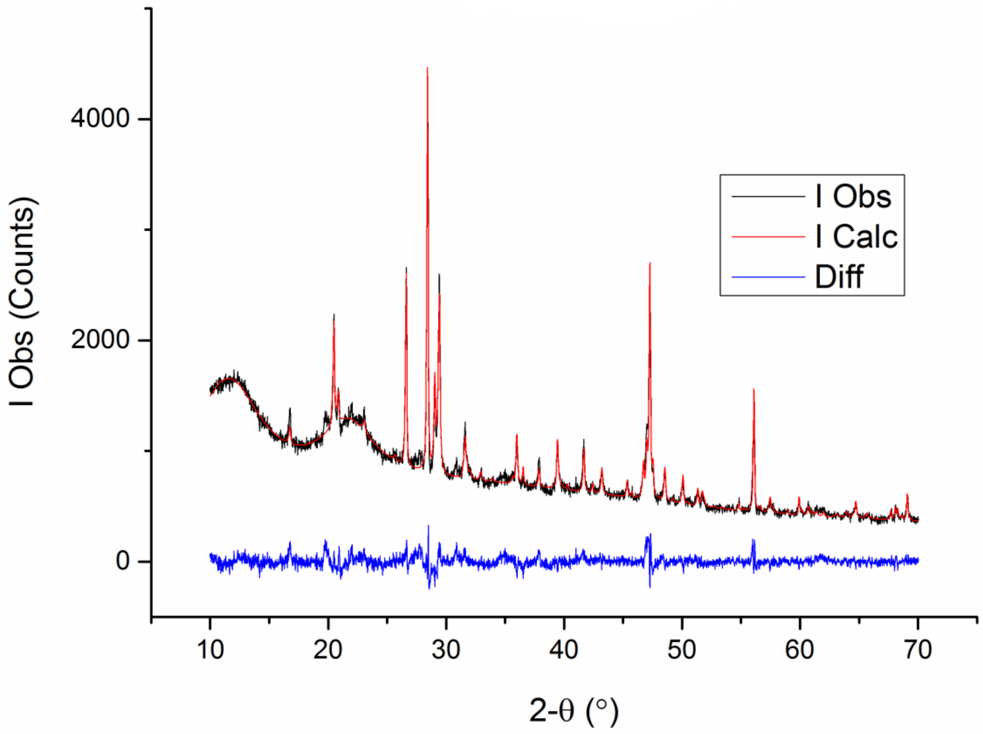
Supplemental Figure S 4. Rietveld plot for fish sludge, replicate 1. I Obs = Intensity observed, I Calc = Intensity calculated, Diff = Difference between I Obs and I Calc.



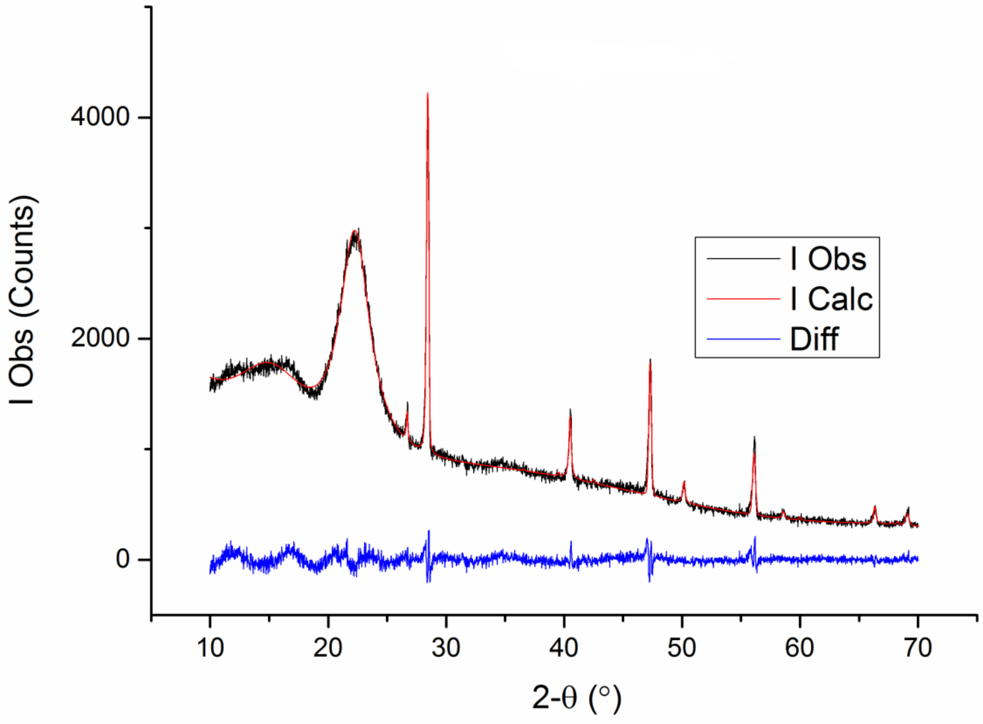
Supplemental Figure S 5. Rietveld plot for catering waste, replicate 1. I Obs = Intensity observed, I Calc = Intensity calculated, Diff = Difference between I Obs and I Calc.



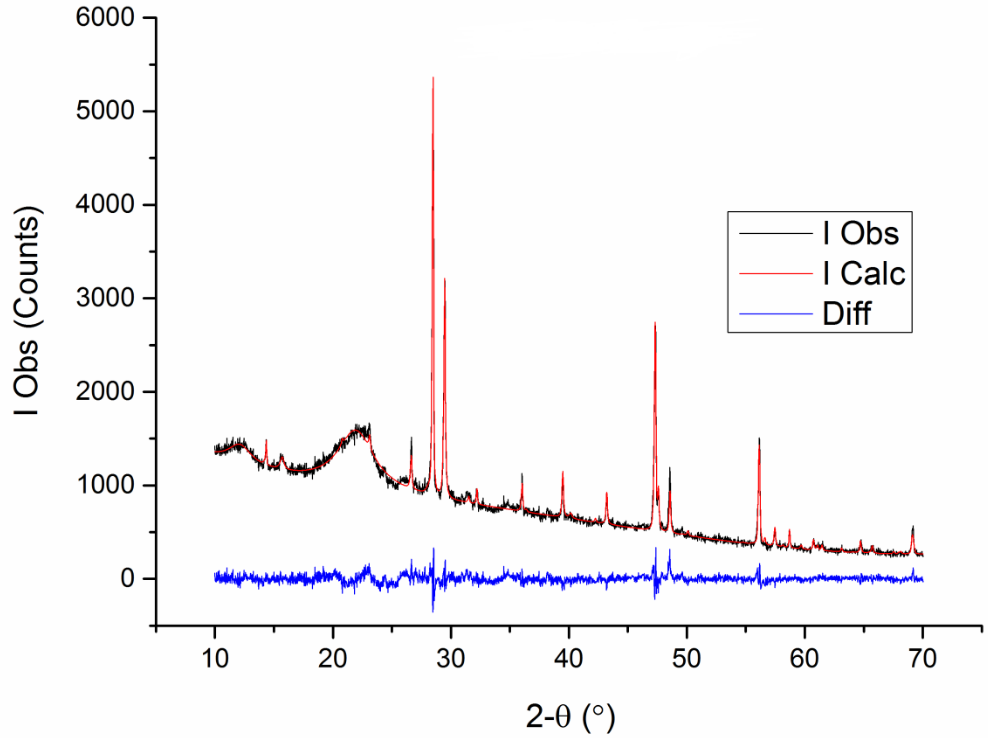
Supplemental Figure S 6. Rietveld plot for liquid digestate, replicate 1. Peak fitted at 21.4 degrees. Effect on QPA negligible. I Obs = Intensity observed, I Calc = Intensity calculated, Diff = Difference between I Obs and I Calc.



Supplemental Figure S 7. Rietveld plot for solid digestate, replicate 1. I Obs = Intensity observed, I Calc = Intensity calculated, Diff = Difference between I Obs and I Calc.



Supplemental Figure S 8. Rietveld plot for dairy manure, replicate 1. I Obs = Intensity observed, I Calc = Intensity calculated, Diff = Difference between I Obs and I Calc.



Supplemental Figure S 9. Rietveld plot for chicken manure replicate 1. Peaks fitted at 14.3, 15.7 and 58.6 degrees. Effect on QPA negligible. I Obs = Intensity observed, I Calc = Intensity calculated, Diff = Difference between I Obs and I Calc.

PAPER IV

Brod E, Øgaard AF, Haraldsen TK, Krogstad T (2015) Waste products as alternative phosphorus fertilisers part II: Predicting P fertilisation effects by chemical extraction. Nutrient Cycling in Agroecosystems 103: 187–199

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ORIGINAL ARTICLE

Waste products as alternative phosphorus fertilisers part II: predicting P fertilisation effects by chemical extraction

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Abstract Knowledge of the relative agronomic P efficiency (RAE) of waste products compared with mineral fertiliser is essential for establishing reliable fertilisation plans. This creates the demand for an evaluation of laboratory methods that can predict RAE of waste products. In this study we compared eight chemical extraction methods in terms of their ability to predict readily-available P and late-season P fertilisation effects of a wide range of waste products (two biomass ashes, meat bone meal, fish sludge, catering waste, two food waste-based digestate products) at two soil pH levels, approximately pH 5.5 (pH level 1) and pH 6.9 (pH level 2). Extractable P was correlated with RAE determined in a bioassay with ryegrass (*Lolium multiflorum*) and a nutrient-deficient sand-peat mixture as model soil. At pH level 1, P extraction with 0.005 M $CaCl_2$ and simultaneous adsorption to iron-oxide impregnated filter paper predicted RAE best at harvest 1 and neutral ammonium citrate predicted RAE best as the sum of harvests 2, 3 and 4. At pH level 2, 0.5 M $NaHCO_3$ (pH 8.5, Olsen P) was the only extraction method that was significantly correlated with RAE at harvest 1 and the sum of harvests 2, 3 and 4, because all other extractions overestimated RAE of waste products containing stable calcium phosphates. The optimum extraction method thus depends on whether readily-available P or late-season P fertilisation effects are being predicted and on the pH of the target soil.

Keywords Prediction · Standard soil test · Soil pH · Meat bone meal · Wood ash · Digestate

Introduction

With rock phosphate becoming an increasingly precious resource, there has been growing interest in the use of waste products as alternative phosphorus (P) fertiliser in agriculture. New types of waste products are continuously being introduced into agriculture, but their P fertilisation effects are not well known and vary widely, even between production plants and batches of a particular product (e.g. Kratz et al. 2010; Cabeza et al. 2011). It is essential to know the relative agronomic P efficiency (RAE) of waste products compared with mineral fertiliser in order to establish fertilisation plans that meet crop demands. Growth experiments are the most reliable method of determining the RAE of waste products, but are too time-consuming and expensive to be used as a standard procedure.

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In Norway, it is common practice to indicate the availability of P in waste products by stating the fraction of AL-extractable P, which is also the standard soil test used in agriculture (Mattilsynet 2013). This involves extraction with a solution containing 0.1 M ammonium lactate and 0.4 M acetic acid adjusted to pH 3.75 according to Egnér et al. (1960). However, the relationship between the fraction of AL-extractable P in waste products and RAE is unknown, and a previous greenhouse study has shown poor relationships between AL-extractable P in meat bone meal and wood ash and P uptake in spring cereals (Brod et al. 2014). In other studies a range of extraction methods, developed for estimation of either readily-available P in soils or the fertilisation effects of mineral P fertilisers, have been compared with plant-available P in waste products as studied in growth experiments, but the results are inconclusive.

Weak extraction solutions have often been shown to result in better relationships with plant-available P in waste products than extraction methods with strong desorption agents. For example, Alt et al. (1994) suggested that extraction with a mixture of $CaCl_2$ and DTPA was better for predicting P concentration and P uptake in plants after application of compost and compost/peat mixtures than extraction with formate, calcium acetate lactate and ammonium acetate. Velthof et al. (1998) found that extraction with 0.01 M $CaCl_2$ with simultaneous adsorption to iron-oxide impregnated filter paper produced better predictions of the RAE of nine different waste products than stronger extraction with NH_4F and HCl (Bray-I) but also than extraction with 0.5 M $NaHCO_3$ at pH 8.5 (Olsen P). However, other studies report good relationships between strong desorption agents and plant-available P. According to Schiemenz et al. (2011), the P fertilisation effects of biomass ash are underestimated by extraction with water and extraction with 2 % citric acid improves prediction. Wang et al. (2012) proposed extracting P in biochar with 2 % formic acid to predict the P fertilisation effects.

These inconclusive results of different studies prove that there is a demand for systematic evaluation of laboratory methods in terms of their ability to predict RAE of new waste products. For example, distinguishing between readily-available P and late-season fertilisation effects might improve the ability of chemical extraction methods to predict P fertilisation effects of waste products. According to Ylivainio et al. (2008), a small fraction of the P in meat bone meal is water-soluble P, whereas 90 % is found in the 1 M HCl-soluble fraction. Accordingly, those authors found that the P fertilisation effects of meat bone meal were poor at the first harvest of a pot experiment and increased only later during the season when roots had developed. Kratz et al. (2010) found a strong relationship between water-soluble P and P uptake at the first harvest of a pot experiment, but concluded that citric acid best predicted P uptake at harvest 3. Another reason for the deviating conclusions in different studies might be the complex chemical composition of P in waste products. The P in mineral fertiliser is usually present as simple, water-soluble compounds (e.g. monocalcium phosphate). Also the P in waste products is typically present as inorganic P (Hedley and McLaughlin 2005), but can occur in a wide variety of compounds with Ca, Fe or Al that are characterised by variable solubility in soil, also depending on soil pH (Lindsay 1979). Therefore, the prediction of RAE of waste products by chemical extraction might be improved by considering the pH of the target soil.

The objective of the present study was thus to evaluate various extraction methods in terms of their ability to predict readily-available P and late-season P fertilisation effects of waste products at two different soil pH levels. We applied eight standard extraction methods and modified versions of two of these to seven P-rich waste products and two manure products. Extractable P in waste products was compared with plant-available P as studied by a bioassay with ryegrass (*Lolium multiflorum*). With the aim of predicting the RAE of waste products, we identified the extraction methods resulting in the best relationship between extractable P and RAE by simple linear regression.

