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From lab to field - investigations of phosphorus availability from sugarcane bagasse ash to soybeans

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As scientists, we step on the shoulders of science, building on the work that has come before us - aiming to inspire a new generation of young scientists to continue once we are gone.

Stephen William Hawking
⋆1942 †2018

Summary

Every year the Brazilian sugarcane industry generates up to 10 million tons (dry matter) of ash from the combustion of sugarcane bagasse, which is a fibrous plant material that remains after sugarcane juice extraction. Bagasse ash is alkaline and contains minerals, including phosphorus (P) and potassium (K). Bagasse ash as a P-fertilizer has been poorly investigated. The experiments conducted and presented in this dissertation were aimed at investigating the fertilization effects of bagasse and bagasse-based ashes following a "From Lab to Field" approach. Soybean (*Glycine max*) was chosen as a model plant because of its suitability for greenhouse and field experiments, relevance for the Brazilian economy and use as rotation crop after sugarcane harvest. The aims of the present study were i) to investigate the efficiency of bagasse ash as a fertilizer for soybeans, ii) to identify factors integral to its fertilization success, and iii) to analyse the potential of bagasse ash as an additive to conventional and organic fertilizers under field conditions.

In greenhouse pot experiments on nutrient-poor substrate, soybeans fertilized with bagasse ashes accumulated significantly less dry matter and took up less P and K than from triple-superphosphate (TSP) and potassium sulphate. The fertilization effects of bagasse ashes were driven by the availability of P, a result supported by solubility analyses in various extraction solutions. The application of large dosages of ash produced comparable amounts of biomass as TSP-fertilized plants, but the uptake of P remained significantly lower than that from TSP. This is potentially related to suboptimal concentrations and ratios of minerals supplied by large dosages of ash. Co-combustion of bagasse with chicken manure, which is poor in Fe and Al but rich in alkali and alkaline earth metals, altered the physical and chemical composition of the ashes and their thermochemical products. The resulting increase in availability of P to soybeans appears to be due to the formation of Ca-alkali phosphates, as determined by X-ray diffraction analyses. Under greenhouse conditions in Oxisol soil, the P-fertilization effects of bagasse-based ash and its thermochemical products on soybean remained significantly lower than from TSP. This was probably due to fewer soluble P-forms in ash as compared to TSP, an increase in pH and suboptimal nutrient ratios and nutrient concentrations; all of which affected the ability of this fertilizer to meet the P demand for soybeans. Under field conditions (Goiânia, Brazil), co-fertilization of soybeans with bagasse ash and TSP (70% of P from bagasse ash) allowed a reduction in the P_2O_5 consumption from TSP by more than 50% and increased the grain yield by 11%. TSP-free fertilization of soybeans with 80 kg P_2O_5 in form of bagasse ash and cattle manure compost (50% of P from bagasse ash) increased the grain yield by 13%. Both increases are in comparison to control fertilization using TSP alone.

Overall, fertilization effects of bagasse and bagasse-based ashes cannot compete with TSP. However, using bagasse ash as an additive to conventional and organic fertilizer has the potential to reduce the consumption of rock-P based fertilizer for soybeans in the field.

Zusammenfassung

Die brasilianische Zuckerrohrindustrie erzeugt jährlich bis zu 10 Millionen Tonnen Asche aus der Verbrennung von Zuckerrohrbagasse, der faserigen Pflanzenmasse, die nach der Extraktion des Zuckerrohrsaftes zurückbleibt. Bagasseasche ist alkalisch und enthält Mineralien, darunter Phosphor (P) und Kalium (K). Bagasseasche als P-Düngemittel ist bisher nur wenig untersucht worden. Daher zielen die in dieser Dissertation durchgeführten Experimente darauf ab, die Düngewirkung von Bagasse und Bagasse-basierter Asche auf die Modellpflanze Soja, zu untersuchen. Unter Verwendung der Modellpflanze Soja (*Glycine max*), waren die Ziele der vorliegenden Studie i) die Effizienz von Bagasseasche als Düngemittel für Sojapflanzen zu untersuchen, ii) Faktoren zu identifizieren, die für den Düngeerfolg entscheidend sind, und iii) das Potential von Bagasseasche als Zusatz zu konventionellen und organischen Düngemitteln unter Feldbedingungen zu analysieren.

In Gewächshausversuchen unter Verwendung von nährstoffarmem Substrat akkumulierten die mit Bagasseasche gedüngten Sojapflanzen signifikant weniger Trockensubstanz und nahmen weniger P und K auf als aus Triple-Superphosphat (TSP) und Kaliumsulfat. Die geringe Düngewirkung von Bagasseasche beruht hierbei auf der Pflanzenverfügbarkeit von P, die auch nach erhöhter Gabe von Aschen nicht mit der von TSP vergleichbar war. Durch die Co-Verbrennung von Bagasse mit Hühnertrockenkot, der reich an Alkali- und Erdalkalimetallen ist, konnte die physikalische und chemische Zusammensetzung der Asche und ihrer thermochemischen Produkte verändert werden. Hierdurch konnte auch ein Anstieg der P-Verfügbarkeit erreicht werden, was auf die Bildung von Ca-Alkaliphosphaten zurückgeführt wird, die mittels Röntgenstrukturanalysen identifiziert werden konnten. Auch in Topfversuchen unter Verwendung von Oxisol Boden, blieb die P-Düngewirkung von Bagasse-basierten Aschen und ihren thermochemischen Produkten signifikant hinter der TSP Düngung zurück. Dies lässt sich neben den weniger löslichen P-Formen in der Asche im Vergleich zu TSP auf einen Anstieg des Boden pH-Wertes und suboptimale Nährstoffverhältnisse und -konzentrationen zurückführen; all dies wirkte sich negativ auf die Fähigkeit dieser Düngemittel aus, den P-Bedarf der Sojapflanzen zu decken. Unter Feldbedingungen ermöglichte die Misch-Düngung von Sojapflanzen mit Bagasseasche und TSP (70% P aus Bagasseasche) jedoch eine Reduzierung des P_2O_5-Bedarfs aus TSP um mehr als 50% und erhöhte den Bohnenertrag um 11%. Die gänzlich TSP-freie Düngung von Sojapflanzen mit 80 kg P_2O_5 in Form von Bagasseasche und Rinderkompost (50% P aus Bagasseasche) erhöhte den Bohnenertrag um 13%. In beiden Fällen diente die Düngung mit TSP als Positivkontrolle.

Insgesamt können die Düngewirkungen von Bagasse und Bagasse-basierter Asche nicht mit dem hochwertigen TSP konkurrieren. Die Verwendung von Bagasseasche als Zusatz zu konventionellem und organischem Dünger hat jedoch das Potenzial, den Verbrauch von Düngern auf Rohphosphat-Basis für Sojapflanzen auf dem Feld zu reduzieren.

Abbreviations

BA$_G$	**b**agasse **a**sh sourced from unknown factories in **G**oiás state
BA$_{NG}$ ash	**b**agasse **a**sh sourced from sugarcane factory **N**ova **G**ália
BA$_{NG2}$/ CMC$_{50P:50P}$	blend of **BA$_{NG2}$ ash** with **CMC** (50% of P from BA$_{NG2}$ ash)
BA$_{NG2}$/ TSP$_{70P:30P}$	blend of **BANG2** ash with **TSP** (70% of P from BA$_{NG2}$ ash)
BA$_{SP}$	**b**agasse **a**sh sourced from unknown factories in **S**ão **P**aulo state
CA	**c**itric **a**cid
cBP ash	**c**ombusted **b**agasse **p**ellets ash
cBP/ SSL$_{83:17}$ ash	co-**c**ombusted **BA$_{NG}$**/ **s**ewage **sl**udge (83:17 by dry weight) ash
cBP/ CM$_{80:20}$ ash	co-**c**ombusted **BA$_{NG}$**/ **c**hicken **m**anure (80:20 by dry weight) ash
cBP/ CM$_{60:40}$ ash	co-**c**ombusted **BA$_{NG}$**/ **c**hicken **m**anure (60:40 by dry weight) ash
cBP/ CM$_{60:40}$+Na product	thermochemically treated **cBP/ CM$_{60:40}$** ash with **Na$_2$SO$_4$**
cBP/ CM$_{60:40}$+Na/K product	thermochemically treated **cBP/ CM$_{60:40}$** ash with **Na$_2$SO$_4$/K$_2$SO$_4$**
cBP/ TSP$_{45P:55P}$	blend of **cBP** ash with **TSP** (45% of total P from cBP ash)
CM	**c**hicken **m**anure
CMC	**c**attle **m**anure **c**ompost
d	**d**ay
EC	**e**lectric **c**onductivity
FA	**f**ormic **a**cid
gBP ash	**g**asified **b**agasse **p**ellets ash
gBP/ CM$_{85:15}$	co-**g**asified **b**agasse **p**ellets/ **c**hicken **m**anure (85:15 by dry weight)
GI	**g**ermination **i**ndex
GR	**g**ermination **r**ate
h	**h**our
ha	**h**ectares
K	**p**otassium (**K**)
L	**l**itre
Mt	**m**illion **t**onnes
N	**n**itrogen
NAC	**n**eutral **a**mmonium **c**itrate
P	**p**hosphorus
P$_{H2O}$	**p**hosphorus soluble in water (**H$_2$O**)
P$_{NH4Cl}$	**p**hosphorus soluble in 1 M **NH$_4$Cl**
P$_{NaOH}$	**p**hosphorus soluble in 0.1 M **NaOH**
P$_{residual}$	**p**hosphorus insoluble in any extraction solution (**residual**)
RAE	**r**elative **a**gronomic **e**ffectiveness
RE	**r**adicle **e**longation
RLF	**r**oot **l**ength **f**raction
RMF	**r**oot **m**ass **f**raction
RPE	**r**elative **p**hosphorus **e**ffectiveness
t	**t**onnes
TDM	**t**otal plant **d**ry **m**atter
TSP	**t**riple **s**uper**p**hosphate
WHC	**w**ater **h**olding **c**apacity
WIT	**w**ater **i**nfiltration **t**ime
WRaI	**w**ater **r**etention **a**fter **i**ncubation