Materials and methods

Waste and manure products

Waste and manure products are described in Table 1, while Table 2 gives an overview of their selected chemical properties, determined as described in Part I of this study (Brod et al. 2015). Based on their heavy metal contents, under Norwegian regulations (Norwegian Ministry of Agriculture and Food 2003) all waste

Table 1 Description of waste and manure products

No.	Waste/manure product	Description
1	Wood ash	Bottom ash from a grate-fired boiler system at the Moelven Østerdalsbruket AS mill, Norway. Parent material is timber unsuitable for industrial use
2	Cereal ash	Bottom ash from a grate-fired boiler system in the Eidsiva Bioenergi district heating system in Kongsvinger, Norway. Parent material is timber unsuitable for industrial use and cereal residues from the local mill
3	Meat bone meal	Stabilised and sanitised slaughterhouse waste from the slaughterhouse in Hamar, Norway
4	Fish sludge	Faeces and feed residues from the Åsen settefisk salmon hatchery, Norway, treated in a reactor developed by the company Global Enviro after separation of water by mechanical filtering
5	Catering waste	Source-separated catering waste from Rica Sunnfjord Hotel, Norway, treated in a reactor developed by the company Global Enviro after separation of grease and water by steam and pressure
6	Liquid digestate	Untreated digestate based on anaerobic treatment of source-separated household waste collected at the Mjøsanlegget biogas plant, Norway
7	Solid digestate	Solid phase after centrifugation of liquid digestate based on anaerobic treatment of source-separated household waste collected at the Mjøsanlegget biogas plant, Norway
8	Dairy manure	Slurry (faeces and urine) of dairy cows collected at the Norwegian University of Life Sciences, Norway
9	Chicken manure	Stabilised, sanitised and pelletised chicken manure produced by Norsk Naturgjødsel, Norway

Table 2 Selected chemical properties of waste and manure products

	Wood ash	Cereal ash	Meat bone meal	Fish sludge	Catering waste	Liquid digestate	Solid digestate	Dairy manure	Chicken manure
pH	13	9.6	6.2	5.4	5.8	7.0	7.7	7.0	6.7
SSA (m^2 g^{-1})	0.6	4.5	0.1	0.1	0.1	0.1	2.8	0.8	0.4
DM (g 100 g^{-1})	99.6	97.6	96.2	95.0	94.5	2.4	25.0	5.8	93.3
OM (g 100 g^{-1} DM)	0.0	17.0	66.6	87.6	81.1	64.6	66.2	81.6	81.7
P (g kg^{-1} DM)	17	51	54	21	10	12	15	6	10
Pi (% of total P)	103	100	98	86	93	74	78	76	47
C (g kg^{-1} DM)	19	111	368	503	449	412	400	470	427
N ([a]g kg^{-1} DM, [b]g L^{-1})	0[a]	4[a]	86[a]	71[a]	50[a]	2.3[b]	56[a]	3.1[b]	45[a]
Nmin ([a]g kg^{-1} DM, [b]g L^{-1})	n.d.	n.d.	5.0[a]	2.6[a]	5.1[a]	1.7[b]	7.2[a]	1.3[b]	0.041[a]
K (g kg^{-1} DM)	56	93	4	3	6	45	6	42	25
S (g kg^{-1} DM)	34	2	34	48	35	59	65	42	58
Ca (g kg^{-1} DM)	310	27	110	37	59	33	62	11	44
Mg (g kg^{-1} DM)	25	26	3	3	2	8	5	6	6
Al (g kg^{-1} DM)	19.1	3.8	0.2	0.3	0.1	8.6	13.8	0.4	0.5
Fe (g kg^{-1} DM)	7.6	4.3	0.5	0.7	0.4	2.8	5.9	1.3	1.0

SSA specific surface area, *DM* dry matter, *OM* organic matter, *Pi* inorganic P, *Nmin* mineral N (NO_3^- and NH_4^+)

products included in the study could be applied as fertiliser to agricultural land.

P extractions

In total eight chemical extraction methods (Table 3), both standard soil extractions and standard fertiliser extractions, were applied to the dried (55 °C) and sieved (≤2 mm) waste and manure products. All extractions were conducted in duplicate, with three blanks for each extraction method, in end-over-end shakers except for FeOH extraction, which was conducted in a horizontal shaker. All extracts except FeOH were filtered through Whatman blue ribbon filters (589/3), pore size 2 µm. After appropriate dilution of extracts with deionised H_2O, ortho-P was

Table 3 Description of chemical extraction methods applied to waste and manure products

Code	Method	Time (h)	Ratio (g mL^{-1})	References
H_2O	Extraction with deionised H_2O	1	1:20 1:50 1:100 1:200	
FeOH	Extraction with 0.005 M $CaCl_2$ by simultaneous adsorption to 5 (2 by 10 cm) iron-oxide impregnated filter papers (0.6 M $FeCl_3$). Extraction of P adsorbed to filter papers in 40 mL 0.2 M H_2SO_4 for 4 h and 2 × washing of filter papers with 20 mL	24	1:80	Kuo (1994)
Olsen P	Extraction with 0.5 M $NaHCO_3$ adjusted to pH 8.5. Active coal was added to each sample before extraction to reduce background colour in the extract	0.5	1.5:30	Olsen et al. (1954)
AA 7	Extraction with 1 M ammonium acetate adjusted to pH 7	1.5	1:20	Adapted according to Sumner and Miller (1996)
AA 4.65	Extraction with 1 M acetic acid and 0.5 M ammonia adjusted to pH 4.65	1.5	1:20	Adapted according to Vuorinen and Mäkitie (1995)
AL	Extraction with 0.1 M ammonium lactate and 0.4 M acetic acid adjusted to pH 3.75	1.5	1:20 1:50 1:100 1:200	Egnér et al. (1960)
CA	Extraction with 2 % citric acid	0.5	2:200	European Union (2003)
AC	Extraction at 65 °C with ammonium citrate adjusted to pH 7	1	1:100	European Union (2003)

Table 4 Chemical properties of the model soil used in the bioassay

pH	P-AL (mg P 100 g^{-1})	Olsen P (mg P kg^{-1})	K-AL (mg P 100 g^{-1})	Mg-AL (mg P 100 g^{-1})	Ca-AL (mg P 100 g^{-1})
5.2–5.3	1.1–1.2	4.5–4.8	1.0–1.1	1.8–1.9	11

AL = extraction with 0.1 M ammonium lactate and 0.4 M acetic acid adjusted to pH 3.75 (Egnér et al. 1960) and analysis on ICP-OES, Olsen P = extraction with 0.5 M $NaHCO_3$ adjusted to pH 8.5 (Olsen et al. 1954) and colorimetric analysis (Murphy and Riley 1962)

determined by colorimetric analysis by the molybdenum blue method according to Murphy and Riley (1962). In addition, H_2O and AL extractions were applied at three modifications to study the effect of increasing sample:solution ratio on extractable P (1:50, 1:100 and 1:200) when standard soil extraction methods are applied to waste products. Increasing sample:solution ratio of AL was also applied to a soil (25 % clay, 40 % silt and 35 % sand) with a low content of plant-available P. Ortho-P was determined as described, and pH was measured in AL extracts.

Bioassay experiment

Readily-available and late-season P fertilisation effects of waste and manure products at two soil pH levels were determined by a bioassay, which was conducted in 5-L pots filled with a nutrient-deficient 8:2 (v/v) blend of sand and sphagnum peat (6.37 kg pot^{-1}). This model soil was chosen to avoid P fertilisation effects of fertiliser treatments being masked by soil P. Table 4 shows selected chemical properties of the model soil, which was limed to pH levels 1 and 2 (approximately pH 5.5 and 6.9) by mixing 1.5 and 7.5 g $CaCO_3$ pot^{-1}, respectively, into the soil volume.

Waste product fertilisation rates were calculated based on total P content. Our intention was to apply 75 mg P pot^{-1}, equivalent to 30 kg P ha^{-1} (assuming 20 cm topsoil depth), but actual fertilisation rates deviated slightly from this (75–88 mg P pot^{-1}), as described in Part I of this study (Brod et al. 2015). The fertilisation effects of waste products were compared with those of dairy and chicken manure, for which fertilisation rates were calculated as for waste products, with a treatment receiving no P fertilisation (NoP) and a mineral control treatment (MinP) that received $Ca(H_2PO_4)_2$ at a rate of 75 mg P pot^{-1}. To study the response of ryegrass to P fertilisation on the model soil, $Ca(H_2PO_4)_2$ was also applied at a rate of 37.5 and 112.5 mg P pot^{-1}, equivalent to 15 and 45 kg P ha^{-1}, respectively. All other nutrients were applied in sufficient amounts. Mineral N in waste products was assumed to become plant-available during the growing season. Organic N was assumed to be immediately available at a rate of 30 % for dairy manure, chicken manure, solid and liquid digestate, 80 % for meat bone meal and fish sludge and 50 % for catering waste (Jeng et al. 2004; Bioforsk 2014; Brod et al. 2014). The remaining N to give 500 mg N pot^{-1} in total was applied as $Ca(NO_3)_2$. In addition, 500 mg K pot^{-1} was applied as K_2SO_4 and 62.5 mg Mg pot^{-1} was applied as $MgSO_4$ together with Fe, Mn, Cu, Mo, B and Zn. After each harvest, 250 mg N and 250 mg K pot^{-1} were applied as $Ca(NO_3)_2$ and K_2SO_4, respectively. Due to a calculation error, the solid digestate treatments initially received too little mineral N fertiliser and an extra 350 mg N were applied 12 days before harvest 1. There were three replicates per treatment at each pH level studied.

Waste and manure products, lime and additional mineral fertiliser were applied 5 days before sowing in May 2013. Waste products were applied after drying (55 °C) and sieving ($\leq$2 mm). Only liquid and solid digestate and dairy manure were applied without previous drying. Ryegrass (*Lolium multiflorum* var. Macho) was sown at a rate of 0.5 g seeds pot^{-1}. The pots were placed side by side on a table outdoors under a transparent glass roof, where the plants were protected from precipitation but otherwise exposed to daylight and outdoor climate. Due to wind protection provided by the north and east side of the roof, the mean temperature was somewhat higher than the mean outdoor temperature in the area, which was lowest in September (11.1 °C) and highest in July (17.5 °C) (Meteorologisk Institutt 2013). The pots were watered based on weight to 60 % of water holding capacity every day, with differences in biomass production between treatments assumed to be negligible in relation to total pot weight. Pot positions were randomised twice a week. Six and 11 days after harvest 3, all plants were successfully treated with Thiacloprid (Bayer) against aphids (*Acyrthosiphon pisum*). Aboveground biomass was harvested at a height of 5 cm above the soil surface four times throughout the season, 6 weeks after sowing and then every 4 weeks. Harvested biomass was dried at 55 °C before weighing and milling. Total N concentration in the material was determined by the Dumas method (EN 13654-2 2001). Concentrations of all other nutrients in plant biomass were determined by the same method as described for the waste products. Plant P uptake (mg pot^{-1}) was computed by multiplying concentration by aboveground yield for each replicate.

To compare the fertilisation effects of waste products with those of MinP, RAE was calculated. RAE is defined as the relative P fertilisation effect of waste products compared with MinP, measured as P uptake in aboveground biomass. RAE was calculated separately for harvest 1 (readily-available P) and for the sum (Σ) of harvest 2, 3 and 4 (late-season P fertilisation effects) as

$$\mathrm{RAE} = 100 \times \frac{X_1}{\mathrm{P\ applied}} \tag{1}$$

$$X_1 = \frac{(Y_1 - b)}{a} \tag{2}$$

Where for harvest 1: P applied = Amount of P applied with waste product (mg P pot^{-1}). X_1 = Amount of MinP (mg P pot^{-1}) to which P uptake in aboveground biomass after fertilisation with the waste product is equivalent. Y_1 = P uptake (mg pot^{-1}) at harvest 1 obtained after application of waste product. a and b = slope and intercept obtained from linear regression with Y = P uptake (mg pot^{-1}) at harvest 1 obtained after application of MinP and X = P application rate of MinP (0, 37.5, 75 and 112.5 mg P pot^{-1}). For the parameters a and b and an example of calculation of RAE, see Fig. 1a. And where for Σ harvest 2, 3 and 4: P applied = Amount of P applied with waste product (mg P pot^{-1}) minus P uptake at harvest 1. Y_1 = P uptake (mg pot^{-1}) as Σ harvest 2, 3

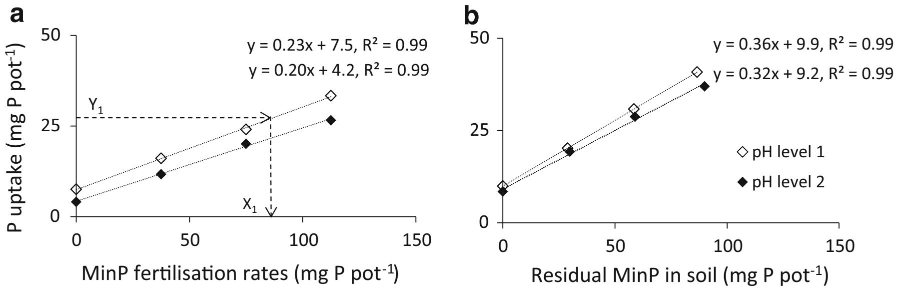

Fig. 1 P uptake in aboveground biomass as an effect of increasing MinP fertilisation rates (0, 37.5, 75 and 112.5 mg P pot^{-1}) at **a** Harvest 1 and **b** Σ Harvest 2, 3 and 4. Example: $Y_1 = 27$ mg P pot^{-1} and $X_1 = 85$ mg P pot^{-1} are the values used for the calculation of RAE at harvest 1 = 111 % of replicate 1 of dairy manure at pH level 1

and 4 obtained after application of waste product. a and b = slope and intercept obtained from linear regression with Y = P uptake (mg pot^{-1}) as Σ harvest 2, 3 and 4 obtained after application of MinP and X = P application rate of MinP (0, 37.5, 75 and 112.5 mg P pot^{-1}) minus P uptake at harvest 1 (see Fig. 1b).

Data analysis

One-way analysis of variance (ANOVA) was conducted to study the effect of extraction methods on extractable P, pH in the extraction solution and the effect of waste products on RAE for each soil pH level in the bioassay. RAE was also analysed by two-way ANOVA to study the effect of the factors fertiliser, soil pH level and their interaction. To perform multiple comparisons, Tukey's honestly significant difference (HSD) test ($\alpha = 0.05$) was used. To study the ability of extraction methods to predict readily-available and late-season fertilisation effects of waste products, simple linear regression with Y = RAE at harvest 1 and at Σ harvest 2, 3 and 4 at each soil pH level and X = extractable P as % of total P (mean of duplicates) was applied. Furthermore, two-way ANOVA with the factors extractable P (mean of duplicates), time point (RAE 1 = 1, RAE as Σ harvest 2, 3 and 4 = 0) and their interaction was used to study whether the slope and intercept of simple linear regression lines differed.

Results

Extractable P in waste and manure products

There were large variations in the amount of P extracted from waste and manure products by the eight different extraction methods (Tables 5, 6). For example, 0–14.8 % of total P was soluble in H_2O, while 45.4–80.4 % of total P was extracted by AC. Most P was extracted from dairy manure or fish sludge, whereas there was no clear trend in which material released least P when all extraction methods were taken into account.

Increasing the sample:solution ratio from 1:20 to 1:200 increased the amount of P extracted by H_2O from all waste and manure products except wood ash, from which no P was extracted by H_2O (Table 6). Moreover, the amount of P extracted from waste and manure products by AL increased with increasing sample:solution ratio. This effect was most pronounced for wood ash, where 4.9 % of total P was extracted at the ratio 1:20 and 89.3 % of total P was extracted at the ratio 1:200. Increasing the sample:solution ratio of AL also increased the amount of P extracted from the soil sample.

The AL solution was buffered to pH 3.75. The pH in the AL extract of the soil sample was 3.81 at the sample:solution ratio 1:20, whereas it was 4.05–4.48 for all waste and manure products except wood ash (Table 7). For wood ash, the pH in the extract was 6.33 at ratio 1:20. Increasing the sample:solution ratio had no effect on pH in the AL extract when the soil was extracted. When the waste products were extracted, increasing sample:solution ratio always decreased the pH in the AL extract. This effect was strongest for wood ash. At ratio 1:200, pH 4.11 in the extract from wood ash was significantly lower than pH 4.39 at ratio 1:100 but still significantly higher compared with all other materials.