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1 Introduction

1.1 Agriculture in Brazil

Brazil is an undisputed main player in the production of sugarcane and second largest producer of soybeans worldwide. Averaging over five years, i.e. 2014 to 2018, the Brazilian sugarcane and soybean industries produced more than 750 million tonnes (Mt) of sugarcane and 100 Mt of soybean on an area of around 10 and 3.5 million hectares (ha), respectively (FAOSTAT, 2020). These production amounts accounted for 40% of global sugarcane yield (FAOSTAT, 2020, Cherubin et al., 2019) and 30% of soybean yield (FAOSTAT, 2020). In the season 2018/ 2019, soybean production increased to more than 120 Mt, which was 37% of the global yield (Kuepper et al., 2019b). While sugar and ethanol, primarily fuel-ethanol, are produced from sugarcane (Sant'Anna et al., 2016, Cherubin et al., 2019), soybeans are used as animal feed and are processed to soymeal, edible oil and biodiesel (Kuepper et al., 2019b). Production of sugar, ethanol and soybean are not only aimed at internal markets but are exported across the world, including Europe (Sant'Anna et al., 2016, Kuepper et al., 2019b). Europe imported 17% of Brazilian soybeans in 2019 (Kuepper et al., 2019b) and is expected to increase the imports of sugar and ethanol once the free trade agreement between European Union and Mercosur states of South America is implemented (USDA, 2019). Subsequently, the sugarcane industry is of huge value to the Brazilian economy.

While 90% of Brazilian sugarcane is cultivated within the central-southern region of Brazil (Cherubin et al., 2015), around 40% of all soybeans are produced in the central region of Brazil (Kuepper et al., 2019b). In this region, the soils are mainly classified as Oxisol and Ultisol soils (Freitas et al., 2013), which are highly weathered (Schaefer et al., 2008, Freitas et al., 2013, Cherubin et al., 2015), acidic (Schaefer et al., 2008, Fageria and Baligar, 2008, Cherubin et al., 2015), rich in aluminium (Al) and iron (Fe) oxides (Schaefer et al., 2008, Freitas et al., 2013, Fageria and Baligar, 2008, Vendrame et al., 2011) and poor in native fertility. This is true especially for phosphorus (P), calcium (Ca) and magnesium (Mg) (Schaefer et al., 2008, Fageria and Baligar, 2008). Phosphorus occurs as bioavailable $H_2PO_4^-$ between pH levels 5.0 and 6.0 (Schachtman et al., 1998), and forms very stable and insoluble aggregates with Al and Fe oxides at more acidic soil conditions (Fageria and Baligar, 2008, Sanz-Saez et al., 2017). Subsequently, the soils need proper management, including liming and the application of fertilizers (Ferreira et al., 2012, Cherubin et al., 2015, Withers et al., 2018). Nonetheless, about 70-90% of P-fertilizers are absorbed and immobilized without any contribution to crop production (Fageria and Baligar, 2008, Sanz-Saez et al., 2017). In the case of sugarcane, farmers apply twice as much phosphorus fertilizer as would be required based on soil P content to maximize the yield (Withers

et al., 2018). Subsequently, the consumption of P-fertilizers in Brazil increased from 0.4 Mt in the 1960s when the sugarcane era began, to about 2.2 Mt in 2016 (Withers et al., 2018). In 2016 over 98% of P-fertilizers were attributed to fertilization of sugarcane, soybeans and maize (Withers et al., 2018). Successful recycling of P from agricultural waste streams would offer enormous economic benefits, as Brazilian agriculture imports about 60% of its P-fertilizers (Withers et al., 2018).

1.2 Sugarcane industry and its by-products

The sugarcane industry in Brazil produces huge amounts of waste, including bagasse, which is the lignocellulosic biomass remaining after sugarcane juice extraction (George Ochoa et al., 2010), vinasse, which is a liquid by-product of ethanol production (Prado et al., 2013), and filter cake, that precipitates during the clarification process of sugarcane juice (George Ochoa et al., 2010, Prado et al., 2013). Both vinasse and filter cake are considered fertilizers and are distributed onto the fields (Prado et al., 2013).

According to Withers et al. (2018), the sugarcane grows for 5 to 7 years and is harvested annually. In the past, the sugarcane fields were burned to remove leaf material, which greatly facilitated harvest, transportation, and processing of sugarcane stalks. Due to economic, environmental and legal issues, mechanized harvesting without burning of sugarcane is now widely adopted (Galdos et al., 2009). Subsequently, heavy machines harvest the sugarcane stalks and leave the tops starting from first fully formed node (Naseeven, 2001) as well as the leaves of the plants on the field (Figure 1 A and B i). Around 80% of sugarcane biomass arrives at the sugarcane mill (Almazan et al., 1998, Figure 1 B ii) and is crushed for sugarcane juice extraction (Figure 1 B iii). The lignocellulosic biomass remaining after sugarcane juice is extracted, the bagasse, accounts for around 23% of shoot biomass (Almazan et al., 1998, Figure 1 B iv and v). After juice clarification and ethanol production, the filter cake and vinasse that are produced are used as fertilizer (Prado et al., 2013, Figure 1 vi and vii). Bagasse is mainly combusted (Figure 1 B viii) to provide energy both for on-site use and for being sold to local grids (George Ochoa et al., 2010, Rocha et al., 2015, Andreão et al., 2019). After combustion, the bagasse ashes are washed out of the boilers and piped to settling tanks (Figure 1 B ix). After setting of the ash, water is removed (Figure 1 B ix) and the ashes are stored in the open environment (Figure 1 B x). In total, the amount of bagasse ash may account for up to 6 wt% of bagasse (Rocha et al., 2015). As such, on average 10 Mt of bagasse ash (dry weight) were produced annually between 2014 and 2018 (calculation was based on FAOSTAT, 2020, Almazan et al., 1998, Rocha et al., 2015). Biomass ashes are very poor in N (Knapp and Insam, 2011) but still contain varying amounts of other minerals, including K, P, Mg, Ca, which are relevant for the nutrition of plants (Demeyer et al., 2001, Knapp and Insam, 2011, Ferreira et al., 2012, Freire et al., 2015, Sharma et al., 2015, Thind et al., 2017, Gonfa et al., 2018), as well as toxic elements, such as Al (Freire et al., 2015). The concentration of P in bagasse ash was reported to range between 0.42 wt% to 2.14 wt% (Lopez et al., 2009, Ferreira et al., 2012, Pita et al., 2012, Akkajit et al., 2013, Sharma et al., 2015, Thind et al., 2017). Subsequently, in the period from 2014 to 2018, between 4