RAE of waste and manure products

There was a clear response of ryegrass to P application on the model soil and P uptake in aboveground

Table 5 Extractable P in waste and manure products expressed as % of total P

Waste/manure product	FeOH	Olsen P	AAc 7	AAc 4.65	CA	AC
Wood ash	0.1	13.2	0.2	15.8	77.9	76.5
Cereal ash	5.5	10.2	4.2	41.8	51.7	59.7
Meat bone meal	1.8	1.9	1.5	17.2	69.7	72.7
Fish sludge	8.4	6.0	6.9	34.3	77.6	80.4
Catering waste	2.2	1.4	2.5	28.3	49.4	50.8
Liquid digestate	6.6	5.3	5.5	15.5	46.9	70.3
Solid digestate	2.5	3.5	1.4	14.1	50.1	74.0
Dairy manure	19.8	25.2	18.2	62.2	66.3	74.9
Chicken manure	6.9	6.7	5.4	33.5	43.2	45.4
SEM	0.2	0.4	0.1	0.8	0.7	1.6
HSD	0.9	2.2	0.3	4.7	4.1	9.1

SEM is the pooled standard error of the mean, HSD the honestly significant difference with $p < 0.05$ between waste and manure products according Tukey's test after one-way ANOVA. For H_2O and AL see Table 6. Abbreviations of the extraction methods are explained in Table 3

Table 6 P extracted by H_2O or AL in waste and manure products and the soil as effect of increasing sample:solution ratio (1:20, 1:50, 1:100 and 1:200) expressed as % of total P

Waste/manure product	H_2O					AL				
	1:20	1:50	1:100	1:200	HSD	1:20	1:50	1:100	1:200	HSD
Wood ash	0.0	0.0	0.0	0.0	0.0	4.9	50.9	71.3	89.3	4.0
Cereal ash	4.4	8.2	9.7	12.1	2.6	45.2	51.9	56.4	53.7	4.9
Meat bone meal	1.8	2.5	2.4	3.7	0.6	31.6	54.7	69.0	71.6	6.6
Fish sludge	9.0	11.5	11.5	18.9	0.6	50.2	69.2	75.8	77.0	4.6
Catering waste	2.8	3.7	4.1	4.7	0.2	36.3	48.6	51.3	54.1	7.7
Liquid digestate	4.1	5.0	5.8	10.3	0.4	21.6	34.9	41.1	52.5	6.1
Solid digestate	0.5	0.9	1.2	2.5	0.1	19.9	38.1	45.6	55.5	6.2
Dairy manure	14.8	24.0	30.6	42.1	2.6	53.8	67.0	64.5	70.9	4.0
Chicken manure	9.3	14.0	15.1	16.0	1.4	33.8	42.3	43.5	43.1	2.6
SEM	0.1	0.2	0.4	0.2		0.5	0.9	0.7	1.4	
HSD	0.3	1.0	2.2	0.9		2.5	5.1	4.0	7.9	
Soil (mg P 100 g^{-1})						3.5	5.1	7.4	9.9	0.8

SEM is the pooled standard error of the mean, HSD is the honestly significant difference with $p < 0.05$ between waste and manure products and between sample: solution ratios according Tukey's test after one-way ANOVA. Abbreviations of the extraction methods are explained in Table 3

biomass was a linear function of increasing MinP application rates (0, 37.5, 75 and 112.5 mg P pot^{-1}; Fig. 1). Thus, by calculating RAE, slightly variable P application rates of waste and manure products could be accounted for. For biomass production as a result of fertiliser treatments, see Part I of this study (Brod et al. 2015).

At pH level 1, the RAE of fish sludge was at the same level as that of dairy manure at both harvest 1 and Σ harvest 2, 3 and 4 (Table 8). At harvest 1, all other waste products including chicken manure resulted in significantly lower RAE than dairy manure. Catering waste resulted in the lowest RAE, at the same level as solid digestate. At Σ harvest 2, 3 and 4, all waste products including chicken manure resulted in significantly lower RAE than dairy manure and fish sludge. At Σ harvest 2, 3 and 4, catering waste again resulted in the lowest RAE, at the same level as solid digestate and chicken manure. For wood ash, RAE at Σ harvest 2, 3 and 4 was clearly higher than RAE at harvest 1.

At pH level 2, both RAE at harvest 1 and RAE at Σ harvest 2, 3 and 4 were generally lower than at pH level 1. At harvest 1, meat bone meal and fish sludge resulted

Table 7 pH in AL-extract after extraction of P in waste and manure products and the soil at increasing sample:solution ratio (1:20, 1:50, 1:100 and 1:200)

Waste/manure product	1:20	1:50	1:100	1:200	HSD
Wood ash	6.33	4.78	4.39	4.11	0.25
Cereal ash	4.28	3.98	3.92	3.83	0.06
Meat bone meal	4.25	3.99	4.03	3.85	0.20
Fish sludge	4.05	3.87	3.90	3.83	0.08
Catering waste[a]	4.11	3.89	3.91	3.83	
Liquid digestate	4.34	3.98	4.04	3.85	0.19
Solid digestate	4.38	4.00	3.94	3.84	0.01
Dairy manure	4.08	3.86	3.88	3.81	0.03
Chicken manure	4.24	3.99	3.99	3.83	0.12
SEM	0.03	0.02	0.03	0.01	
HSD	0.16	0.08	0.16	0.04	
Soil[a]	3.81	3.81	3.81	3.81	

SEM is the pooled standard error of the mean, HSD is the honestly significant difference with $p < 0.05$ between waste and manure products and increasing sample: solution ratios according Tukey's test after one-way ANOVA

[a] ANOVA was not possible due to lack of differences within replicates

in significantly lower RAE at pH level 2 than at pH level 1. At Σ harvest 2, 3 and 4, meat bone meal, fish sludge and catering waste resulted in significantly lower RAE and dairy manure in significantly higher RAE at pH level 2 than at pH level 1. At pH level 2, all waste products resulted in significantly lower RAE than dairy manure, both at harvest 1 and at Σ harvest 2, 3 and 4. At harvest 1, chicken manure, liquid digestate, wood ash and cereal ash were the waste products with the highest RAE. At Σ harvest 2, 3 and 4, wood ash and liquid digestate were the waste products with the highest RAE.

As described in Part I of this study (Brod et al. 2015), observed fertilisation effects were ascribed to P supplied by the different treatments rather than N because the N:P ratios in plant biomass (g kg^{-1}) were ≥9 (Liebisch et al. 2013) and growth limitation by other nutrients was excluded (Bergmann 1993).

Prediction of RAE of waste products by extractable P

For prediction of RAE by chemical extraction, only waste products were considered. When predicting RAE (Y) of the waste products by extractable P (X), separating readily-available P (RAE at harvest 1) and late-season P fertilisation effects (RAE at Σ harvest 2, 3 and 4), as well as splitting the dataset in the two soil pH levels, resulted overall in better model fits than when the whole dataset was included in the regression analyses (results not shown).

At pH level 1 and harvest 1, there were significant positive relationships between RAE and H_2O (1:20, 1:50 and 1:200), FeOH, AA7 and AC (Table 9). FeOH resulted in the best fit and explained 56 % of the

Table 8 Relative agronomic efficiency (RAE) expressed as % for harvest 1 and Σ harvest 2, 3 and 4 at pH levels 1 and 2

Waste/manure product	Harvest 1	Σ Harvest 2, 3 and 4
pH level 1		
Wood ash	51de	85b
Cereal ash	76bc	73bc
Meat bone meal	67cd	71c
Fish sludge	91ab	99a
Catering waste	28f	53d
Liquid digestate	81bc	75bc
Solid digestate	45ef	66cd
Dairy manure	111a	100a
Chicken manure	67cd	62cd
SEM	4	3
HSD	22	13
pH level 2		
Wood ash	70b	93b
Cereal ash	62bc	68cd
Meat bone meal	11d	9e
Fish sludge	47c	59d
Catering waste	12d	17e
Liquid digestate	74b	78bc
Solid digestate	47c	60d
Dairy manure	127a	121a
Chicken manure	72b	77c
SEM	3	3
HSD	17	16
2-Way ANOVA, source of variation		
Fertiliser	***	***
pH level	***	***
Fertiliser × pH level	***	***

SEM is the pooled standard error of the means, HSD is the honestly significant difference with $p < 0.05$ between treatments according Tukey's test after one-way ANOVA and letters indicate significant differences between treatments

*, **, *** Represent significance at $p < 0.05$, 0.01 and 0.001 probability level, respectively

variation in RAE at harvest 1 (Fig. 2a). For Σ harvest 2, 3 and 4, there were significant positive relationships between RAE and all extractions except H_2O (1:100), AA 4.65 and AL (1:20). AC resulted in the best fit and explained 56 % of the variation in RAE for Σ harvest 2, 3 and 4 (Fig. 2b). At pH level 2, the only significant positive relationship was between RAE and Olsen P, both at harvest 1 and at Σ harvest 2, 3 and 4 (Table 9). Olsen P explained 57 % of the variation in RAE at harvest 1 and 66 % of the variation in RAE at Σ harvest 2, 3 and 4. There were no significant differences between the slope and intercept of the simple linear regression lines at the two time points RAE 1 and RAE for Σ harvest 2, 3 and 4. Therefore, in Fig. 2c only the simple linear regression line with X = Olsen P extractable P and Y = RAE as sum of all harvests is presented.

Increasing the sample:solution ratio did not influence the ability of H_2O to predict RAE at harvest 1 or at Σ harvest 2, 3 and 4 at any pH level. However, increasing sample:solution ratio of AL changed the ranking of waste products in terms of extractable P and therefore influenced the prediction ability of AL (Tables 6, 9).

Discussion

Prediction of RAE by chemical extraction

The results of this study suggest that chemical extraction of new waste products with unknown P fertilisation effects can potentially predict their RAE compared with mineral P fertiliser. Figure 2 shows the simple linear regression models that gave the best fits for X = extractable P and Y = RAE during this study. We identified two requirements for the RAE prediction by chemical extraction: (1) Distinguishing readily-available P and late-season fertilisation effects of waste products and (2) considering the pH in the soil to be fertilised.

Readily-available P and late-season fertilisation effects of waste products

Only at pH level 1, distinguishing between readily-available P and late-season fertilisation effects improved the ability of chemical extraction methods to predict P fertilisation effects of waste products.

Table 9 Results of simple linear regression analyses with Y = RAE at harvest 1 or as Σ harvest 2, 3 and 4 and X = extractable P (% of total P)

	Harvest 1		Σ Harvest 2, 3 and 4	
	R^2	*p* value	R^2	*p* value
pH level 1				
H_2O 1:20	0.45	***	0.28	*
H_2O 1:50	0.45	***	0.22	*
H_2O 1:100	0.44	**	0.17	0.06
H_2O 1:200	0.55	***	0.27	*
FeOH	0.56	***	0.21	*
Olsen P	0.05	0.31	0.27	*
AA7	0.50	***	0.19	*
AA4.65	0.09	0.19	0.02	0.50
AL 1:20	0.16	0.08	0.01	0.64
AL 1:50	0.12	0.12	0.33	**
AL 1:100	0.07	0.24	0.40	**
AL 1:200	0.02	0.59	0.42	**
CA	0.07	0.25	0.50	***
AC	0.23	*	0.56	***
pH level 2				
H_2O 1:20	0.01	0.76	0.00	0.97
H_2O 1:50	0.01	0.66	0.00	0.91
H_2O 1:100	0.02	0.53	0.00	0.81
H_2O 1:200	0.04	0.38	0.01	0.63
FeOH	0.09	0.18	0.04	0.40
Olsen P	0.57	***	0.66	***
AA7	0.06	0.29	0.02	0.56
AA4.65	0.00[a]	0.87	0.01[a]	0.76
AL 1:20	0.12[a]	0.12	0.18[a]	0.06
AL 1:50	0.07[a]	0.23	0.04[a]	0.36
AL 1:100	0.03[a]	0.49	0.00[a]	0.79
AL 1:200	0.01	0.71	0.05	0.34
CA	0.00[a]	0.93	0.01	0.69
AC	0.12	0.12	0.16	0.07

Dairy and chicken manure were not included in the analyses. Abbreviations of the extraction methods are explained in Table 3

*, **, *** Represent significance at $p < 0.05$, 0.01 and 0.001 probability level, respectively

[a] Negative relationship

In the first period after sowing and at pH level 1, the RAE of waste products was best predicted by FeOH. When root systems are still small, P uptake by plants relies to a great extent on diffusion in addition to mass

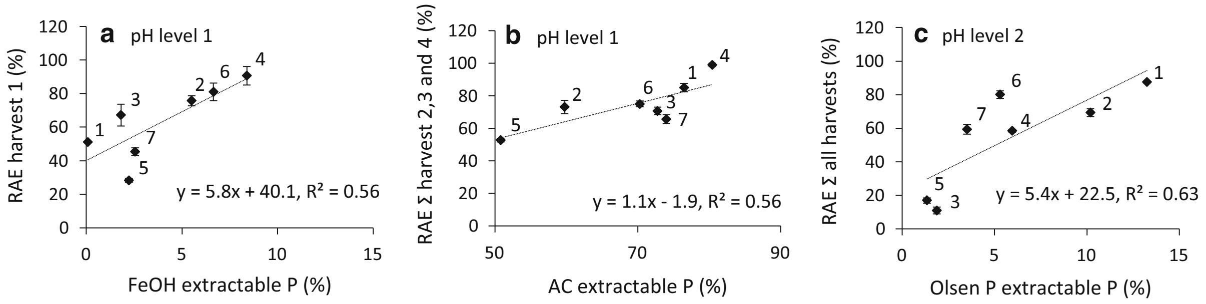

Fig. 2 Distribution of waste products on simple linear regression lines with the best fit for **a** Harvest 1 at soil pH level 1, **b** Σ Harvest 2, 3 and 4 at soil pH level 1 and **c** Σ All harvests at soil pH level 2, where 1 = Wood ash, 2 = Cereal ash, 3 = Meat bone meal, 4 = Fish sludge, 5 = Catering waste, 6 = Liquid digestate and 7 = Solid digestate. Abbreviations of the extraction methods are explained in Table 3

flow to the roots. The higher the soil solution P concentration, the more P will reach the roots by these processes. The fraction of readily-available P in waste products indicates the product's ability to increase soil solution P concentration and to replenish the P concentration after plant P uptake. This is measured by the FeOH method, which was developed with the aim of imitating equilibrium processes in soils following plant P uptake (Kuo 1994). Iron-oxide impregnated filter papers act as a sink, adsorbing P extracted with 0.005 M $CaCl_2$. The P concentration in the extraction solution is thus kept low, resulting in continuous release of readily-available P from the sample. Good relationships between weak extraction methods and P fertilisation effects of waste products in the first period after sowing are in accordance with previous findings by Velthof et al. (1998) and Kratz et al. (2010). From wood ash, however, almost no P was extracted by FeOH despite its clear fertilisation effect. This low solubility of wood ash P in FeOH might have resulted from the highly alkaline waste product causing a greater increase in pH in the extraction solution compared with the soil pH increase one would expect after application to soil. Implications of increased pH in the extraction solution on extractability are discussed below.

Later during the season, strong desorption agents correlated best with P fertilisation effects at pH level 1. When plant roots are well developed, in addition to diffusion and mass flow, P availability in soil is influenced by root exudates such as low molecular weight organic acids and protons which mobilise P (Hinsinger 2001). These processes seem to be best reflected by strong desorption agents. AC and CA resulted in the best correlation with RAE at Σ harvest 2, 3 and 4 at pH level 1 because they were the methods that extracted most P from wood ash and fish sludge, the waste products with the best late-season P fertilisation effects. AC predicted late-season fertilisation effects better than CA because it extracted more P from liquid and solid digestate. In Part I of this study we found that liquid and solid digestate were in addition to fish sludge the only waste products that contained noteworthy amounts of NaOH-soluble P, which is defined as Fe-/Al-bound P (Brod et al. 2015). Braithwaite (1987) concluded that AC extracts more Fe-/Al phosphates than CA in single superphosphate. Therefore, we recommend extracting P in waste products with AC to estimate late-season fertilisation effects in acid soils.

Effect of pH in the target soil

Our results also indicate that the best prediction of RAE of waste products will be given by different chemical extraction methods depending on the pH in the target soil. This is because the P fertilisation effects of some waste products are strongly dependent on soil pH, while the effects of others are independent of soil pH. Meat bone meal, fish sludge and catering waste resulted in significantly lower RAE at pH level 2 than at pH level 1. The main P compound in meat bone meal and catering waste was found to be stable Ca phosphates with a molar Ca:P ratio >1, mainly hydroxy- or chlor-fluorapatite. Fish sludge also contained stable Ca phosphates (Brod et al. 2015). Olsen P, which is a quite weak extraction, was the only extraction method that significantly correlated with

RAE at pH level 2, because it extracted less P from meat bone meal and catering waste than the other extraction methods. Olsen P was buffered at pH 8.5, where the solubility of stable Ca phosphates is strongly reduced (Lindsay 1979). Olsen P extracted more P from wood ash, where less P was identified as being present as stable Ca phosphates, than from meat bone meal and catering waste (Brod et al. 2015), as also reflected by better availability to ryegrass in the bioassay. At pH level 2 and harvest 1, all other extraction methods than Olsen P, including FeOH, probably extracted relatively too much P from stable Ca phosphates and therefore overestimated the RAE of meat bone meal and catering waste, while they underestimated the RAE of wood ash. Our results indicate that at pH level 2, the solubility of stable Ca phosphates in the whole soil volume was of greater importance than rhizosphere effects as reflected by AC for RAE at Σ harvest 2, 3 and 4 at pH level 1.

In the bioassay, we included only two pH levels and therefore we cannot deduce the RAE of waste products containing large fractions of stable Ca phosphates at other soil pH levels. With reference to Lindsay (1979), the decrease in RAE with increasing soil pH can be assumed to be log-linear, but we cannot specify the pH level at which to switch from extraction with FeOH and AC to Olsen P.

Methodological limitations

Applicability of standard soil extraction methods to waste products

We included modifications of H_2O and AL to study the applicability of standard soil extraction methods to waste products. Chemical extraction methods for soils were originally developed for agricultural soils with total P contents of 0.2–5 g P kg^{-1} and with an average of 0.6 g P kg^{-1} (Lindsay 1979). The waste products in the present study contained 6–54 g P kg^{-1}. The pH in some waste products was also considerably higher than expected for agricultural soils.

Increasing sample:solution ratios of H_2O resulted in significantly increased fractions of extractable P in the waste products (Table 6), as described previously for soils and explained by equilibrium reactions (Øgaard 1995). However, increasing sample:solution ratio had no effect on the prediction ability of H_2O, because the ranking between the different waste products was not changed.

Increasing sample:solution ratio of AL also resulted in significantly increased fractions of extractable P in the waste products and in the soil sample, which again can partly be explained by equilibrium reactions. However, for AL, increasing sample:solution ratio considerably influenced the prediction ability of AL because the ranking of the waste products was changed. The main explanation for the varying prediction ability of AL depending on sample:solution ratio was probably the varying effect of the waste products on the pH of the extraction solution. The increase in extraction solution pH was most pronounced for wood ash at a sample:solution ratio of 1:20, and it was less at higher sample:solution ratios for all waste products. Similar results have been found in extraction of sewage sludges, which raised the pH in AL (1:20) to 5.8 (unpublished data by Øgaard). For the soil sample, in contrast, increasing soil:sample ratio had no effect on the pH in the AL extract. In Part I of this study (Brod et al. 2015), we showed that inorganic P in the waste products was mainly present as different Ca phosphates. The solubility of Ca phosphates, and hence their extractability, decreases with increasing pH (Lindsay 1979). Similarly, Bøen (2013) found that increasing pH in AL before extracting meat bone meal significantly reduced P extractability. Therefore, the increase in extracted P at wider sample:solution ratios of AL was most pronounced for wood ash. Wood ash was the waste product with the highest RAE at Σ harvest 2, 3 and 4 in addition to fish sludge at pH level 1. Thus, there were no significant relationships between AL (1:20) and RAE at any pH level or time point, but increasing sample:solution ratio improved the prediction of RAE for Σ harvest 2, 3 and 4 at pH level 1. As in earlier studies (Brod et al. 2014), we conclude that the Norwegian standard soil test (AL) is not suited for prediction of the P fertilisation effects of waste products.

Practical applicability and further development of prediction models

This study provided an indication of which extraction methods are most suitable for predicting the P fertilisation effects of waste products. However, the models presented in Fig. 2 still need validation before they can be used to establish reliable fertilisation plans with waste products. For example, the RAE of the studied waste products should be confirmed after application to different soil types and arable crops, as well as under field conditions. Furthermore, the applicability of the models to unstudied waste products should be tested.

In agriculture, many waste products will commonly be applied as N fertiliser, often resulting in considerable overapplication of P due to low N:P ratio compared with crop demands (Table 2). Within a crop rotation, waste products may still provide sufficient P for the following crops if additional N is applied. The present experiment did not allow us to study long-term P fertilisation effects of waste products after harvest 4, which should also be considered in further development of prediction models.

Conclusions

Based on systematic evaluation of eight extraction methods and a bioassay, we suggest that chemical extraction of phosphorus (P) in waste products can be used to predict their relative agronomic efficiency compared with mineral P fertiliser. The optimum chemical extraction method depends on two conditions: (1) whether readily-available P or late-season fertilisation effects are of interest and (2) the pH in the target soil. The simple linear regression lines with the best model fits are presented here, but will have to be validated before they can be used to establish reliable fertilisation plans for P-rich waste products.

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PAPER V

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Drivers of phosphorus uptake by barley following secondary resource application

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Abstract

Minable rock phosphate is a finite resource. Replacing mineral phosphorus (P) fertilizer with P-rich secondary resources is one way to manage P more efficiently, but the importance of physicochemical and microbial soil processes induced by secondary resources for plant P uptake are still poorly understood. Using radioactive labelling techniques, the fertilization effects of dairy manure, fish sludge, meat bone meal and wood ash were studied as P uptake by barley after 44 days and compared with those of water-soluble mineral P (MinP) and an unfertilized control (NoP) in a pot experiment with an agricultural soil containing little available P at two soil pH levels, approximately pH 5.3 (unlimed soil) and pH 6.2 (limed soil). In a parallel incubation experiment, the effects of the secondary resources on physicochemical and microbial soil processes were studied. The results showed that the relative agronomic efficiency compared with MinP decreased in the order: manure ≥ fish sludge ≥ wood ash ≥ meat bone meal. The solubility of inorganic P in secondary resources was the main driver for P uptake by barley (*Hordeum vulgare*). The effects of secondary resources on physicochemical and microbial soil processes were of little overall importance. Application of organic carbon with manure resulted in microbial P immobilisation and decreased uptake by barley of P derived from the soil. On both soils, P uptake by barley was best explained by a positive linear relationship with the H_2O + $NaHCO_3$-soluble inorganic P fraction in fertilizers, or by a linear negative relationship with the HCl-soluble inorganic P fraction in fertilizers.

1 Introduction

Minable rock phosphate is a finite resource. However, industrialized agroecosystems are today far from managing phosphorus (P) efficiently and Europe's food production is largely dependent on imports of mined rock phosphate (van Dijk *et al.*, 2015). The greatest reductions in P imports could be achieved by replacing mineral fertilizer with recycled P from secondary resources (Schoumans *et al.*, 2015). In food systems, the accumulated P content in secondary resources is often of the same order of magnitude as that in mineral fertilizer, as shown for Europe (Withers *et al.*, 2015). In Norway, the total amount of P in secondary resources (27,700 Mg P yr^{-1}) actually greatly exceeds the amount of P applied to soil with mineral fertilizer (8400 Mg P yr^{-1}) and the amount of P removed by crops (11,000 Mg P yr^{-1}) (Hamilton *et al.*, 2015). The Norwegian secondary resources containing the largest amounts of P are manure (11,000 Mg P yr^{-1}), fish excrement and feed losses from salmon and trout farming in open cages in fjords (fish sludge, 9000 Mg P yr^{-1}), meat bone meal (2100 Mg P yr^{-1}), and sewage (3100 Mg P yr^{-1}) (Hamilton *et al.*, 2015). Wood ash, a residue from bioenergy plants and industrial timber production, also contains considerable amounts of secondary P (800 Mg P yr^{-1}) (Norwegian Environment Agency, 2015).

The P recycling potential of secondary resources is determined by, among other parameters, the solubility of the P species they contain (Nanzer *et al.*, 2014b; Brod *et al.*, 2015a). Phosphorus in secondary resources is generally present as a complex mixture of inorganic P species, predominantly calcium (Ca) phosphates with differing solubility but also amorphous aluminium (Al)- or iron (Fe)- bound P, while organic P usually represents a small fraction (Hedley and McLaughlin, 2005). The P fertilization effects of secondary resources can be considerably affected by the pH in the target soil (Brod *et al.*, 2015a), as the solubility of Ca phosphates decreases with increasing soil pH, whereas the solubility of Al-/Fe-bound P decreases with decreasing soil pH (Lindsay, 1979). The best method for predicting the P fertilization effects of secondary resources also depends on the pH in the target soil (Brod *et al.*, 2015b). In a previous bioassay with ryegrass (*Lolium multiflorum*) grown in a sand-peat substrate containing little available P, the apparent P use efficiency of nine different secondary resources with predominantly Ca-bound P was best explained by a positive relationship with H_2O-soluble inorganic P in acid soil and a negative relationship with HCl-soluble inorganic P in a near-neutral soil (Brod *et al.*, 2015a).

Plant P uptake following secondary resource application can also be influenced by their effects on microbial or physicochemical processes in the soil. Many secondary resources contain organic matter, meaning that organic carbon (C) is applied to the soil when they are used as alternatives to mineral fertilizers (e.g. manure, fish sludge and meat bone meal). Organic C application can trigger microbial activity, which may result in immobilisation of soil P and fertilizer P (McLaughlin and Alston, 1986; Oberson and Joner, 2005) and in microbes competing with plants for available P. Microbial activity can also increase P availability by affecting physicochemical processes. Low molecular weight organic acids excreted by microorganisms during the breakdown of organic C have been shown to reduce phosphate retention on soil particles (Øgaard, 1996). Furthermore, these acids can solubilize P by complexing metal cations such as Al, Fe and Ca that associate with P in insoluble forms, or by decreasing soil pH (Jakobsen *et al.*, 2005). Other secondary resources contain inorganic C, for example in the form of calcium carbonate ($CaCO_3$) (e.g. wood ash), which neutralizes pH in acid soils. Moreover, the solubility of applied fertilizer P can affect physicochemical soil processes depending on equilibrium processes in the target soil, since increased phosphate concentration in the soil solution can in turn result in reduced phosphate release from the soil (Achat *et al.*, 2014a).

To date, the P fertilization effects of fish sludge, meat bone meal and wood ash have only been studied by the difference method (Erich, 1991; Jeng *et al.*, 2006; Ylivainio *et al.*, 2008; Brod *et al.*, 2015a). This method compares the P uptake by a plant fertilized with the secondary resource with the P uptake by a plant receiving no P fertilizer. The difference in P uptake between the two treatments is defined as the P fertilization effect of the secondary resource. The underlying assumption in the difference method is that unfertilized and fertilized plants take up the same amount of P from the soil, i.e. that the secondary resource does not affect soil P availability. However, the effects of secondary resources such as fish sludge, meat bone meal and wood ash on microbial and physicochemical soil P processes are still poorly understood and it is not known whether the difference method actually reflects the net P fertilization effects of these secondary resources. Understanding the effects of complex secondary resources on soil processes is therefore important for a holistic evaluation of their fertilization effects.

Using radioisotopes of P in growth and incubation experiments provides the possibility to study P processes in soil/plant systems. In growth experiments, labelling soil with radioisotopes of P before application of secondary resources is a way to differentiate P taken up by the plants deriving from the fertilizer and from the soil (Morel and Fardeau, 1989; Frossard *et al.*, 1996; Frossard *et al.*, 2011). In incubation experiments, isotopic dilution can be used to study the incorporation of fertilizer into different soil P pools (Nanzer *et al.*, 2014a), or to quantify the amount of isotopically exchangeable phosphate (E-value) as affected by fertilizer application (e.g. Frossard *et al.*, 1996; Achat *et al.*, 2014a).

The aim of this study was to determine the main drivers of plant P uptake following secondary resource application. To this end, the P fertilization effects of dairy manure (manure), fish sludge, meat bone meal and wood ash were compared with those of water-soluble mineral P fertilizer (MinP) in a pot experiment with barley (*Hordeum vulgare* var. Heder) by ^{33}P labelling of a loam soil at two soil pH levels (unlimed and limed). In parallel, soil incubation experiments were conducted to study the effects of the secondary resources on physicochemical and microbial soil processes and to explore their importance for plant P uptake.

2 Material and methods

2.1 Secondary resources

The secondary resources are described in Table 1, while Table 2 gives an overview of their selected chemical properties. The secondary resources were also analysed for heavy metal concentrations by ICP-OES after digestion in concentrated nitric acid in an ultraclave (EN ISO 11885, 2009) (results not shown). Based on their heavy metal concentrations and Norwegian regulations (Norwegian Ministry of Agriculture and Food, 2003), all the secondary resources studied here were eligible for application as fertilizer to agricultural land.

2.2 Experimental soil

The experimental soil originated from plots in a long-term field experiment in Norway (59°39'48.0"N 10°45'40.8"E) that has received 0 kg P yr^{-1} and 0 or 5 kg potassium (K) yr^{-1} since 1966. It is classified as an Albeluvisol in the World Reference Base for Soil Resources (NIBIO, 2015) and contains 27% clay, 40% silt and 33% sand. Selected chemical characteristics of the soil are presented in Table 3. This soil was chosen because of its low content of plant-available P (measured as ammonium lactate-extractable P (P-AL)), in order to avoid P fertilization effects being masked by soil P. Before the soil was sampled at the end of the growing season in November 2013, barley, wheat and oats were grown in rotation for 16 years, with the last year of grass production being in 1997. After harvest of the cereals, including the straw, the soil was usually ploughed in autumn. During sampling, random soil cores were taken from the 0-20 cm horizon in the middle of the plots. The soil was air-dried before sieving at mesh width 5 mm. To study the effect of soil pH on P uptake following secondary resource application, one part of the soil was limed with 2 g $CaCO_3$ kg^{-1} soil dry matter (DM). Then both the unlimed and the limed soils were incubated in portions of 15 kg at 60% of water-holding capacity (WHC, 100% WHC = 447 g H_2O kg^{-1} soil) for 2.5 months in the dark before drying at 40°C. After transportation to Switzerland, the soil was again sieved at mesh width 5 mm, carefully rewetted in portions of 1 kg soil DM and incubated at 40% of WHC for at least 3 weeks. The pre-incubation aimed at reaching constant microbial activity, in order to minimize a microbial boost during set-up of the experiment. When the experiments were set up, soil pH (measured in H_2O) was 5.3 and 6.2 in the unlimed and limed soil, respectively.