x 10^4 and 2 x 10^5 t of P remained annually in bagasse ash. There is no common use of bagasse ash and it is either used as partial replacement for cement (Andreão et al., 2019), as fertilizer, or as waste (George Ochoa et al., 2010).
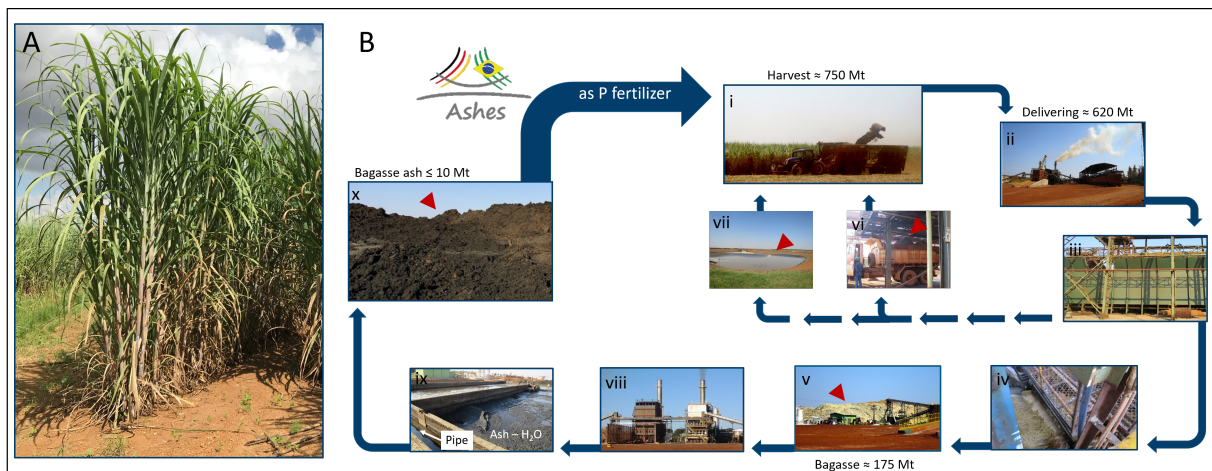
Figure 1: From sugarcane (A) to bagasse ash. (B) Process of sugarcane harvest, processing and production of by-products. (i) Harvest, (ii) delivering at sugarcane factory, (iii) crashing of sugarcane stalks, (iv) sugarcane juice extraction, (v) bagasse as by-product (red arrow points at bagasse), (vi) filter cake as by-product (red arrow points at filter cake), (vii) vinasse as by-product (red arrow points at vinasse), (viii) bagasse combustion for energy production, (ix) settling tanks of bagasse ash after removal from the boiler, and (x) bagasse ash as final by-product of sugarcane processing (red arrow points at bagasse ash).

1.3 Ash as fertilizer for plants

Biomass ash is known more as a potassium (K) rather than as a P-fertilizer, because of their contrasting solubilities (Demeyer et al., 2001). Li et al. (2017) investigated the solubility of K from varying ashes and biochars and concluded that these can be used as K fertilizer, albeit with slightly lower short-term availability than from KCl. However, Tran et al. (2018) reported comparable availability of K from cattle manure ash for Guinea grass as from KCl. Contrary to K, only 1% and 40% of total P was soluble in water and in citric acid, respectively (Tran et al., 2018). Phosphorus soluble in water and citric acid represent the available pool of P from the fertilizer(s) to plants (EU, 2003). The availability of P from cattle manure ash for Guinea grass was very low after 25 d and increased after 25 d and 50 d of growth. Guinea grass plants took up significantly less P from cattle manure ash than from triple-superphosphate after 100 d growth (Tran et al., 2018).

Studies of bagasse ashes as fertilizers are limited. Positive effects due to fertilization with bagasse ash and bagasse-based ashes were reported for lettuce (Lopez et al., 2009), corn (Pita et al., 2012), sugarcane (Akkajit et al., 2013), wheat (Thind et al., 2017, Gonfa et al., 2018) and bean (Webber III et al., 2017). These were grown in varied rooting

media, such as greenhouse substrate, nitisol, loamy sand, substrate consisting of soil and compost. Independent of the plant and growing medium, applications of bagasse ash and bagasse-based ashes significantly increased the uptakes of P and K, and/ or the biomass accumulations and yields of grains compared to unfertilized plants. The fertilization effects increased with the dosage of bagasse ashes applied (Webber III et al., 2017, Gonfa et al., 2018). However, all ashes were produced under undefined conditions from uncharacterized bagasse and biomass feedstocks. Furthermore, no controls with mineral P-fertilizers were carried out. Comparison between these studies is confounded by ash type, experimental setup, and plant species.

1.3.1 Factors affecting the solubility and availability of P from ashes for plants

The solubility and availability of P from ash for plants depends on various factors, including biomass feedstock (Demeyer et al., 2001, Schiemenz and Eichler-Löbermann, 2010) and its chemical composition (Smith et al., 2002, Wang et al., 2012, Müller-Stöver et al., 2018), thermal conditions of the fuel processing environment, and collection and storage of the ashes (Demeyer et al., 2001). Subsequently, the availability of P from the ashes for the plants can be manipulated in different ways.

During thermal processing of biomass, P oxides condense at temperatures between 400 and 600 °C and can remain as P oxide or, more frequently, form orthophosphoric acid, H_3PO_4, in the presence of water (Beck and Unterberger, 2006). Orthophosphoric acid is soluble in water and forms orthophosphate, $H_2PO_4^-$, at certain pH levels (Schachtman et al., 1998). Orthophosphate is the P-form taken up by the plants (Schachtman et al., 1998, Hawkesford et al., 2012, Młodzińska and Zboińska, 2016, Zogli et al., 2017). At higher processing temperatures, vaporized P oxides undergo various chemical reactions with other metal vapours, (including Zn, Fe, alkali (Na, K), and earth alkaline metals (Ca, Mg)), and homo- and heterogeneous coagulation to form a mixture of their oxides and phosphates (Tan and Lagerkvist, 2011). Ca, as well as Fe and K, have strong tendencies to form phosphates (Tan and Lagerkvist, 2011) with decreasing solubility and availability to plants (Qian et al., 2009, Qian and Jiang, 2014). Furthermore, sintering (Wang et al., 2011) and agglomeration of melted particles may occur (Tan and Lagerkvist, 2011, Wang et al., 2011). This increases the size of the particles, which negatively influences the availability of P from the ashes for plants (Tan and Lagerkvist, 2011). According to Tan and Lagerkvist (2011), large particles composed of apatite, which has low solubility in water and is poorly available to plants (Tan and Lagerkvist, 2011). Apatite is formed at combustion temperatures above 700 °C (Thygesen et al., 2011), and may be made up of 80% of all P in the ash (Tan and Lagerkvist, 2011). However, the physical environment, including O_2 partial pressure and temperature, inside and around individual biomass particles may vary during the combustion process (Boström et al., 2011), which complicates the control of combustion environment. Mixing of biomasses can prevent the agglomeration up to above 1020 °C (Tan and Lagerkvist, 2011) increasing the availability of P to plants. Ferreira et al. (2012) investigated the effect of co-combustion of bagasse

with bovine residues and reported a significant increase in soluble P due to co-processing of the feedstocks.

In a thermochemical post-treatment, the ashes are combusted with additives, such as Na_2SO_4 and K_2SO_4, at temperatures between 900-1000 °C, which alters the composition of crystalline P compounds (Herzel et al., 2016, Steckenmesser et al., 2017, Vogel et al., 2018). Thermochemical post-treatments were reported to increase the solubility and subsequently the availability P from the ashes for plants (Severin et al., 2014, Steckenmesser et al., 2017, Vogel et al., 2018).

1.4 Phosphorus as a nutrient for plants

Phosphorus is the second most frequently limiting macronutrient for plant growth (Schachtman et al., 1998). It is used to generate energy rich compounds, such as ATP and $NADP^+$, is a compound of nucleic acids and phospholipids, and regulates enzyme activity and various metabolic and catabolic reactions (Vance et al., 2003, Hawkesford et al., 2012). Energy rich compounds are also needed to assimilate nutrients, such as N and S (Hawkesford et al., 2012), and are needed for fixation of N from atmospheric N_2 (Zogli et al., 2017).

At luxury conditions where more P is available than needed for plant growth, plants accumulate and store it, allowing the use of these P stores at deficient conditions, thus ensuring continued functionality of P dependent processes in the plant (Hawkesford et al., 2012). At P deficient conditions, the uptake of P decreases (Gutiérrez-Boem and Thomas, 1999), affecting metabolism of P and energy dependent processes, including assimilation of N and S (Zogli et al., 2017, Cooper and Scherer, 2012, Młodzińska and Zboińska et al., 2012, Hachiya and Sakakibara, 2017), and the vegetative and generative growth (Gutiérrez-Boem and Thomas, 1999, Hawkesford et al., 2012). Plants respond to this deficiency with alterations in morphology to ameliorate the uptake of P. Leaf surface area and the number of leaves decrease due to limited availability of P (Gutiérrez-Boem and Thomas, 1999, Hawkesford et al., 2012), while roots continue to grow, resulting in larger biomass allocation to the roots compared to shoot organs (Vance et al., 2003, Gutiérrez-Boem and Thomas, 1999, Hawkesford et al., 2012). The primary root length, root branching, number and length of lateral roots, and the formation of root hairs increase (Zogli et al., 2017). This increases the root length density (Vance et al., 2003, Schachtman et al., 1998, Gutiérrez-Boem and Thomas, 1999) and increases the surface of exchange between the roots and the rhizosphere (Zogli et al., 2017). The adaptation of root morphology at P deficient conditions is in response to the slow diffusion of P in soil (Schachtman et al., 1998). In addition to these morphological changes, plants exude protons and acidify the soil, as well as phosphatases and organic acids which pass into the rhizosphere to mobilize fixed P directly or indirectly by recruiting microorganisms (Haynes, 1983, Schachtman et al., 1998, Hocking, 2001, Hawkesford et., 2012, Tawaraya et al., 2014). All these plant responses can be monitored or used as markers for plant P uptake or availability. This is especially important when dealing with a P-source with unknown or not well known P-forms, such as calcium phosphate silicate, sodium calcium

magnesium phosphate, and hydroxyapatite in wood ash or calcium dipotassium diphosphate struvite-K, ß-Lomonosovite and whitlockite in cereal ash (Brod et al., 2015).

1.5 Objective of the research

The aim of this thesis was to investigate the availability of P from differently processed bagasse and bagasse-based ashes to soybeans. Based on the assumption that the availability of P from bagasse ash can be modified by manipulating the processing environment, modifying the biomass and by employing thermochemical post-treatments of the ash, the collaborative research project "ASHES", comprising partners from Brazil and Germany, aimed to produce a bagasse-based P fertilizer. This was done with the goal of decreasing the dependency on imported P and, ideally, closing the local nutrient cycle of P.

Soybean was chosen as a model crop because of its rapid growth, ability to grow under greenhouse conditions in Germany and field conditions in Brazil, and great value for Brazilian economy (see above). In Brazil, soybeans are planted every 5 – 7 years between sugarcane rotations to restore soil fertility (Bordonal et al., 2018, Withers et al., 2018). Germany depends almost entirely on imported soybeans (FAOSTAT, 2020) using them, among other purposes, for livestock and biodiesel production (Kuepper and Riemersma, 2019a).

The "From Lab to Field" approach was chosen to analyse the bagasse ash and bagasse-based ashes as potential fertilizers for soybean. The experiments are to be performed under highly controlled and reproducible conditions in the greenhouse using both a P-poor substrate as well as Oxisol soil from field sites in Brazil (Figure 2 A-B). The insights gained from experiments are then to be evaluated in experiments under field conditions in Brazil (Figure 2 C). Analyses of P-form diversity and solubility from field and lab experiments, will be instrumental in understanding the mechanisms of P availability from ashes to soybeans.

Partners from research project ASHES combusted (FRAUNHOFER UMSICHT, Sulzbach-Rosenberg, Germany) and gasified (CUTEC, Clausthal-Zellerfeld, Germany) sugarcane bagasse pellets under defined thermal conditions. This was undertaken with bagasse either as solitary fuel or in combination with chicken manure or sewage sludge to produce bagasse-based ashes and gasification residues. Chicken manure is a practical co-processing feedstock because Brazil is the largest chicken producer in South America (FAOSTAT, 2020). In Brazil, chicken manure is applied to the soils as organic fertilizer (Rogeri et al., 2016, Withers et al., 2018), and has been co-pyrolysed with various mineral sources of P and Mg to produce a slow-release fertilizer for tropical soils (Lustosa Filho et al., 2017). As Brazil produces around 60% of all cattle in South America (FAOSTAT, 2020), another nutrient rich material in Brazil is cattle manure compost. This is to be used as organic co-fertilizer of bagasse ash under field conditions. Project partners from FRAUNHOFER UMSICHT (Sulzbach-Rosenberg, Germany) and BAM (Berlin, Germany) produced fertilizer products based on the information gained from field experiment, which in this study are tested under greenhouse conditions.
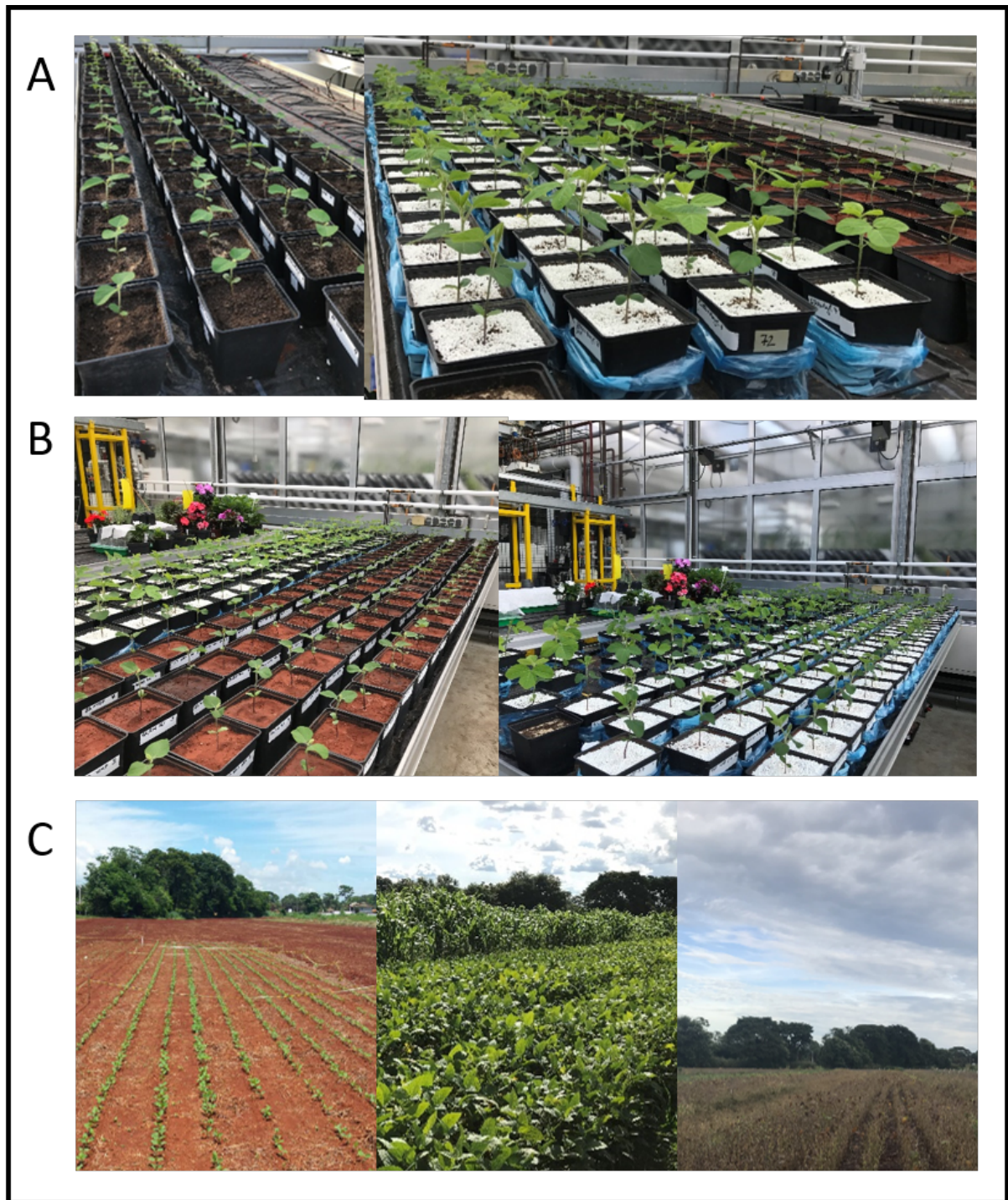
Figure 2: "From Lab to Field" approach. (A) Soybean growth on nutrient-poor substrates and (B) Oxisol soil under greenhouse conditions at Forschungszentrum Jülich, IBG-2: Plant Sciences, and (C) soybean growth in the field at Brazilian Federal University of Goiás (**U**niversidade **F**ederal de **G**oiás, UFG, Goiânia, Brazil).

Objectives of the present thesis are:

 (i) To identify factors determining fertilization effects of various bagasse and bagasse-based ashes to soybean in a nutrient-poor substrate under greenhouse conditions.

 (ii) To investigate the effects of both co-processing bagasse with nutrient-rich residues and performing subsequent thermochemical treatments on the availability of P to soybeans in an Oxisol soil environment under greenhouse conditions.

(iii) To evaluate the fertilizer potential of bagasse ash as additives to mineral (triple-superphosphate) and organic (cattle manure compost) fertilizers under field conditions in Brazil.

1.5.1 Hypotheses

The hypotheses are as follows:

 (i) The fertilization effects of bagasse ash are driven by the P and K available to soybeans. The relative agronomic effectiveness of P and K from the ash (uptake of P and K from the ash relative to P and K uptakes from TSP and potassium sulphate) will be comparable to P and K from TSP and potassium sulphate.

 (ii) Modifying processing conditions of bagasse ash production will also modify plant-available P. As bagasse has a low total P content, this is expected to be insufficient to produce fertiliser.

(iii) The total P in ash and P-forms can be modified by co-processing bagasse with nutrient rich residues and / or employing thermochemical treatments. This is expected to result in increases in P availability and, therefore, improved fertilizer efficiency.

(iv) Application of bagasse ash, along with its blends containing P-rich sources will allow a decrease in the use of conventional P-fertilizers under field conditions. This is expected due to higher pH in Oxisol soil and longer P-availability due to slow release of P from the ash.