2.3 Pot experiment

The P fertilization effects of secondary resources were studied in a pot experiment using indirect labelling with ^{33}P (Frossard *et al.*, 2011). Pre-incubated portions of 1 kg soil DM were mixed with carrier-free ^{33}P-orthophosphate at a rate of 1.1 MBq kg^{-1} soil, which was added after dilution in H_2O by 10 mL kg^{-1} soil. The soil was transferred into pots with sealed bottoms and again incubated at 16-18°C for 10 days to reach near-equilibrium conditions for the pools of plant-available ^{31}P and ^{33}P in the soil. Pots containing the same amount of unlabelled soil were also mixed and kept under the same conditions. The fertilization effects of manure, fish sludge, meat bone meal and wood ash (all dried at 55°C and sieved at ≤ 2 mm) were compared with those of a treatment receiving no P fertilizer (NoP) and a treatment receiving water-soluble mineral P (MinP, $Ca(H_2PO_4)_2*H_2O$ in aqueous solution). For the purposes of methodological control, the fertilization effect of MinP was also studied using direct labelling (MinPdir). MinPdir was produced by labelling $Ca(H_2PO_4)_2*H_2O$ in aqueous solution with specific activity (SA) 40 kBq mg P^{-1} and applied corresponding to 1.2 MBq kg^{-1} soil. All fertilizers were applied based on a total P content equivalent to 30 mg P kg^{-1} and mixed into the whole soil volume. This P dose corresponded to 5.09 g manure kg^{-1} soil, 1.48 g fish sludge kg^{-1} soil, 0.57 g meat bone meal kg^{-1} soil and 1.76 g wood ash kg^{-1} soil. To study the response of the soil to P fertilization, unlabelled MinP was also applied at rates of 15 and 45 mg P kg^{-1} soil. At the same time, all pots received a P-free nutrient solution containing 75 mg N ($Ca(NO_3)_2*4H_2O$), 75 mg K (K_2SO_4), 15 mg magnesium (Mg; $MgSO_4*7H_2O$), 0.1 mg molybdenum (Mo; $Na_2MoO_4*2H_2O$), 1 mg zinc (Zn; $ZnSO_4*7H_2O$), 1 mg Fe (Fe-chelate), 1 mg boron (B; H_3BO_3), 2 mg copper (Cu; $CuSO_4*5H_2O$) and 2 mg manganese (Mn; $MnSO_4*H_2O$) per kg soil. There were four replicates per treatment. Seven barley seeds (*Hordeum vulgare*, var. Heder) were sown per pot and thinned out to five plants after germination. Seventeen days after set-up of the experiment, when plants had developed 3-4 leaves, all pots were also given 75 mg N and 209 mg K as KNO_3. All plants were watered with distilled water by weighing to 70% of WHC until germination and thereafter to 60% of WHC every two or three days and daily towards the end of the experiment. Growing conditions in the greenhouse were set to 16 hours photoperiod with artificial lights turning on when daylight <20 klx. Atmospheric humidity and mean temperature were set to 65% and 20°C during the day and 72% and 16°C at night. Pot positions were randomized three times a week. Forty-four days after set-up of the experiment, when the first awns were visible (development stage varying between Zadoks 35-50; Zadoks, 1974), aboveground biomass was harvested by cutting the plants with scissors at 2 cm above the soil surface. Plant material was dried at 55°C for 48 h, DM production per pot was recorded and the plant material was milled in a Retsch ZM 200 mill (≤0.2 mm). For determination of P concentration in the plant tissue, 250 mg were incinerated at 550°C for 3 h and extracted with 3 mL concentrated, hot HNO_3 (adapted according to Nanzer *et al.*, 2014a). The P in the diluted filtrate (0.2 µm pore size) was determined colorimetrically according to Ohno and Zibilske (1991). The P uptake per kg soil was computed by multiplying DM production by plant tissue P concentration. The ^{33}P beta emissions in the labelling solutions and the extracts were measured in 1 mL sample after addition of 5 mL appropriate scintillation liquid (PerkinElmer Ultima Gold or PerkinElmer Ultima Gold AB) by liquid scintillation counting (TRI-CARB 2500 TR, liquid scintillation analyser, Packard Instruments, Meriden, CT) and corrected for radioactive decay back to the day when the soil was labelled. The N concentration in plant tissue was determined using a Thermo Electron FlashEA 1112 Automatic Elemental Analyser. Soil samples were taken in each pot and soil pH was measured in a solid-solution-ratio of 1:2.5 (v/v) in H_2O after drying soil samples at 55°C and sieving at mesh width ≤ 2 mm.

2.4 Seed P experiment

An additional experiment was conducted to determine the contribution of barley seed P to P uptake in aboveground biomass in response to increasing fertilization rate when the indirect method was used (Pypers *et al.*, 2006; Nanzer *et al.*, 2014a). Sand (0.7-1.2 mm) was washed in 2% HCl before thorough rinsing with distilled H_2O. Afterwards, the pH of the sand was 4.97 (solid-solution-ratio of 1:2.5 (v/v) in H_2O). Portions of 1 kg sand DM were then fertilized with 0, 7.5, 15, 22.5 or 30 mg P kg^{-1} sand. The P fertilizer ($Ca(H_2PO_4)_2*H_2O$ in aqueous solution) was labelled with ^{33}P. The pots received 720 kBq kg^{-1} sand with the fertilizer, i.e. the specific activity of the P fertilizer was 96, 48, 32 and 24 kBq mg^{-1} P, respectively. The same P-free nutrient solution as given in the pot experiment was used. There were four replicates per treatment. Seven barley seeds were sown per pot and thinned out to five plants after germination. With five barley seeds, 0.71±0.08 mg P were applied per kg soil, as determined by the average weight of five barley seeds (0.20±0.02 g DM kg^{-1}, n = 20) and P concentration (3.49±0.04 mg P g^{-1} DM, determined by colorimetric analysis after microwave digestion in concentrated H_2O_2 and HNO_3, n = 4). During the first 19 days, the plants were watered up to 130 g H_2O kg^{-1} sand, after which the water ratio was increased to 220 g H_2O kg^{-1} sand. Plants were harvested by cutting with scissors at 2 cm above the sand surface 50 days after set-up of the experiment when the first awns were visible (Zadoks 35-49). Plant material was analysed in the same way as described for the pot experiment. During the seed P experiment, any isotopic dilution of the ^{33}P in the shoot was caused by seed P, since this was the only non-labelled source. This experiment therefore allowed the P contribution from the seed and that from the fertilizer to be distinguished.

2.5 Calculations for pot and seed P experiment

When labelled fertilizer was applied to the soil (direct method in pot study and seed P experiment), P derived from the fertilizer (Pdf fertilizer, mg P kg^{-1} soil) was calculated as:

$$\text{Pdf fertilizer} = \frac{\text{SA}_{\text{plant}}}{\text{SA}_{\text{fert}}} \times \text{P uptake}_{\text{P+}} \quad (1)$$

where SA_{plant} (Bq mg^{-1} P) is the specific activity in the plant amended with the labelled fertilizer, SA_{fert} (Bq mg^{-1} P) is the specific activity in the fertilizer and P $uptake_{P+}$ (mg P kg^{-1} soil) is the amount of P taken up by the fertilized plant in aboveground biomass. In the seed P experiment, P derived from the seed (Pdf seed) was calculated as the difference between P uptake and Pdf fertilizer.

When the pool of plant-available P in the soil was labelled before application of an unlabelled fertilizer (indirect method), Pdf fertilizer was calculated as:

$$\text{Pdf fertilizer} = \text{P uptake}_{\text{P+}} - \text{Pdf soil}_{\text{P+}} - \text{Pdf seed}_{\text{P+}} \tag{2}$$

where Pdf $\text{soil}_{\text{P+}}$ is the amount of P derived from the soil (mg P kg^{-1} soil) in the fertilized plant, which was calculated as:

$$\text{Pdf soil}_{\text{P+}} = \frac{\text{SA}_{\text{plant P+}}}{\text{SA}_{\text{plant NoP}}} \times (\text{P uptake}_{\text{P+}} - \text{Pdf seed}_{\text{P+}}) \tag{3}$$

where $\text{SA}_{\text{plant P+}}$ (Bq mg^{-1} P) is the specific activity in the fertilized plants, $\text{SA}_{\text{plant NoP}}$ (Bq mg^{-1} P) is the average specific activity in the plants receiving no P fertilizer with P uptake corrected for Pdf seed, and Pdf $\text{seed}_{\text{P+}}$ is P derived from the seed (mg P kg^{-1} soil) in the fertilized plants, which was calculated from the seed P experiment as follows:

$$\text{Pdf seed}_{\text{P+}} = \text{a} \times \text{P uptake}_{\text{P+}} + \text{b} \tag{4}$$

where a and b are the slope and intercept of the function presented in Figure 2.

Moreover, fertilizer recovery (%) was calculated as the ratio between Pdf fertilizer and the amount of P applied with the fertilizer (P fert, mg P kg^{-1}):

$$\text{Fertilizer recovery } = \frac{\text{Pdf fertilizer}}{\text{P fert}} \times 100 \tag{5}$$

Relative agronomic efficiency (RAE, %) was calculated as the ratio between the fertilizer recovery of each secondary resource (P+) and the fertilizer recovery of MinP applied at the same rate (30 mg P kg^{-1} soil) as the secondary resource:

$$\text{Relative agronomic efficiency } = \frac{\text{Fertilizer recovery}_{\text{P+}}}{\text{Fertilizer recovery}_{\text{MinP}}} \times 100 \tag{6}$$

2.6 Incubation experiment

2.6.1 Set-up of the incubation experiment

In parallel, soil-fertilizer incubations were conducted to study physicochemical and microbial soil processes affected by the secondary resources in comparison with MinP and NoP on the unlimed and limed soil. Again, the soil was pre-incubated with distilled H_2O at 40% of WHC to minimize a microbial boost during set-up of the experiments. Three weeks after pre-incubation, aliquots of 1.2 kg soil were mixed with ^{33}P corresponding to 5.2 MBq kg^{-1} soil that was added after dilution in H_2O by 10 mL kg^{-1} soil for an incubation experiment with soil P labelling (indirect method). As for the pot experiment, the soil was kept in a controlled environment to reach near-equilibrium conditions for ^{31}P and ^{33}P for 10 days. A soil-fertilizer incubation experiment with no soil or fertilizer P labelling (termed unlabelled incubation) was also set up to determine isotopically exchangeable P as affected by P fertilizer (method described below). During the set-up of both incubations, aliquots of 300 g soil were watered to 50% of WHC and mixed with fertilizer corresponding to 30 mg P kg^{-1} soil, but in contrast to the pot experiment, no P-free nutrient solution was added. Sub-aliquots of 100 g soil corresponding to three time points were placed in sealed plastic bags and kept in the dark under identical experimental conditions as in the pot experiment until analysis. There were four replicates per treatment. The effect of fertilizers on soil pH was studied after 7, 21 and 42 days in the incubation experiment with soil P labelling as described above.

2.6.2 P concentration in soil solution and isotopically exchangeable P

The effects of fertilizers on the P concentration in the soil solution (C_P, mg P L^{-1}) and isotopically exchangeable P (E_{1min}, in mg P kg^{-1}) (Fardeau *et al.*, 1996) were determined after 21 days in soil sampled in the unlabelled incubation experiment. The E_{1min} comprises the Pi in the soil solution and Pi adsorbed to soil particles, which is exchangeable within the first minute of isotopic exchange kinetics (Frossard *et al.*, 2011). Incubated soil samples were dried at 40°C before extraction of 10 g soil in 99 mL H_2O by end-over-end-shaking. Isotopic exchange kinetics analyses were carried out after 16 h of shaking based on the assumption that steady-state equilibrium was reached, i.e. that C_P was constant. The suspensions were then stirred at 300 rpm on a magnetic plate when 1 mL carrier-free ^{33}P solution with a known amount of radioactivity was added to result in R = 600-900 Bq mL^{-1} in the sample. The soil:solution ratio was then 1:10. The suspensions were sampled with plastic syringes at 1, 4, 10, 30, 60 and 90 min after ^{33}P addition (t). The remaining ^{33}P in the filtrate (r(t), 0.2 µm pore size) was determined using scintillation counting as described above. After the last sampling, C_P was determined colorimetrically (Ohno and Zibilske, 1991). The E_{1min} was calculated based on the assumption that R was evenly diluted with all inorganic P fractions having the same isotopic composition, according to:

$$E_{1min} = 10 \times C_P \times \frac{R}{r(1)} \qquad (7)$$

$$\frac{r(t)}{R} = m \times \left[t + (m)^{\frac{1}{n}}\right]^{-n} + \frac{10 \times C_P}{Pi} \qquad (8)$$

where Pi is the sum of Pi in the experimental soil (Total P minus Po; Table 3) and the fertilization rate 0 or 30 mg P kg^{-1}. The isotopic dilution parameters m and n were calculated from a non-linear regression between r(t)/R and t before statistical refinement. The isotopic dilution parameter m is a measure of the remaining radioactivity in the solution after 1 min and n is a measure of how fast the radioactivity is disappearing from the solution.

2.6.3 Resin-extractable P

The effects of fertilizers on resin-extractable P (Resin P) were studied after 7 and 21 days on soil sampled in the indirectly labelled incubation experiment. Moist samples equalling 2 g soil DM were extracted in 30 mL H_2O upon horizontal shaking at 160 rec min^{-1} for 16 h with simultaneous adsorption to anion-exchange resin membranes (BDH 55164 2S, 6x2 cm) that had been shaken in advance twice in 0.5 M $NaHCO_3$ for 1h. The P adsorbed to resin membranes was extracted by 0.1 M NaCl/0.1 M HCl. The P concentrations and radioactivity were determined as described above. The indirect labelling allowed estimation of the fraction of Resin P deriving from the fertilizer (Pdff Resin P, %) according to:

$$\text{Pdff Resin P} = \left(1 - \frac{SA_{P+}}{SA_{NoP}}\right) \times 100 \qquad (9)$$

where SA_{P+} (Bq mg^{-1} P) is the specific activity in the soil amended with fertilizer and SA_{NoP} (Bq mg^{-1} P) is the specific activity in the soil receiving no P fertilizer.

2.6.4 Microbial P

The effects of fertilizer on P in microbial biomass(Pmic) were estimated after 7 and 21 days in the indirectly labelled incubation experiment as the difference between extracted P with simultaneous adsorption to anion-exchange resin membranes from fumigated and non-fumigated soil samples (Resin P). For the fumigation-extraction, moist soil equalling 2 g soil DM was extracted in 30 mL H_2O with 1 mL hexanol for 16 h. As a methodological control, 2 g soil DM were extracted in 30 mL H_2O using anion exchange resin membranes after addition of a P spike of 10 µg P g^{-1} soil for 16 h. The test showed that released P was effectively sorbed to the anion-exchange resin membranes and it was not necessary to correct microbial biomass P for sorption to soil of P released during the fumigation-extraction.

2.7 Statistical analysis

Equation (8) was adjusted using a non-linear procedure. Two-way ANOVA was applied to test the effect of the factors fertilizer treatment and pH level and their interaction on parameters studied in the pot and incubation experiment. Data sets were also analysed using one-way ANOVA within the unlimed and the limed soil, respectively. Directly labelled treatments were excluded from the variance analyses and presented separately, including the standard deviation of four replicates. Analysed data were checked for normal distribution (normal quantile plots) and homogeneity of variance (residual versus fitted plots), and log transformed if indicated. For pair-wise comparisons, Tukey’s HSD test or t-tests were used at significance level $\alpha = 0.05$. Moreover, simple linear regressions were run with selected parameters of the pot experiment as response variables and the parameters of the incubation experiment as explanatory variables, which were averaged over the four replicates. All statistical analyses were performed with JMP Pro 11.1.1 (SAS Institute Inc., 2013).

3 Results

3.1 P concentration, dry matter production and total P uptake

There was a clear response of barley to P application on the experimental soil, as shown by linear increases in P uptake in aboveground biomass as a function MinP application rate (0, 15, 30, 45 mg P kg^{-1} soil) on both the unlimed and limed soil. The slopes of the response curves for the two soils were not significantly different (p=0.75), while the intercept was significantly higher on the limed than on the unlimed soil (p<0.01) (see Supplementary material). Phosphorus concentration in plant biomass ranged from 1.6-2.2 mg P g^{-1} DM. The P nutrition index for temperate grasses, calculated according to Liebisch *et al.* (2013), clearly indicated P limitation in all fertilizer treatments (results not shown). Nitrogen concentrations (3.0-4.4 g $100g^{-1}$ DM, results are not shown) were clearly above critical levels in temperate grasses as calculated according to Lemaire *et al.* (2008), and observed differences between fertilizer treatments were therefore ascribed to P fertilization effects rather than N fertilization effects.