2 Material and Methods

2.1 Fertilizers

Four lots of **b**agasse **a**shes (BA) from Brazilian sugarcane factories were used. Two of these lots were sourced from unknown factories located in the states of **G**oiás (BA$_G$) and **S**ão **P**aulo (BA$_{SP}$), Brazil, whereas the other two were sourced from a sugarcane factory named Usina **N**ova **G**ália Ltda. (Paraúna, Brazil). Subsequently the ashes are named BA$_{NG1}$ and BA$_{NG2}$. At the sugarcane factory Usina Nova Gália Ltda., bagasse was combusted at around 1050 °C under standard steam pressure of 41.16 bar. The combustion capacity of the boiler was 130 t h^{-1}. Bagasse ash and the remains from the combustion were washed out of the boiler with water and piped into sedimentation tanks (Figure 1 B ix). After settling, the water was removed and bagasse ash was stored on the heap in an open environment (Figure 1 B ix and x). The BA$_{NG1}$ ash was dried at 60 °C (TR 1050, Nabertherm GmbH, Lilienthal, Germany) until it reached a constant weight prior to using it as fertilizer in experiments under greenhouse conditions. The BA$_{NG2}$ ash was used directly on the field in Brazil without any processing (around 40 wt% water). The ashes contained 0.38 wt% and 0.94 wt% P, 1.21 wt% and 2.20 wt% K, and 5.05 wt% and 41.00 wt% carbon, respectively (for more information see 1).

The biomass feedstocks, i.e. bagasse pellets, chicken manure pellets and sewage sludge, used for the production of bagasse and bagasse-based ashes are described in Table S1. Bagasse pellets (Usina Nova Gália Ltda., Paraúna, Brazil), chicken manure pellets (Jürgen Abeler GmbH & Co. KG, Nordwalde, Germany) and sewage sludge (Hohenburg, Landkreis Amberg-Sulzbach, Germany) contained 7 wt%, 10 wt% and 14 wt% water, respectively, and were stored sheltered from the rain. In a grate furnace (HDG Compact 100 (100 kW thermal output), HDG Bavaria GmbH, Massing, Germany), the **b**agasse **p**ellets were **c**ombusted as single fuel (cBP) and as blends (co-combustion) with **c**hicken **m**anure pellets (CM) in ratios of 80:20 (cBP/ CM$_{80:20}$) and 60:40 (cBP/ CM$_{60:40}$), as well as with **s**ewage **sl**udge in a ratio of 83:17 (cBP/ SSL$_{83:17}$) by dry weights (Martin Meiller, FRAUNHOFER UMSICHT, Sulzbach-Rosenberg, Germany). The retention time in the grate was around 45 minutes. The temperature on the fire bed ranged from 400 to 800 °C under oxidizing conditions ($\lambda = 2.2$). The ashes contained 0.24 wt% to 4.00 wt% P and 1.30 wt% to 3.15 wt% K. The ashes contained 0.62 wt% to 2.80 wt% carbon (for more information see 1). In agreement with Wang et al. (2011), the ashes were coarse and contained sintering and melting compounds (Figure 15 A).

Based on Vodegel and Müller (2019), **b**agasse **p**ellets (BP) and the blend with **c**hicken **m**anure (CM) pellets in a ratio of 85:15 by dry weight (BP/ CM$_{85:15}$) were **g**asified in a circulating fluidized bed gasifier (ArtFuel-Gasifier, T & M Engineering, Bad Frankenhausen,

Table 1: Chemical compositions of bagasse and bagasse-based ashes. Elementary analyses of ashes from 1-3 and 5-9 were conducted by Felix Müller (CUTEC, Clausthal-Zellerfeld, Germany), 10-13 by Hannes Herzel (BAM, Berlin, Germany) and 4 by project partners from Universidade Federal de Goiás (UFG, Goiânia, Brazil). Bagasse ashes 1 and 2 were sourced from unknown factories located in Goiás (BA_G) and São Paulo (BA_{SP}) states, bagasse ashes 3 and 4 from a sugarcane factory named Usina Nova Gália Ltda. (BA_{NG}), 5 and 6 from combustion and gasification of bagasse pellets (cBP and gBP), respectively, ash 7 was sourced from co-gasification of bagasse pellets with chicken manure in a ratio of 85:15 by dry weight (gBP/ $CM_{85:15}$), ashes 8, 9 and 10 were sourced from co-combustion of bagasse pellets with sewage sludge (83:17 by dry weight, cBP/ $SSL_{83:17}$), and chicken manure (80:20 by dry weight, cBP/ $CM_{80:20}$, and 60:40 by dry weight cBP/ $CM_{60:40}$), cBP/ $CM_{60:40}$ ash was thermochemically treated with Na_2SO_4 (cBP/ $CM_{60:40}$+Na) and Na_2SO_4/ K_2SO_4 (cBP/ $CM_{60:40}$+Na/K). The ashes were measured for total carbon (C), nitrogen (N), phosphorus (P), potassium (K), copper (Cu), manganese (Mn), sodium (Na), sulphur (S), zinc (Zn), calcium (Ca), magnesium (Mg), iron (Fe), aluminium (Al), silicon (Si), extractable P in citric acid (CA), and pH values in 0.01 M $CaCl_2$ solution (1:2.5, w/v). Total and extractable P as well as pH value in 0.01 M $CaCl_2$ solution (1:2.5, w/v) were measured in triple-superphosphate (TSP).

Fertilizer	C wt%	N wt%	P wt%	K wt%	Cu wt%	Mn wt%	Na wt%	S wt%	Zn wt%	Ca wt%	Mg wt%	Fe wt%	Al wt%	Si wt%	pH	Extractability of P in CA % of total P
1. BA_{SP}	6.68		0.38	2.01	0.01	0.08	0.13	0.12	0.01	1.25	0.70	1.40	1.49	34.18		
2. BA_G	36.48		0.34	1.21	0.01	0.06	0.05	0.12	0.01	1.14	0.44	5.65	3.46	18.42		
3. BA_{NG1}	5.05	0.10	0.38	1.21	0.01	0.07	0.10	0.16	0.01	1.26	0.46	5.38	3.86	14.62	7.12	56
4. BA_{NG2}	41.00	1.06	0.94	2.20				0.51		1.60	0.17				7.04	
5. cBP	0.62	0.04	0.24	1.30	0.01	0.09	0.04	0.01	0.00	0.93	0.52	3.80	2.76	28.93	9.12	19
6. gBP	53.70	0.05	0.41	1.04	0.01	0.05	0.04	0.04	0.04	0.99	0.57	3.32	2.98	0.56	9.91	86
7. gBP/ $CM_{85:15}$	40.52	0.08	1.66	2.41	0.01	0.11	0.19	0.07	0.04	5.15	1.14	2.46	2.72	0.72	11.45	92
8. cBP/ $SSL_{83:17}$	0.21	0.02	3.02	0.64	0.14	0.06	0.11	0.15	0.11	3.99	0.67	3.86	4.18	0.01	7.72	38
9. cBP/ $CM_{80:20}$	1.07	0.03	2.05	3.15	0.01	0.10	0.24	0.31	0.03	9.12	1.01	1.44	1.14	0.04	12.68	83
10. cBP/ $CM_{60:40}$	2.80		4.00	5.2			0.20	0.40		15.20	1.80	2.00	1.60	17.00	12.70	92
11. cBP/ $CM_{60:40}$+Na	0.00		3.20	4.40			3.10	4.60		12.40	1.50	1.50	1.30	15.80	9.50	100
12. cBP/ $CM_{60:40}$+Na/K	0.00		3.10	6.80			2.50	5.00		11.80	1.40	1.40	1.20	14.90	9.30	100
13. TSP			19.86												2.60	94

Germany, modified by CUTEC, Clausthal-Zellerfeld, Germany) at around 800 °C under reducing conditions ($\lambda = 0.4$; Felix Müller, CUTEC, Clausthal-Zellerfeld, Germany). The resulting gasification residues (gBP and gBP/ CM$_{85:15}$), further named ashes, contained 0.41 wt% and 1.66 wt% P, 1.04 wt% and 2.41 wt% K, and 53.70 wt% and 40.52 wt% carbon, respectively (for more information see Table 1).

Ash from co-combustion of bagasse pellets with chicken manure, i.e. cBP/ CM$_{60:40}$, was thermochemically post treated with Na$_2$SO$_4$ and Na$_2$SO$_4$/K$_2$SO$_4$ (Hannes Herzel, BAM, Berlin, Germany). cBP/ CM$_{60:40}$ ash was mixed with 23 wt% milled chicken manure as a carbon source to achieve the reducing atmosphere required for the thermochemical process (Stemann et al., 2015, Herzel et al., 2016). Thus, 30 g of **cBP/ CM$_{60:40}$** ash and 9 g of chicken manure were either mixed with 13.5 g **Na$_2$**SO$_4$ (cBP/ CM$_{60:40}$+Na) or with 11 g **Na$_2$**SO$_4$ and 4.5 g **K$_2$**SO$_4$ (cBP/ CM$_{60:40}$+Na/K) and treated in corundum crucibles with lids at 1000 °C for 30 minutes in a stationary furnace (LH 15/14, Nabertherm GmbH, Lilienthal, Germany). cBP/ CM$_{60:40}$ ash and cBP/ CM$_{60:40}$+Na and cBP/ CM$_{60:40}$+Na/ K thermochemical products contained 3.10 wt% to 3.20 wt% P and 4.40 wt% to 6.80 wt% K (for more information see 1).

Bagasse-based fertilizer products for soybeans were produced by project partners (Hannes Herzel, BAM, Berlin, Germany, and Martin Meiller, FRAUNHOFER UMSICHT, Sulzbach-Rosenberg, Germany). Bagasse ash, which was combusted at 400 - 800 °C (data not shown) was mixed with chicken manure in a ratio of 90:10 by dry weight and either pelletized (PP120B, qteck GmbH, Germany) or granulated (R02 Maschinenfabrik Gustav Eirich GmbH & Co KG, Germany). Water was used as binder solution. While granule products were dried at 110 °C over night and sieved to reach sizes between 2 mm and 6.3 mm, the pellet products were dried at room temperature and were ≤16 mm x 0.6 mm. Final granules and pellets contained 0.70 wt%, 0.40 wt% and 0.80 wt% N, P and K, respectively. The ratio of P to K was similar to the optimum nutrient ratio for soybeans growing in Oxisol soils in the region of central-west of Brazil (FAO, 2004). Bagasse ash/ chicken manure-based pellets were more stable to mechanical disruption than granules, i.e. 88% and 37.3% of all pellets and granules presented the initial shapes after mechanical disruption, respectively (Stahl et al., 2018).

While all ashes were milled (≤250 μm, Retsch Z 1, Retsch GmbH, Haan, Germany) prior to application as fertilizer, bagasse-based granules and pellet products were used without processing.

Triple-superphosphate (TSP, Triferto Fertilizers, Doetinchem, Netherlands), potassium-sulphate (K$_2$SO$_4$, Carl Roth GmbH + Co. KG, Karlsruhe, Germany) and ammonium nitrate, Carl Roth GmbH + Co. KG, Karlsruhe, Germany) were used as P-, K- and N-fertilizers under greenhouse conditions and K$_2$SO$_4$ (Haifa SOP, São Paulo, Brazil) and urea (unknown producer) were used under field conditions in Brazil. In one of the experiments, modified 1/3-strength Hoagland's solution, which contained all plant-relevant minerals but no P, was used as additional mineral source (Table S2).

2.2 Phosphorus analyses in the ashes

Phosphorus analyses were based on powder X-ray diffraction (XRD) measurements, ^{31}P NMR spectroscopy, sequential extraction of P and extractions using varying solutions, including formic acid, neutral ammonium citrate and/ or citric acid. X-ray diffraction measurements and the extractions of P in citric acid, formic acid and neutral ammonium citrate were conducted by Hannes Herzel (BAM, Berlin, Germany). ^{31}P NMR spectroscopy and sequential extraction of P were conducted in collaboration with Dr. Sabine Willbold and Dr. Volker Nischwitz (ZEA-3, FZJ, Jülich, Germany). The term "P-forms" includes different crystalline P compounds, P-species and interactions with other ions, such as Ca, Mg, Fe and Al, which were determined by XRD, ^{31}P NMR and sequential extractions, respectively.

2.2.1 X-ray diffraction spectroscopy

Powder X-ray diffraction measurements were based on the protocol described by Herzel et al. (2020). In brief, the measurements of the ashes were performed in Bragg-Brentano geometry over a 2θ range from 5° to 80°, with a step size of 0.02° (D8 Advance, Bruker AXS, Germany). The diffraction patterns were collected using Cu Kα1 and Cu Kα2 (λ1 = 1.54056 Å, λ2 = 1.54443 Å) radiation. The diffraction patterns were recorded with a Lynxeye detector.

2.2.2 ^{31}P NMR spectroscopy

The P-species were analysed using ^{31}P NMR spectroscopy (Bruker 600-MHz spectrometer), which was based on the protocol published by Vestergren et al. (2012). Briefly, P was extracted by shaking 1.5 g of dry BA_{NG1}, cBP, cBP/ $CM_{80:20}$, and cBP/ $SSL_{83:17}$ ashes and 1.0 g of gBP and gBP/ $CM_{85:15}$ ashes in 30 ml extraction solutions (0.25 M NaOH and 50 mM Na_2EDTA,) for 16 h. Lower amounts of gasified ashes, i.e. gBP and gBP/ $CM_{85:15}$ ashes, were due to larger volumes compared to combusted BA_{NG1}, cBP, cBP/ $CM_{80:20}$, and cBP/ $SSL_{83:17}$ ashes.

Afterwards, the samples were centrifuged (30 min, 14000 g) and the supernatants were frozen at 80 °C and lyophilized. 200 mg of freeze-dried solids were dissolved in 500 μl of a mixture of NaOD and D_2O at pH 13, and 100 μl of methylenediphosphonic acid (MDPA, 0.84 mg/mL in D_2O) was added as internal reference. The concentration of MDPA was kept at a similar order of magnitude as the signals in the spectra (Cade-Menun and Liu, 2014). ^{31}P NMR spectra were obtained using a Bruker Avance III 600 MHz spectrometer (Bruker Biospin, Rheinstetten, Germany). The NMR parameters used were: 32 K data points, 1 s repetition delay, 0.7 s acquisition time, 30° pulse width and 24576 scans (Jiang et al., 2017). The ^{31}P NMR spectroscopy was conducted in collaboration with Dr. Sabine

Willbold (ZEA-3, FZJ, Jülich, Germany).

2.2.3 Sequential P extraction

Sequential extractions of P from BA_{NG1}, cBP, gBP, gBP/ $CM_{85:15}$, cBP/ $CM_{80:20}$ and cBP/ $SSL_{83:17}$ ashes followed the protocol reported by Hieltjes and Lijklema (1980) as modified by Qian et al. (2009). To do this, P from 500 mg of the ash was sequentially extracted with 20 ml of DDI water (P_{H2O}), 1 M NH_4Cl at pH 7 (P_{NH4Cl}), 0.1 M NaOH (P_{NaOH}) and two times with 0.5 M HCl (P_{HCl}), respectively. The samples were shaken for 2, 2, 17, and 2x 24 h, respectively, and centrifuged at 15000 g for 5 min before transferring the supernatants in new tubes. Phosphorus in supernatants was measured using ICP-OES according to Brod et al. (2015). Insoluble P ($P_{residual}$) was calculated as the difference of total P and the sum of extracted P (Qian et al., 2009). According to Qian et al. (2009), P_{NH4Cl} is thought to represent labile P, which may replace ions lost from the system (Ebelhar, 2008), P_{NaOH} represents Fe-/ Al-bound P, P_{HCl} represents Ca-/ Mg-bound P, and $P_{residual}$ insolubles P, which may also contain organic P-forms. Labile P is available to plants in the short term, whereas Fe-/ Al-bound P is thought to be available to plants bit longer term (Qian and Jiang, 2014). Ca-/ Mg-bound P is very difficult for plants to be absorbed directly (Qian and Jiang, 2014). The analyses were conducted by Dr. Volker Nischwitz (ZEA-3, FZJ, Jülich, Germany).

2.2.4 Solubility of P in citric acid, formic acid and neutral ammonium citrate

Phosphorus soluble in 2% citric acid (CA) was extracted from BA_{NG1}, cBP, cBP/ $CM_{80:20}$, cBP/ $CM_{60:40}$, cBP/ $SSL_{83:17}$, gBP and gBP/ $CM_{85:15}$ ashes as well as TSP for use as reference. Phosphorus soluble in neutral ammonium citrate (NAC) and 2% formic acid (FA) was only extracted from cBP/ $CM_{80:20}$ and cBP/ $CM_{60:40}$ ashes, cBP/ $CM_{60:40}$+Na and cBP/ $CM_{60:40}$+Na/K thermochemical products, and TSP. The extractions were carried out according to the EU regulation No. 2003/2003 (EU, 2003) in duplicates. In brief, the ratios of ash to extraction solutions were 1:100 and the extraction periods were 30 min for the extractions in CA and FA and 60 min in FA (for more information see Herzel et al., 2020).

2.3 Greenhouse experiments

2.3.1 Conditions and location

The experiments were conducted under greenhouse conditions at the Institute of Bio- and Geosciences, Plant Sciences (IBG-2, Forschungszentrum Jülich, Germany, 50°54'36"N, 6°24'49"E) over the period from August 2016 to August 2018. The plants received 16 h light day^{-1} from natural and artificial light sources regulated by an automated light system. The artificial light was produced by sodium vapour lamps (SON-T AGRO 400, Phillips), which ensured a minimum irradiance of 400 μmol s^{-1} m^{-2}. Air temperature, CO_2 and humidity concentrations were 24-25 °C/ 15-20 °C (day/ night), around 420 ppm, and 42-52%/ 59–67% (day/ night). In total, plants received 72-210 mmol day^{-1} m^{-2} light irradiance depending on the experiment. The pots were covered with plastic bags to avoid leaching of nutrients and the nutrient-poor substrate and Oxisol soil were covered with white plastic granulate to reduce evaporation of water. The plant locations were randomized every week.