All secondary resources resulted in equally high P concentration as MinP on both soils except manure, which resulted in significantly lower P concentration than MinP on the limed soil. Aboveground DM production ranged from 3.5-5.2 g kg^{-1} soil and was equally high on the unlimed and limed soil (Table 4). None of the secondary resources increased DM compared with NoP on either soil, while MinP significantly increased DM compared with NoP on both soils. Phosphorus uptake in aboveground biomass ranged from 5.7-10.8 mg P kg^{-1} soil and was as result of slightly higher P concentration on the limed soil on average 0.5 mg P kg^{-1} soil greater on the limed than on the unlimed soil. All secondary resources resulted in significantly lower P uptake than MinP on both soils, except fish sludge, which resulted in equally large P uptake as MinP on the limed soil (Table 4).

3.2 P uptake from different sources

Phosphorus derived from soil was the most important P source for barley plants with all fertilizer treatments on both soils (Figure 1). All treatments resulted in equally large Pdf soil, except manure, which resulted in significantly smaller Pdf soil than all other treatments on the unlimed soil and in smaller Pdf soil than NoP on the limed soil.

Phosphorus derived from fertilizer was significantly smaller after application of secondary resources than after MinP on both soils (Figure 1). Only manure resulted in equally large Pdf fertilizer as MinP on the limed soil. Fish sludge and wood ash resulted in equally large Pdf fertilizer as manure, while meat bone meal resulted in significantly smaller Pdf fertilizer than manure on both soils. The Pdf fertilizer accounted for 40-44% of P uptake in plants after application of MinP or manure, and in significantly smaller fractions after application of the other secondary resources compared with MinP (Table 4). Fertilizer recovery was 14-16% of applied MinP and was significantly lower after application of all secondary resources on both soils. The RAE decreased in the order manure ≥ fish sludge ≥ wood ash ≥ meat bone meal on both soils, but only meat bone meal resulted in significantly lower RAE than manure. Fish sludge and wood ash resulted in equally high RAE as manure.

The MinP treatment resulted in significantly smaller Pdf soil than MinPdir, the average difference being 1 mg P kg^{-1}, and in significantly larger Pdf fertilizer than MinPdir, the difference being 0.7 mg P kg^{-1} (two-sided t-tests over both soils, $n = 8$). The variability between replicates was also generally lower for MinPdir than for MinP and all other treatments.

The Pdf soil and Pdf fertilizer were corrected for Pdf seed as estimated from the relationship between P uptake and Pdf seed studied in the seed P experiment (Figure 2). In the seed P experiment, Pdf seed significantly increased with increasing P fertilization rate. The P uptake by the highest P fertilization rate (1.04±0.15 mg P kg^{-1}) in aboveground biomass was higher than the average amount of P applied with the seeds (estimated to be 0.71±0.08 mg P pot^{-1}). With a and b from Figure 2, 0.65-0.95 mg P pot^{-1} in aboveground biomass was estimated to derive from seeds, representing 8.6-10.7% of P uptake in the pot experiment. The ranking of Pdf soil and fertilizer among treatments did not change when not corrected for Pdf seed (see Supplementary material), emphasising the low importance of Pdf seed in the present study.

3.3 Physicochemical and microbial soil processes

On the unlimed soil, all fertilizers initially increased available P over NoP in the incubation experiment, as reflected by Resin P, measured 7 days after application (Table 5). Seven days after fertilizer application, Pdf fertilizer in the Resin P pool ranged from 25-38%, but there were no significant differences between treatments. Twenty-one days after application, Resin P was on average 4.6 mg P kg^{-1} soil lower than 7 days after fertilizer application (two-sided t-test over both time points, $n = 48$), and only MinP resulted in higher Resin P than NoP. The Pdf fertilizer in the Resin P pool ranged from 19-52%, again without significant differences between treatments. Twenty-one days after application, all secondary resources increased C_P over NoP and resulted in equally high E_{1min} as MinP.

Drivers of secondary P recycling

On the limed soil, meat bone meal and fish sludge did not increase available P over NoP as reflected by C_P, E_{1min} and Resin P at any time point. Wood ash did not increase Resin P over NoP at any time point on the limed soil, but resulted in equally high C_P and E_{1min} as MinP. Seven days after fertilizer application, there were no differences in Pdf fertilizer in the Resin P pool between fertilizer treatments, with values ranging from 29-49%. Twenty-one days after fertilizer application, meat bone meal was the only secondary resource that resulted in significantly lower Pdf fertilizer in the resin-extractable P pool (12%) than MinP (41%).

In the pot experiment, wood ash increased soil pH compared with NoP on both soils and meat bone meal resulted in significantly lower soil pH than NoP on the limed soil, while the other fertilizers had no significant effect on soil pH compared with NoP in the pot experiment (Table 5). In the incubation experiment, there were no differences in soil pH between the three time points, but soil pH was generally 0.4 and 0.2 pH units lower than in the pot experiment on the unlimed and limed soil, respectively. In the incubation experiment, wood ash resulted in significantly higher soil pH than NoP on both soils and the effects of fertilizer treatments generally followed a similar pattern as in the pot experiment. The results are therefore not shown.

Phosphorus uptake in microbial biomass (Table 5) was of the same order of magnitude as P uptake in plants (Table 4). It was generally higher on the limed than on the unlimed soil, the average difference being 8.4 mg P kg^{-1} soil at 7 days and 3.1 mg P kg^{-1} soil at 21 days after fertilizer application. On the unlimed soil there were no differences in Pmic between treatments, except an increase over NoP following meat bone meal application 21 days after fertilizer application. On the limed soil at 7 days after fertilizer application, only MinP had significantly increased Pmic over NoP. However, this effect was transient, as 21 days after fertilizer application Pmic of MinP was significantly lower than at 7 days after fertilizer application, and MinP and meat bone meal resulted in equally low Pmic as NoP. In contrast, manure and fish sludge had significantly increased Pmic over NoP.

3.4 Drivers of P uptake by barley

Phosphorus uptake by barley was best explained by the solubility of inorganic P in fertilizers, whereas additional effects of fertilizers on physicochemical and microbial soil processes were of little overall importance. This is shown by linear positive relationships between P uptake in barley and the H_2O + $NaHCO_3$-soluble inorganic P (Pi) fraction in fertilizers and linear negative relationships between P uptake and the HCl-soluble Pi fraction in fertilizers on both soils (Figure 3 and Table 6). According to the sequential fractionation based on Hedley *et al.* (1982), the H_2O + $NaHCO_3$-soluble Pi fraction is operationally defined as readily available and labile Pi, while the HCl-soluble Pi fraction is defined as the slowly soluble Ca-P fraction. Phosphorus uptake by barley could further be explained by C_P, m and Resin P measured 21 days after fertilizer application on both soils, which all represent measures for the solubility of fertilizer P applied to the soil. The Pmic measured 7 days after fertilizer application resulted in significant relationships with P uptake by barley. However, while the relationship was negative on the unlimed soil, it was positive on the limed soil. Soil pH was unable to explain the variation in P uptake by barley between fertilizer treatments.

4 Discussion

4.1 Effects of inorganic P species in secondary resources on P uptake by barley

The P uptake by barley following secondary resource application was mainly affected by the solubility of the inorganic P species the resource contained. The indirect effects of the secondary resources studied on P uptake through their influences on physicochemical and microbial soil processes were generally of less importance.

Poor P uptake following meat bone meal application can be explained by its large fraction of Ca-bound P such as hydroxyapatite and chlorfluorapatite (Brod *et al.*, 2015a) with low solubility, especially in soils with pH >6.5 (Morel and Fardeau, 1990). Similar results have been reported by Ylivainio *et al.* (2008) and Brod *et al.* (2015a) after application of meat bone meal to ryegrass.

Fish sludge was the secondary resource that tended to result in the highest P uptake, probably because a considerable P fraction in fish sludge is readily available and labile, i.e. soluble in H_2O and $NaHCO_3$ (Table 2). However, fish sludge also contains apatite (Brod *et al.*, 2015a), which can explain why neither meat bone meal nor fish sludge increased C_P, E_{1min} and Resin P over NoP in the limed soil of the incubation experiment.

Wood ash resulted in equally high P uptake as the other secondary resources, as expected from its large fraction of labile Pi, in addition to slowly soluble Ca-P (mainly $(Ca_2(SiO_4))_6(Ca_3(PO_4)_2)$) (Brod *et al.*, 2015a). The P uptake following wood ash application was equally high on both soils, but on the limed soil of the incubation experiment wood ash surprisingly increased C_P and E_{1min} to the same level as MinP. Similarly, Brod *et al.* (2015a) found increasing P fertilization effects of the same wood ash with increasing soil pH and attributed this effect to the likely presence of $NaHCO_3$-soluble P adsorbed to Al-/Fe-(hydr)oxides. Wood ash was the secondary resource with the largest $NaHCO_3$-soluble Pi fraction among all products studied. Phosphorus adsorbed to Al-/Fe-(hydr)oxides is characterized by increasing solubility with increasing soil pH (Lindsay, 1979). However, increased C_P and E_{1min} values after wood ash application on the limed soil could also be a methodological artefact if the magnetic stirrer mechanically destroyed $Ca_2(SiO_4))_6(Ca_3(PO_4)_2$, thereby solubilising phosphate, while the same stable Ca-P was already solubilized in the unlimed soil. Similarly, Sinaj *et al.* (1994) found that silicato-calcium phosphate present in Thomas slag quickly solubilized after application to an acidic soil with pH 6.2. A methodological artefact during determination of E_{1min} would explain why increased wood ash P solubility on the limed compared with the unlimed soil was not reflected by increased Pdf fertilizer in the pot experiment or elevated Resin P on the limed soil.

Manure resulted in lower P uptake by barley than MinP, even though 75% of P in manure was present as readily available and labile Pi (Table 2). Phosphorus uptake following manure application tended to be lower than expected from the linear regression lines with the H_2O + $NaHCO_3$-soluble or HCl-soluble Pi fractions as explanatory variables (Figure 3). Oberson *et al.* (2010) also reported lower P fertilization effects of cow faeces than di-ammonium phosphate after a pot experiment using indirect labelling and soils with different fertilization histories.

4.2 Effects of microbial soil processes on P uptake by barley

Our results indicate that organic C applied with manure may have resulted in microbial immobilisation of soluble P, since Pdf soil (mg P kg^{-1}) in barley was significantly lower after manure application than after NoP on both soils (Figure 1). This is also in agreement with manure increasing Pmic, compared with MinP, on both soils after 21 days (Table 5). Oberson *et al.* (2010) reported microbial P immobilisation following cow faeces application to soils with different fertilization histories, and Bünemann *et al.* (2004b) describe increases in Pmic as a result of glucose addition during an incubation experiment with a P-deficient tropical soil. On the limed soil in the present study, total P uptake by barley was lower for the manure than the MinP treatment, but Pdf fertilizer (mg P kg^{-1}) after manure application was equally high as after MinP. Therefore, the difference method would have underestimated the fertilization effect of manure because of microbial immobilisation of soluble P in this soil and thereby a lower contribution from soil P.

Even though organic C also was applied with fish sludge and meat bone meal, microbial P immobilisation seems not to have been a major competitor to barley plants in these cases, and Pdf soil (mg P kg^{-1}) was equally high as after MinP. At the same fertilization rate of P, only 557 and 152 mg organic C kg^{-1} were applied with fish sludge and meat bone meal, compared with 2008 mg organic C kg^{-1} with manure (Table 2). In the incubation experiment, however, there were still signs of P immobilisation, indicated as increased Pmic over NoP 21 days after application of fish sludge on the limed and meat bone meal on the unlimed soil (Table 5). This can be explained by a larger fraction of C in fish sludge and meat bone meal being soluble in H_2O than the C in manure (Table 2). Accordingly, Bünemann *et al.* (2004a) point out a strong impact of C quality on microbial P immobilisation. Still, due to too few products included in this study, we cannot specify the quantity and quality of organic C in secondary resources at which P uptake by plants might be negatively affected by activated microbial activity. Furthermore, it remains unknown whether the effect of secondary resources on microbial soil processes is also negligible for plant P uptake on soils with high microbial activity, because in the present study Pmic in both soils was overall rather low (Frossard *et al.*, 2015).

4.3 Effects of physicochemical soil processes on P uptake by barley

Phosphorus uptake by barley was higher on the limed than on the unlimed soil (Figure 1), probably because growing conditions were better overall. Barley is known to be sensitive to low soil pH (e.g. Bona *et al.*, 1993), which is often associated with high concentrations of soluble Al. However, higher P uptake on the limed than on the unlimed soil could also be due to higher P availability, as supported by higher P concentration in plants receiving no P fertilizer (two-sided t-test, n = 8) (Table 4). On the unlimed soil, more P was probably adsorbed to Al-/Fe-(hydr)oxides because of increased positive surface charges at lower soil pH. The increased importance of P adsorption on the unlimed soil was also indicated by a decrease in Resin P over time, which was not observed in the limed soil (Table 5).

Wood ash caused a significant increase in soil pH on both soils as result of the liming effect of $CaCO_3$. Increasing effects of wood ash on soil pH are well known (e.g. Demeyer *et al.*, 2001). However, the effect of wood ash on pH was far too small to significantly influence P availability in the soil (Table 6). Equally high Pdf soil (mg P kg^{-1}) after wood ash application and NoP showed that soil P availability was not affected by the pH-increasing effect of wood ash within the pH range in the present experiment. With wood ash, only 0.1 g $CaCO_3$ kg^{-1} soil was applied (8 wt-% $CaCO_3$ in wood ash according to Brod *et al.*, 2015a), in comparison with 2 g $CaCO_3$ kg^{-1} soil applied to the limed soil.

4.4 Methodological considerations

Conducting the isotope dilution technique allowed us to conclude that the difference method would have led to similar results for P fertilization effects in this case, because P uptake by barley after secondary resource application was overall little affected by their influence on physicochemical and microbial soil processes. The underlying assumption in the difference method that fertilized and unfertilized treatments take up the same P amount from the soil would only have been violated after manure application due to microbial immobilisation. Therefore, the results are also in agreement with those of a previous experiment in which a sand-peat mixture was used as a model soil (Brod *et al.*, 2015a), even though natural soil processes could not be studied. Thus the results of the present study indicate that the difference method is reliable for secondary resources with low ratios of OM to P. However, these results should be confirmed with different soil types and extended with several secondary resources with a wide range of organic C content compared with P.

The indirect labelling method is based on the assumptions that plant-available soil P is homogeneously labelled and that dilution of the radioisotope is only due to the unlabelled fertilizer. The internal control treatment MinPdir resulted in significantly higher Pdf soil and lower Pdf fertilizer than MinP according to a comparison of the two treatments over both soils. The difference between the directly and indirectly labelled mineral control treatment suggests that unlabelled soil P contributed to the dilution of the specific activity in plants in the MinP treatment, e.g. via the mineralisation of organic or microbial P (Oberson *et al.*, 2010). This means that fertilization effects might have been slightly overestimated when the indirect method was used. In fact, significantly increased Pmic over NoP after MinP application on the limed soil after 7 days indicates modified microbial activity also in the mineral control treatment, even though no organic C was applied with MinP. In that case, soil microbes were probably stimulated as a methodological artefact by introducing oxygen when mixing the experimental soil, as suggested by Oberson *et al.* (2001) and Bünemann *et al.* (2007). Twenty-one days after fertilizer application, the microbial boost could no longer be observed.

Another methodological inconsistency was observed in the seed P experiment, where our results incorrectly indicated that the amount of P derived from the seed was larger than the amount applied with seed. It is possible that the acid-washed sand still contained P, some P added with the two removed seeds had leaked into the soil, or P applied with the seed was underestimated because the seeds sown per pot were not weighed. In addition, large variation between the replicates of Pdf seed (Figure 2) and relatively low transfer from the seed compared with total P uptake might have contributed to the inconsistency. However, the actual reasons could not be identified. An alternative to the approach used here is to estimate Pdf seed by comparing the P content in the seeds at seedling stage and the remaining P content in the seeds at harvest, as suggested by Achat *et al.* (2014b). Several studies have pointed out the importance of correcting for seed P contribution when the indirect method is applied (e.g. Pypers *et al.*, 2006; Achat *et al.*, 2014b; Nanzer *et al.*, 2014a) to avoid overestimation of P derived from the unlabelled fertilizer. Here, the fraction of Pdf seed in total P uptake was small in comparison with e.g. results reported by Achat *et al.* (2014b), who used ryegrass and fescue as the experimental crop and suggested that 15-50% of P uptake at the first cut was Pdf seed. The difference between the different indirectly labelled treatments was also small and the relative fertilization effect of the secondary resources was not affected by Pdf seed.