2.3.2 Plants

Two plant species, i.e. garden cress (*Lepidium sativum*) and soybeans (*Glycine max* (L.) *Merr.* var. RGT Shouna), were used as model plants in greenhouse experiments. RGT Shouna is assigned to maturity groups 5 and was expected to complete the life cycles 140 days (d) after sowing (Thüringer Landesamt für Landwirtschaft und Ländlichen Raum, 2019). Rapid development during the juvenile stage is characteristic of RGT Shouna (Thüringer Landesamt für Landwirtschaft und Ländlichen Raum, 2019). Soybeans were selected twice before starting the fertilization treatments. First, soybeans germinated on moist filter paper in the dark (covered with aluminium foil) at 24–25 °C for 3 d. Afterwards, seedlings with comparable radicle lengths were transplanted in nutrient-poor substrate. In the stage of fully expanded unifoliate leaves, the roots were washed and only plantlets with comparable heights and leaf areas were inoculated with N_2 fixing *Bradyrhizobium japonicum* (NPPL HI Stick, BASF SE, Ludwigshafen, Germany) and transplanted in pots containing different fertilization treatments. Inoculation of soybean roots with rhizobia after and not prior pre-cultivation was because the nutrient-poor substrate was removed during transplantation process. Soybeans were selected twice before starting fertilization treatments because different germination of soybeans in treated rooting media would lead to different phenotypes of the plantlets from beginning of the experiments.

2.3.3 Rooting media

Filter papers (Whatman, type 589), sand (as used in a previous study: particle size ≤ 1 mm and no detectable amounts of plant nutrients ; Nabel et al., 2018), blends of sand and garden substrate (substrate type: "*Null-Erde*", Balster Einheitserdewerk, Fröndenberg, Germany) mixed in a 1:1 ratio by volume, Oxisol$_1$ soil (46.3 wt% clay ($<$0.002 mm), 10.8 wt% silt (0.00-0.063 mm), and 42.8 wt% sand (0.063-2.0 mm)), and Oxisol$_2$ soil (38.8 wt% clay, 16.8 wt% slit and 44.5 wt% sand) were tested (2.3.3.1) or used as rooting media in greenhouse experiments (2.3.4.2, 2.3.5 and 2.3.6). The Oxisol soil sourced from natural and unfertilized area at Brazilian Federal University of Goiás (Universidade Federal de Goiás, UFG, Goiânia, Brazil) and was sieved to select particles ≤ 3 mm before using.

"*Null-Erde*" substrate was shredded (ES 300-E Roxor, FREYMATIC AG, Domat/Ems, Switzerland) and sieved (Berkili Siebmaster 1, SM1E, 18 x 8 mm sieve, HOWA parts, Warstein, Germany) prior blending it with sand in a concrete mixer (ATIKA 322050, Altrad Lescha Atika GmbH, Burgau, Germany). Subsequently, the particles were ≤ 1.44 cm^2 and the blend of sand and "*Null-Erde*" substrate, further named nutrient-poor substrate, contained $<$0.03 wt% P and 1.27 wt% K (Table 2). The pH averaged 6.4 in nutrient-poor substrate and 4.5 and 5.8 in Oxisol$_1$ and Oxisol$_2$ soils, respectively (Table 2). The concentrations of P and K in Oxisol$_1$ and Oxisol$_2$ soils were between 0.04 and 0.05 wt%, and 0.06 and 0.07 wt%, respectively (Table 2). Contrary to the nutrient-poor substrate, the Oxisol soils contained up to 6 and 11 fold more Al and Fe, respectively (Table 2).

Table 2: Elementary compositions of nutrient-poor substrate and three Oxisol soils. The pH values of all samples were determined in 0.01 M CaCl$_2$ solution (ratio 1:2.5, w/v).

Elements	Abbrev. (wt%)	Nutrient poor substrate (wt%)	Oxisol$_1$ soil (wt%)	Oxisol$_2$ soil (wt%)	Oxisol$_{Field}$ soil
Nitrogen	N	0.06	0.10	0.10	
Phosphorus	P	$<$0.030	0.040	0.047	0.0472
Potassium	K	1.272	0.068	0.063	0.0781
Sulphur	S		$<$0.002		
Magnesium	Mg	0.104	0.040	0.022	
Calcium	Ca	0.197	0.003	0.023	
Copper	Cu	$<$0.005	$<$0.001	0.008	
Manganese	Mn	0.012	0.070	0.062	
Molybdenum	Mo	$<$0.003	$<$0.001	$<$0.003	
Zinc	Zn	0.002	0.006	0.001	
Sodium	Na	0.187	0.007	0.007	
Silicon	Si	38	18.6	17.06	
Iron	Fe	0.938	10.2	8.98	
Aluminium	Al	2.125	13.3	11.52	
Chromium	Cr		0.022		
Nickel	Ni	0.009	0.002	0.007	
Titanium	Ti		2.744		
Cadmium	Cd		0.001		
Lead	Pb		0.013		
pH		6.4	5.8	4.5 and 5.3	5.0

2.3.3.1 Effect of bagasse ash on rooting media (Exp. 1)

Application of ash could affect the physical and chemical properties of rooting medium and subsequently impair the growth of soybeans. Rooting media, i.e. sand, nutrient-poor substrate and $Oxisol_1$ soil, were tested for the effects of bagasse ashes (BA_{SP}) on water holding capacity (WHC), infiltration time (WIT), water retention after incubation (WRaI) either at room temperature or at 60 °C to enhance the drying process, pH levels and electric conductivity (EC). Sand, nutrient-poor substrate and $Oxisol_1$ soil were dried at 60° (TR 1050, Nabertherm GmbH, Lilienthal, Germany) for 5 d prior to starting the experiments. Bagasse ash (BA_{SP}) was homogeneously incorporated into rooting media. The rooting media received 0, 2.5, 7.5, 15, and 22.5 g BA_{SP} L^{-1} rooting media. The dosages were equivalent to 0, 5, 15, 30 and 45 t ha^{-1} based on a 20 cm tillage depth.

Five replicates containing 300 g of sand, nutrient-poor substrate and $Oxisol_1$ soil were filled in pots (340 ml), which contained fleece tissue on the bottom to avoid loss of the growing medium. The weights of the pots were measured before and after filling (Figure 3 A). To measure the infiltration rate, the bottom of a 50 ml Falcon tube was removed and the tube was positioned in the growing medium at a depth of 1 cm. Fifty millilitres of water were added to fill the tube and the measurement began after the water level on the falcon tube showed that 5 ml had infiltrated (Figure 3 B). The time was stopped after infiltration of further 40 ml water. The elapsed time was multiplied by 25 to get the water infiltration time (WIT) of 1000 ml (Eq. 1).

Afterwards, the tube was removed and washed above the pot to retain the growing medium in the pot. The pots were then saturated with water (Figure 3 C) and the weights were measured 2 h after saturation (Figure 3 D and E). The calculation of water holding capacity (WHC) was based on Mangrich et al. (2015) and was calculated as described in Eq. 2.

$$WIT \, (\text{sec. } L^{-1}) = time \, (\text{sec.}) \cdot 25 \tag{1}$$

$$WHC \, (\text{wt\%}) = 100 \cdot \frac{Mass_{\text{Wett}} \, (\text{g})}{Mass_{\text{Dry}} \, (\text{g})} \tag{2}$$

$$WRaI \, (\% \text{ of water } T_0) = 100 \cdot \frac{WaterT_{\text{x}} \, (\text{g})}{WaterT_0 \, (\text{g})} \tag{3}$$

To measure the water retention after incubation (WRaI) of the growing medium, it was dried either at room temperature (sand) or at 60 °C to enhance the drying of nutrient-poor substrate and $Oxisol_1$ soil (Figure 3 F). The weight were measured every day (Figure 3 G). In sand, nutrient-poor substrate, and $Oxisol_1$ soil, the water retention after incubation (WRaI) is given by water content 9, 5 and 4 days (d) after drying (water content T_{x}), respectively, relative to the water content measured 2 h after saturation (water content T_0; Eq. 3).