5 Conclusions

This study explored the effects of secondary resources on physicochemical and microbial soil P processes and their importance for plant P uptake at two soil pH levels in the same arable soil. The main driver for P uptake was found to be the solubility of inorganic P species contained in the secondary resources, while indirect effects on P availability via influences on physicochemical and microbial soil processes were of little overall importance. This implies that P uptake following secondary resource application can be sufficiently predicted by intrinsic chemical P characteristics. The P uptake by barley was indeed best explained by a linear positive relationship with the H_2O + $NaHCO_3$-soluble inorganic P fraction and a linear negative relationship with the HCl-soluble inorganic P fraction in fertilizers on both unlimed and limed soil. Organic C resulted in microbial immobilisation of labile P and decreased uptake in barley of P derived from the soil only after manure application. In this arable soil with rather low microbial biomass P, immobilisation of P in microbial biomass could not challenge barley plants as the main P sink after application of fish sludge and meat bone meal. Further studies are needed to identify the critical organic C content in secondary resources at which microbial P processes influence plant P uptake. The significant increase in soil pH as result of wood ash application had no effect on P uptake by barley plants within the pH range in this study.

6 Abbreviations

SA	Specific activity
Pi	Inorganic P
P uptake	P taken up by the fertilized plant in aboveground biomass
Pdf fertilizer	P derived from the fertilizer
Pdf soil	P derived from the soil
Pdf seed	P derived from the seed
P fert	P applied with the fertilizer
RAE	Relative agronomic efficiency
Pdff ResinP	Resin P deriving from the fertilizer

7 Author contribution

EB and AO designed the experiment; EB conducted the experiment; EB analyzed the data with the help of AO and wrote the paper. All authors contributed to the interpretation of the results, and read and approved the manuscript.

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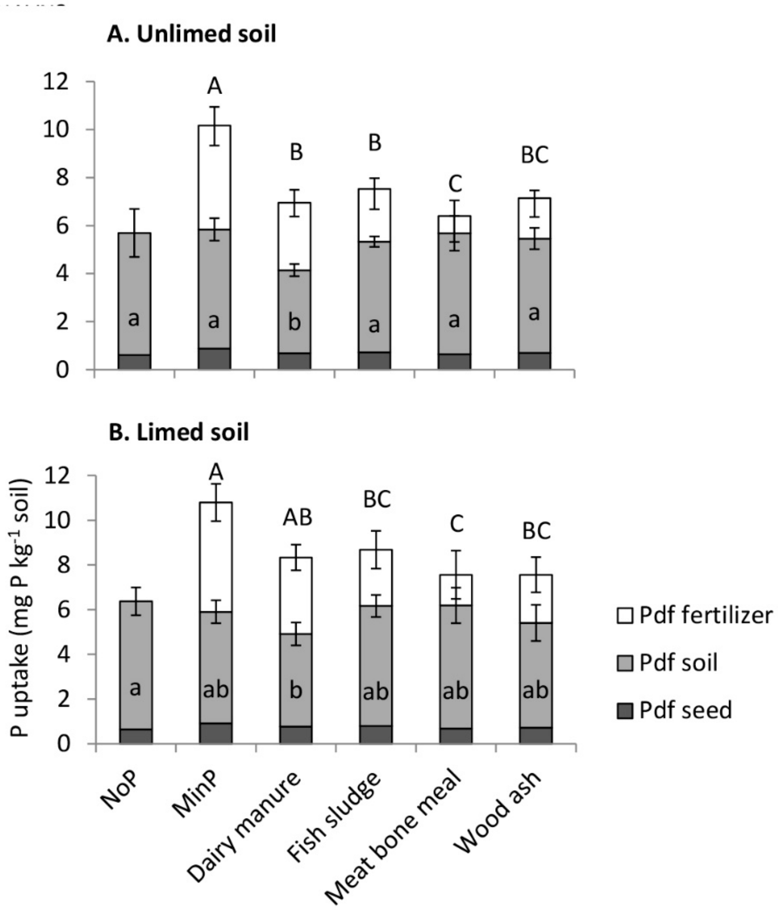

	Pdf fertilizer	Pdf soil	Pdf seed
Two-way ANOVA, source of variation			
Treatment	***	***	***
Soil	*	*	**
Treatment x Soil	n.s.	n.s.	n.s.

Figure 1. Phosphorus derived from fertilizer (Pdf fertilizer), soil (Pdf soil) and seed (Pdf seed) in barley (mg P kg^{-1} soil) as an effect of the different fertilizer treatments on: A) Unlimed soil and B) limed soil. Error bars represent the standard deviation within each treatment. Letters indicate significant differences between treatments according to Tukey's test (one-way ANOVA for each soil); uppercase letters refer to Pdf fertilizer and lowercase letters to Pdf soil. On the unlimed soil, data on Pdf soil were log-transformed for the statistical analysis. On the limed soil, for Pdf fertilizer of meat bone meal only three observations were considered. *, **, * significant at $p < 0.05$, 0.01 and 0.001 probability level.**

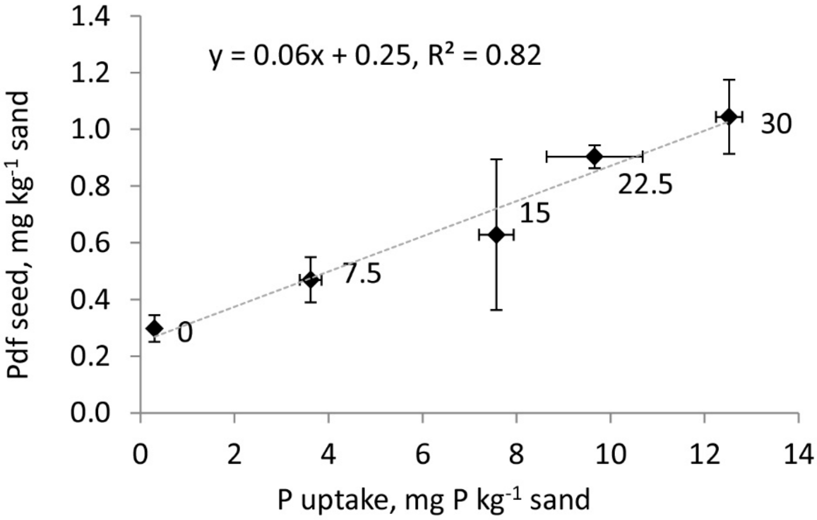

Figure 2. Relationship between P uptake in aboveground biomass (mg P kg^{-1} sand) and P derived from the seed (Pdf seed, mg P kg^{-1} sand) for barley grown on sand and fertilized with increasing rates of MinP labelled with ^{33}P (0, 7.5, 15, 22.5 and 30 mg P kg^{-1} sand as indicated next to the experimental points). Error bars represent standard deviation of the four replicates.

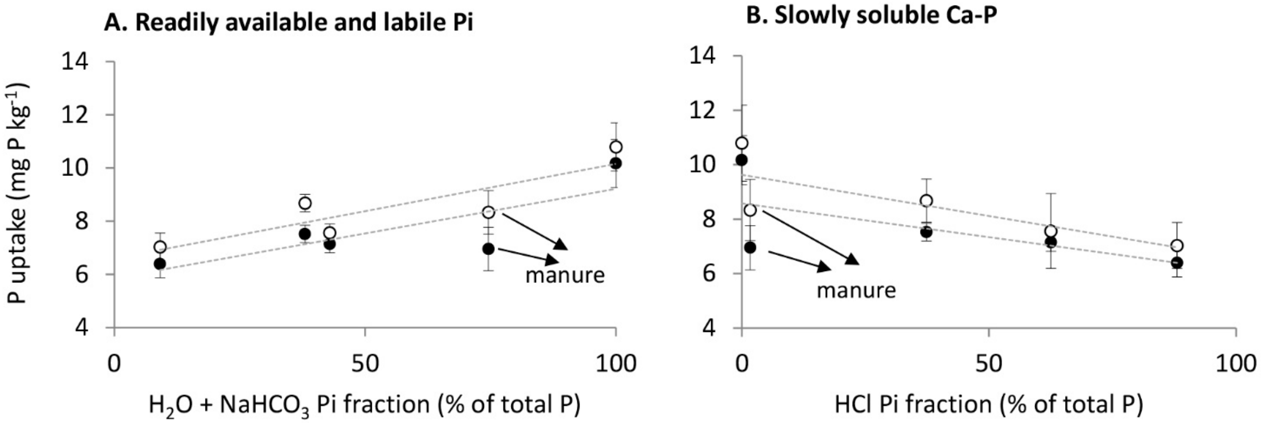

Figure 3. Phosphorus uptake by barley (mg P kg^{-1} soil) as an effect of: A) H_2O + $NaHCO_3$-soluble inorganic P fraction and B) HCl-soluble inorganic P fraction in fertilizers for both soils (● = unlimed soil, ○ = limed soil). All P in MinP was assumed to be present in the H_2O + $NaHCO_3$ Pi fraction. Error bars indicate standard deviation of Pdf fertilizer between replicates.

Table 1. Description of secondary resources and identified P characteristics (Brod *et al.*, 2015a)

Product	Description	Inorganic P characteristics
Manure	Dried slurry (faeces and urine) of dairy cows collected from the barn at the Norwegian University of Life Sciences, Norway.	Mainly readily available (H_2O-soluble) and labile ($NaHCO_3$-soluble) Pi without further speciation.
Fish sludge	Collected from the on-land Åsen settefisk salmon hatchery, Norway. Fish are bred in closed cages until they are approximately 1 year old. Effluent containing faeces and feed residues (mainly fish meal and soya) was mechanically filtered before the material was treated on-site in a reactor developed by the company Global Enviro.	Stable Ca-bound P such as apatite, but also amorphous Ca-bound P.
Meat bone meal	Commercial product originating from a slaughterhouse in Hamar, Norway, and merchandized by Norsk Protein AS. Slaughterhouse waste of category III according to EC (2002), which was stabilized and sanitized at 133°C and 3.0 bar for 20 min.	Stable Ca-bound P, mainly apatite.
Wood ash	Bottom ash from a grate-fired boiler system at the Moelven Østerdalsbruket AS mill, Norway. Parent material was timber unsuitable for industrial use.	Stable Ca-P, mainly calcium phosphate silicate, and amorphous P. May also contain Al-/Fe-bound P.

Table 2. Selected chemical properties of secondary resources. Po = organic P, Nmin = mineral N (NO_3^- and NH_4^+), n.d. = not detectable

		Manure	Fish sludge	Meat bone meal	Wood ash
Dry matter [a]	g $100g^{-1}$	5.8	95.0	96.2	99.6
Organic matter [b]	g $100g^{-1}$ DM	81.6	87.6	66.6	0.0
pH [c]		7.0	5.4	6.2	13
P [d]	g kg^{-1} DM	6	21	54	17
Po [d]	% of P	24	14	2	n.d.
P_{H2O} [e]	% of P	42	19	4	n.d.
P_{NaHCO3} [e]	% of P	33	19	5	43
P_{NaOH} [e]	% of P	4	12	3	n.d.
P_{HCl} [e]	% of P	2	37	88	63
C [f]	g kg^{-1} DM	470	503	368	19
Organic C [g]	g kg^{-1} DM	393	375	266	0.1
Organic C_{H2O} [h]	% of organic C	14	34	41	n.d.
N [i]	g kg^{-1} DM	53	71	86	n.d.
Nmin [j]	g kg^{-1} DM	22	2.6	5.0	n.d.
K [k]	g kg^{-1} DM	42	3	4	56
Mg [k]	g kg^{-1} DM	6	3	3	25
S [k]	g kg^{-1} DM	42	48	34	34
Ca [k]	g kg^{-1} DM	11	37	110	310
Al [k]	g kg^{-1} DM	0.4	0.3	0.2	19.1
Fe [k]	g kg^{-1} DM	1.3	0.7	0.5	7.6

[a] Drying of the original samples at 105°C.

[b] Incineration of the original samples at 550°C.

[c] Measured on dried and sieved (< 2mm) samples in H_2O in a solid:solution ratio of 1:2.5 (v/v).

[d] By ignition method on dried and milled samples before extraction with 6 M H_2SO_4 according to Møberg and Petersen (1982). Colorimetric analysis according to Murphy and Riley (1962).

[e] Sequentially extracted Pi of 1 g dried and milled sample in 200 mL H_2O for 1h, 200 mL 0.5 M NaHCO3, 0.1 M NaOH and 1M HCl for each 16h. Colorimetric analysis according to Murphy and Riley (1962).

[f] C/N elemental analysis (Leco TruSpec CHN) on dried and milled samples.

[g] Analyzed on triplicate dried and milled samples after washing with 2 M HCl solution using a Perkin Elmer 2400 CHN analyzer.

[h] Extraction of 1 g dried and sieved sample in 200 mL H_2O for 2 h, analysed on Shimadzu TOC-V CPN.

[i] Modified Kjeldahl method (EN 13654-1, 2001) analysed on Leco TruSpec CHN. Analyzed on a liquid sample of manure.

[j] Analyzed on Konelab Aqua 60 analyser after extraction with 2 M KCl (Henriksen and Selmer-Olsen, 1970; Selmer-Olsen, 1971). Analyzed on a liquid sample of manure.

[k] Analyzed by ICP-OES after digestion of dried and milled samples with concentrated nitric acid in an ultraclave (EN ISO 11885, 2009).

Table 3. Chemical properties of the soil, Po = organic P

Organic matter [a]	Total P [b]	Po [b]	P-AL [c]	K-AL [c]	Mg-AL [c]	Ca-AL [c]	Ox-Fe [d]	Ox-Al [d]
%	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}
4.5	1024	456	44	138	44	1.3-1.9	4.8	1.9

[a] Incineration at 550°C.

[b] By ignition method after extraction with 6 M H_2SO_4 according to Møberg and Petersen (1982). Colorimetric analysis according to Murphy and Riley (1962).

[c] Extraction with 0.1 M ammonium lactate and 0.4 M acetic acid adjusted to pH 3.75 according to Egnér *et al.* (1960), analysed on ICP-OES.

[d] Extraction with 0.2 M ammonium oxalate in oxalic acid according to van Reeuwijk (1995), analyzed by ICP-OES.

Table 4. Aboveground dry matter production (DM), P concentration and P uptake in aboveground biomass, P derived from fertilizer (Pdf fertilizer, %), fertilizer recovery (%) and relative agronomic efficiency (RAE, %) as an effect of different fertilizer treatments on unlimed and limed soil

Treatment	Dry matter g DM kg^{-1}		P con-centration mg P g^{-1} DM		P uptake mg P kg^{-1} soil		Pdf fertilizer %		Fertilizer recovery %		RAE %	
Unlimed soil												
NoP	3.5	b	1.6	b	5.7	c						
MinP	5.2	a	1.9	a	10.2	a	42.4	a	14.4	a	100[a]	
Manure	4.1	b	1.7	ab	7.0	bc	40.2	ab	8.8	b	60.9	a
Fish sludge	4.0	b	1.9	a	7.5	b	29.0	bc	6.9	b	47.8	a
Meat bone meal	3.5	b	1.8	ab	6.4	bc	11.2	d	2.3	c	16.0	b
Wood ash	3.7	b	1.9	a	7.1	bc	23.6	cd	5.6	bc	38.7	ab
SEM	0.2		0.1		0.4		2.9		0.9		5.6	
HSD	0.8		0.3		1.6		12.8		4.0		23.4	
MinPdir	5.0±0.3		2.1±0.2		10.3±0.6		36.9±2.5		12.7±0.6		n.d.	
Limed soil												
NoP	3.5	b	1.8	ab	6.4	b						
MinP	5.0	a	2.2	a	10.8	a	45.1	a	16.3	a	100[a]	
Manure	4.6	ab	1.8	b	8.3	b	41.0	a	10.7	b	65.7	a
Fish sludge	4.5	ab	1.9	ab	8.7	ab	28.6	b	7.9	bc	48.6	ab
Meat bone meal[b]	3.7	b	1.9	ab	7.0	b	18.8	b	4.4	c	26.9	b
Wood ash	3.9	ab	1.9	ab	7.6	b	28.1	b	7.1	bc	43.8	ab
SEM	0.3		0.1		0.5		2.6		1.2		6.7	
HSD	1.1		0.4		2.4		11.3		5.1		28.6	
MinPdir	4.9±0.4		2.3±0.1		11.2±1.0		33.5±0.2		12.5±1.1		n.d.	
Two-way ANOVA, source of variation												
Treatment	***		***		***		***		***		***	
Soil	n.s.		*		**		n.s.		*		n.s.	
Treatment x Soil	n.s.		n.s.		n.s.		n.s.		n.s.		n.s.	

SEM = pooled standard error of the mean and HSD = Tukey's honest significant difference at each pH level, where values followed by the same letter are not significantly different. For MinPdir mean ± standard deviation of 4 replicates. *, **, *** significant at $p < 0.05$, 0.01 and 0.001 probability level, n.s. = not significant, n.d. = not determined.

[a] By definition set to 100%.

[b] Only three observations due to Pdf fertilizer < 0 for one replicate.

Table 5. pH measured in the pot experiment after harvest, C_P = P concentration in the soil solution, m and n = isotopic dilution parameters, E_{1min} = isotopically exchangeable P within 1 min, Resin P = resin-extractable P, Pdff Resin = P derived from the fertilizer in the resin-extractable P pool (%) and Pmic = microbial P 7 and 21 days after fertilizer application as an effect of different fertilizer treatments on unlimed and limed soil

	pH	C_P [a]	m	n	E_{1min}	Resin P	Resin P [a]	Pdff Resin	Pdff Resin	Pmic[d]	Pmic
		Day 21	Day 21	Day 21	Day 21	Day 7	Day 21	Day 7	Day 21	Day 7	Day 21
Treatment		mg P L^{-1}			mg P kg^{-1}	mg P kg^{-1}	mg P kg^{-1}	%	%	mg P kg^{-1}	mg P kg^{-1}
Unlimed soil											
NoP	5.51 bc	0.09 c	0.27 ab	0.40 a	3.3 b	12.5 b	10.1 b			2.8 n.s.	6.2 bc
MinP	5.49 c	0.15 a	0.32 a	0.38 b	4.8 a	21.6 a	25.2 a	38[b] n.s.	52[b] n.s.	3.7[b] n.s.	3.6[b] c
Manure	5.58 b	0.13 ab	0.29 ab	0.39 ab	4.6 a	23.7 a	13.9 b	32 n.s.	38 n.s.	6.4 n.s.	9.7 ab
Fish sludge	5.45 c	0.12 b	0.27 ab	0.39 ab	4.4 ab	19.1 a	16.6 ab	37[b] n.s.	30 n.s.	5.8 n.s.	3.0 c
Meat bone meal	5.44 c	0.12 b	0.27 ab	0.39 ab	4.5 a	21.5 a	10.1 b	25 n.s.	19 n.s.		9.9 a
Wood ash	5.73 a	0.12 b	0.26 b	0.39 ab	4.4 ab	19.1 a	13.9 b	30 n.s.	40 n.s.	9.2 n.s.	5.0 c
SEM	0.02	0.01	0.01	0.00	0.3	1.1	2.2	7	7	1.6	0.8
HSD	0.09	0.03	0.05	0.02	1.2	4.7	9.9	35	35	7.0	3.7
Limed soil											
NoP	6.25 bc	0.08 b	0.23 bc	0.37 a	3.5 c	10.3 c	11.8 b			9.1 bc	6.8 c
MinP	6.23 bcd	0.15 a	0.27 a	0.35 c	5.4 a	16.7 ab	23.4 a	43 n.s.	41 a	18.8 a	6.1 c
Manure	6.30 b	0.13 a	0.24 abc	0.35 bc	5.3 ab	18.8 a	16.9 ab	49 n.s.	40 a	14.7 ab	15.1 a
Fish sludge	6.17 cd	0.10 b	0.24 ab	0.37 a	4.2 bc	9.7 c	13.2 b	35 n.s.	24 ab	14.8 ab	11.9 ab
Meat bone meal	6.15 d	0.09 b	0.22 c	0.37 ab	4.2 bc	12.5 bc	14.2 b	29[c] n.s.	12 b	7.0 c	6.7 c
Wood ash	6.45 a	0.14 a	0.22 bc	0.34 c	6.1 a	13.7 abc	15.5 b	40 n.s.	43[b] a	13.1 abc	9.8 bc
SEM	0.02	0.01	0.01	0.00	0.3	1.2	1.7	6	5	1.5	0.9
HSD	0.08	0.03	0.03	0.02	1.2	5.4	7.6	26	26	6.9	4.3
Two-way ANOVA, source of variation											
Treatment	***	***	***	***	***	***	***	n.s.	***	*	***
Soil	***	*	***	***	**	***	***	n.s.	n.s.	***	***
Treatment x Soil	n.s.	**	n.s.	**	**	*	**	n.s.	n.s.	*	***

SEM = standard error of the mean and HSD = Tukey's honest significant difference at each pH level, where values followed by the same letter are not significantly different. *, **, *** significant at $p < 0.05$, 0.01 and 0.001 probability level, respectively, and n.s. = not significant. [a]Two-way ANOVA based on log transformation. [b]Only three observations. [c]Only 2 observations. [d]One-way ANOVA without meat bone meal on unlimed soil, two-way ANOVA without meat bone meal

Table 6. Results of simple linear regression with Y = P uptake (mg P kg^{-1} soil) by barley plants on unlimed or limed soil without NoP, and X = explanatory variables, where fertilizer characteristics are sequentially extracted inorganic P (Pi) fractions in secondary P resources (Table 2), C_P = water-soluble P (mg P L^{-1}), m and n = isotopic dilution parameters, E_{1min} = isotopically exchangeable P (mg P kg^{-1} soil), Resin P = resin-extractable P (mg P kg^{-1} soil), Pdff Resin P = P derived from the fertilizer in the resin-extractable P pool (%) and Pmic = microbial P (mg P kg^{-1} soil), measured respectively 7 and 21 days after fertilizer application

	Unlimed soil		Limed soil [a]	
	R^2	p-value	R^2	p-value
Fertilizer characteristics				
H_2O + $NaHCO_3$ soluble Pi	0.55	***	0.47	***
HCl fraction	0.35	**	0.41	**
Physicochemical soil processes				
C_P	0.58	***	0.25	*
m	0.53	***	0.60	***
n	0.83 [c]	***	0.05	n.s.
E_{1min}	0.38	**	0.03	n.s.
Resin P, 7 days	0.00	n.s.	0.08	n.s.
Pdff resin P, 7 days	0.55	***	0.15	n.s.
Resin P, 21 days	0.84	***	0.44	**
Pdff resin P, 21 days	0.58	***	0.14	n.s.
Soil pH (pot experiment)	0.02	n.s.	0.02	n.s.
Microbial soil processes				
P mic, 7 days	0.51 [b, c]	**	0.50	***
P mic, 21 days	0.35	**	0.03	n.s.

*, **, *** significant at $p < 0.05$, 0.01 and 0.001 probability level respectively and n.s. = not significant

[a] Only three replicates of meat bone meal

[b] Without meat bone meal

[c] Negative relationship

Supplementary material

Drivers of phosphorus uptake by barley following secondary resource application

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Appendix A: Response curves

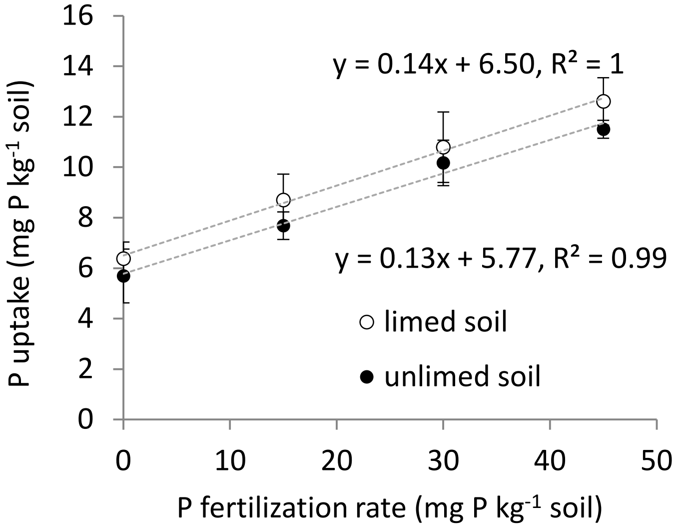

Figure S1. P uptake by barley (mg P kg^{-1} soil) as an effect of P fertilization rate (0, 15, 30, 45 mg P kg^{-1} soil) on unlimed and limed soil. Error bars represent standard deviation of the four replicates.

Appendix B: Seed P correction

B.1. No Pdf seed correction

To study the importance of seed P correction for the results obtained, Pdf soil and Pdf fertilizer as an effect of fertilizer treatment and soil were calculated and analysed without any seed P correction (Table S1). Comparing Pdf soil and Pdf fertilizer, the ranking between fertilizer treatments was not changed. This shows low importance of Pdf seed in the present study.

B.2. Pdf seed correction according to Oberson *et al.* (2010)

An alternative approach to estimating Pdf seed, based on the difference between proportional Pdf fertilizer after MinP and MinPdir application (Oberson *et al.*, 2010), was also used for each soil. Here, Pdf seed was assumed to be equal for all treatments on the respective soil and independent of P uptake. The proportional Pdf fertilizer after MinP and MinPdir was assumed to be identical. Phosphorus derived from the seed does not affect Pdf fertilizer determined using the direct method, because in MinPdir the fertilizer is labelled.

Pdf $\text{fertilizer}_{\text{MinP}}$ (mg P kg^{-1}) after MinP application without seed P correction was calculated as:

$$\text{Pdf fertilizer}_{\text{MinP}} = \left(1 - \frac{\text{SA}_{\text{plant MinP}}}{\text{SA}_{\text{plant NoP}}}\right) \times \text{P uptake}_{\text{MinP}} \qquad (1)$$

where $\text{SA}_{\text{plant MinP}}$ (Bq mg^{-1} P) is the specific activity in the plants fertilized with MinP, $\text{SA}_{\text{plant NoP}}$ (Bq mg^{-1} P) is the average specific activity in the plants receiving no P fertilizer and P uptake (mg P kg^{-1} soil) is the amount of P taken up by the plants fertilized with MinP in aboveground biomass.

Pdf $\text{fertilizer}_{\text{MinP}}$ (mg P kg^{-1}) after MinP application with seed P correction was calculated as:

$$\text{Pdf fertilizer}_{\text{MinP}} - \text{Pdf seed} = \frac{\text{SA}_{\text{plant MinPdir}}}{\text{SA}_{\text{fert}}} \times \text{P uptake}_{\text{MinP}} \qquad (2)$$

where $\text{SA}_{\text{plant MinPdir}}$ (Bq mg^{-1} P) is the specific activity in the plants fertilized with MinPdir and SA_{fert} (Bq mg^{-1} P) is the specific activity in the MinPdir fertilizer.

Pdf seed (mg P kg^{-1} soil) after MinP application was hence calculated as:

$$\text{Pdf seed} = \left[\left(1 - \frac{\text{SA}_{\text{plant MinP}}}{\text{SA}_{\text{plant NoP}}}\right) - \frac{\text{SA}_{\text{plant MinPdir}}}{\text{SA}_{\text{fert}}}\right] \times \text{P uptake}_{\text{MinP}} \qquad (3)$$

With this approach, Pdf seed was again estimated to be larger than the amount of P applied with the seeds (0.71±0.08 mg P kg^{-1}) on both soils. This confirms our assumption that the acid-washed sand still contained P, some P added with the two removed seeds had leaked to the soil, or more P might have been applied with the seeds than was determined. Pdf seed was estimated to be

0.85±0.59 mg P kg^{-1} on the unlimed soil and 1.63±0.42 mg P kg^{-1} on the unlimed soil (Table 1). Here, Pdf seed accounted for 8-15% of total P uptake on the unlimed soil and for 15-25% of total P uptake on the limed soil.

With this estimation approach, the complete difference in Pdf fertilizer between MinPdir and MinP is ascribed to Pdf seed. However, the difference in Pdf fertilizer between MinPdir and MinP could also be explained by unlabelled soil P contributing to the dilution of the specific activity in the plants, e.g. via mineralization of organic or microbial P during the indirect method (Oberson *et al.*, 2010). We assumed greater importance of microbial activity on the limed soil than on the unlimed soil, which could explain the larger Pdf seed on the limed compared with the unlimed soil. With this approach, there was no effect of soil pH on Pdf soil or Pdf fertilizer.

Table S1. Comparison of P derived from soil (Pdf soil) and fertilizer (Pdf fertilizer) without Pdf seed correction and with Pdf seed correction according to Oberson *et al.* (2010)

	No Pdf seed correction			Pdf seed correction according to Oberson *et al.* (2010)		
Treatment	Pdf seed	Pdf soil	Pdf fertilizer	Pdf seed	Pdf soil	Pdf fertilizer
Unlimed soil						
NoP	0	5.7 a		0.9	4.8 a	
MinP	0	5.6 a	4.6 a	0.9	5.6 a	3.8 a
Dairy manure	0	3.9 b	3.1 b	0.9	3.3 b	2.8 ab
Fish sludge	0	5.2 ab	2.3 b	0.9	4.4 ab	2.3 b
Meat bone meal	0	5.6 a	0.8 c	0.9	4.7 a	0.8 c
Wood ash	0	5.3 a	1.8 bc	0.9	4.5 ab	1.8 bc
SEM		0.3	0.3		0.3	0.2
HSD		1.4	1.4		1.4	1.1
MinPdir	0	6.5±0.6	3.8±0.2	0.9	5.7±0.4	3.8±0.2
Limed soil						
NoP	0	6.4 a		1.6	4.7 ab	
MinP	0	5.6 ab	5.2 a	1.6	5.6 a	3.6 a
Dairy manure	0	4.6 b	3.7 ab	1.6	3.4 b	3.3 a
Fish sludge	0	6.0 ab	2.7 bc	1.6	4.4 ab	2.6 ab
Meat bone meal	0	6.1 ab	1.5[a] c	1.6	4.5 ab	1.4[a] b
Wood ash	0	5.2 ab	2.3 bc	1.6	3.9 b	2.1 ab
SEM		0.4	0.4		0.3	0.4
HSD		1.6	1.7		1.5	1.6
MinPdir	0	7.5±0.7	3.8±0.3	1.6	5.9±0.7	3.8±0.3
Two-way ANOVA, source of variation						
Treatment		***	***		***	***
Soil		*	*		n.s.	n.s.
Treatment x soil		n.s.	n.s.		n.s.	n.s.

SEM = pooled standard error of the mean and HSD = Tukey's honest significant difference at each pH level, where values followed by the same letter are not significantly different. For MinPdir mean ± standard deviation of 4 replicates. *, **, *** significant at $p < 0.05$, 0.01 and 0.001 probability level, n.s. = not significant

[a] Only three observations due to Pdf fertilizer < 0 for replicate 4.