The pH levels and electric conductivity measurements were based on 2.3.7.5 and 2.3.7.6.
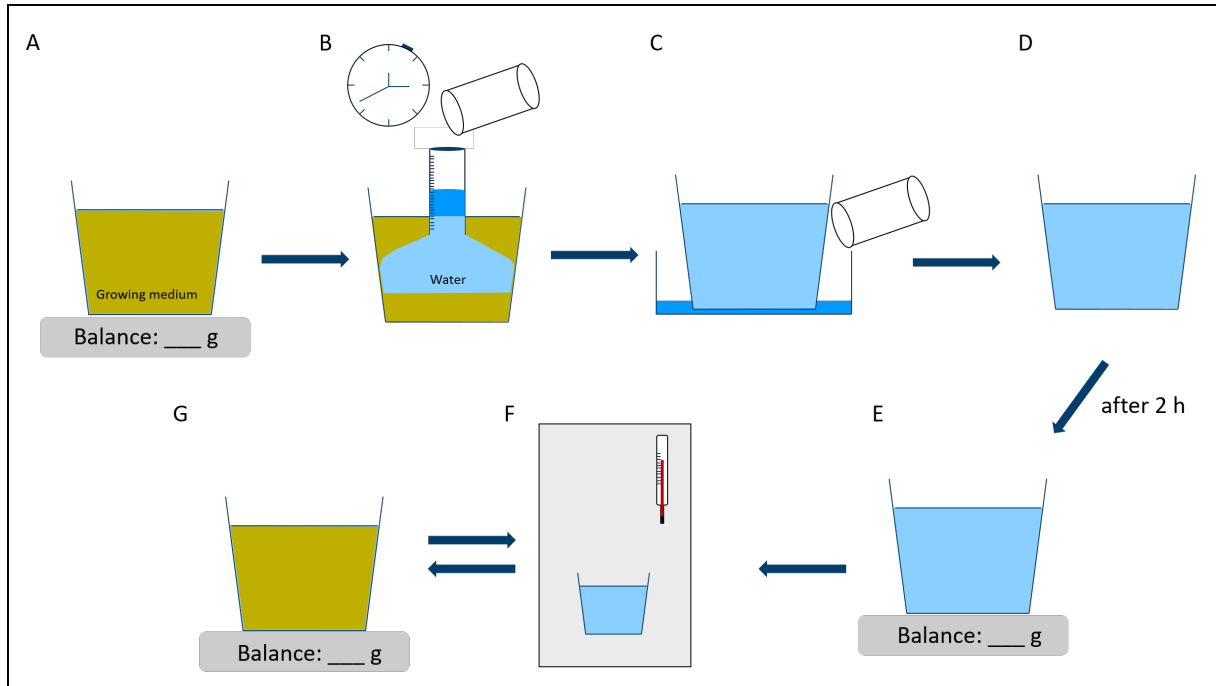
Figure 3: Setup for measurements of water infiltration time (WIT), water holding capacity (WHC) and water retention after incubation (WRaI). (A) Weighing of dry growing medium. (B) Modified Falcon tube (50 ml) was positioned in growing medium at a depth of 1 cm. Timing was begun after the infiltration of 5 ml water until 40 ml had infiltrated (WIT). (C) The growing medium was then saturated with water, (D) left for 2 h, and (E) measured for weight ($Mass_{Wet}$). (F) Sand was dried at room temperature and nutrient-poor substrate and Oxisol soil at 60 °C. (G) Weights ($Mass_{Dry}$) of rooting media were measured every day until they reached a constant weight. (E-G) The weights of water saturated and dry growing media were used to calculating the WRaI and WHC.

2.3.4 Toxicity analyses (Exp. 2)

The toxicity analyses were based on germination rate and the radicle lengths of garden cress seeds (*Lepidium sativum*) and on root growth of pre-cultivated soybean plantlets (*Glycine max* (L.) *Merr.* var. RGT Shouna) in the system of rhizotrons.

2.3.4.1 Germination-Elongation test

A germination-elongation test to investigate the toxicities of BA_{SP} and BA_G ashes (Table 1) to garden cress seeds was based on Paradelo et al. (2010). Stock solutions, which contained 0, 7.5, 15 and 22.5 mg BA_{SP} and BA_G ashes per millilitre of ultra-pure water (Milli-Q Reference, Merck, Darmstadt, Germany), were prepared. Filter papers (Whatman type 589) were placed in petri dishes (9 cm diameter) and received 3 ml of these stock solutions, respectively. Every treatment contained three replicates with 35 garden cress seeds per petri dish. The petri dishes were closed with Parafilm and incubated at around 25 °C in the dark for 9 d.

Germinated seeds with radicle lengths ≥ 1 mm were considered as having germinated ($G_{No.}$ = number of germinated seeds) and were photographed (Canon EOS 450D, EFS-5 18-55 mm). Measurements of radicle elongations (RE) were based on picture analyses (ImageJ 1.46r). Germination rate (GR) and germination index (GI) were based on equations 4 and 5. $G_{No.C}$ and RE_C represent the germination rate and radicle elongation of the control treatment. According to (Zucconi et al. (1981), germination indexes below 50% indicate high phytotoxicity, 50-80% slight toxicity and higher than 80% no phytotoxicity.

$$GR\,(\%) = 100 \cdot \frac{G_{No.}}{35} \tag{4}$$

$$GI\,(\%) = 100 \cdot \frac{G_{No.} \cdot RE\,(\text{cm})}{G_{No.C} \cdot RE_C\,(\text{cm})} \tag{5}$$

2.3.4.2 Root response to bagasse ash as fertilizer

Bagasse ash from controlled combustion was tested (i) for its toxicity to soybeans in a nutrient-poor substrate environment and (ii) as P-fertilizer for soybeans. The analyses were based on the protocol described by Nabel et al. (2018). Bagasse pellets ash (cBP ash) used as P-fertilizer (88 g ash, which contained 213 mg P) and 93 mg P in the form of TSP were localized as depot (6 cm diameter) in nutrient-poor substrates. The depot was located on the opposite side from the plant, at a depth of 13.5 cm and 4 cm from the side border of the rhizotron (Figure 4). A treatment without any P-fertilization was used as the no-P control. Treatments with TSP and no-P received 850 mg K and 67 mg N in forms of potassium sulphate (K_2SO_4) and ammonium nitrate. While cBP ash-fertilized treatments received the same dosages of N as control treatments, only 430 mg K in the form of K_2SO_4 were supplied, as 420 mg was supplied by cBP ash. Soybeans were pre-cultivated in nutrient-poor substrate for 12 d. When they reached the stage of having fully expanded unifoliate leaves, the roots were washed, inoculated with *B. japonicum* (NPPL HI Stick, BASF SE, Ludwigshafen, Germany) and those soybean plantlets with similar morphology were transplanted opposite to the site of the fertilizer depot in the rhizotrons (n = 3). This allowed the soybean roots to grow towards the depot, which contained cBP ash, or to avoid it.

The rhizotrons were inclined 45° with the transparent site downwards. The soybeans grew for 20 d and then photos of root developments were taken (Canon EOS 70D, EF 14 mm) daily. Afterwards, leaflets, stem incl. petioles, roots incl. nodules, were separated, dried at 60 °C for 5 d (TR 1050, Nabertherm GmbH, Lilienthal, Germany) and measured (PG503-S, Mettler Toledo GmbH, Gießen, Germany). Dry weights (DW) of total plants including shoot and root organs (TDW), and biomass partitioning to the roots expressed as root mass fraction (RMF; Eq. 6) were considered. The root growth based on image analyses (PaintRhizo, FZJ, Jülich, Germany). Calculation of root length (RL) fraction in the area of the depot (RLF_{Depot}) is expressed as the length of visible roots relative to total root length (TRL) detected 13 and 20 d after fertilizer application (Eq. 7).
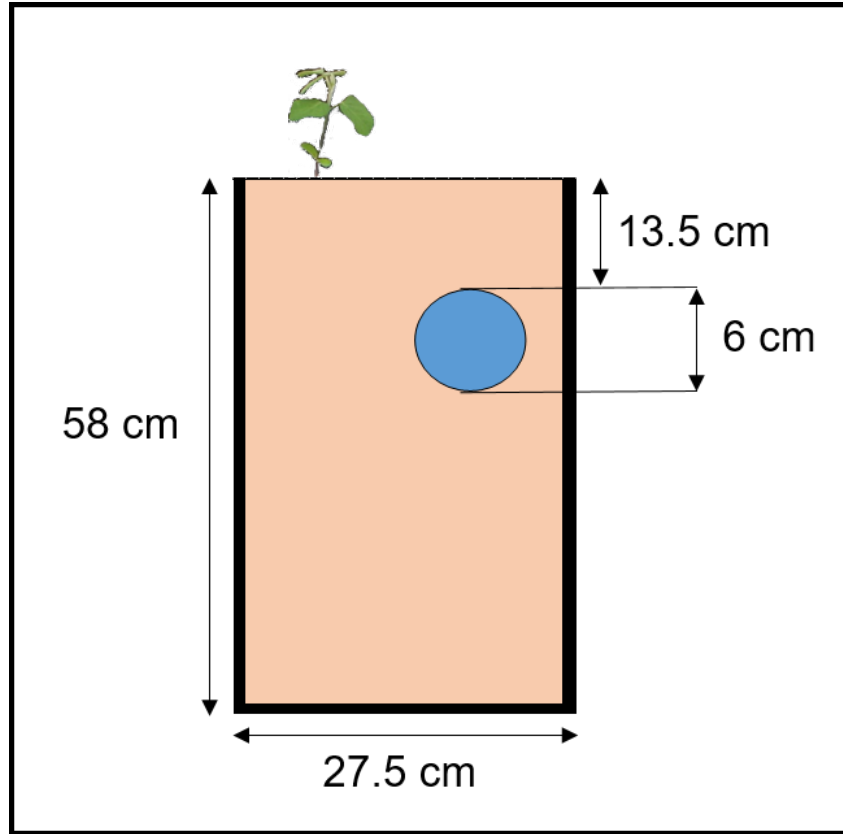
Figure 4: Setup of rhizotron experiment. The soybean treatments were inclined 45° with the transparent site downwards.

$$RMF \, (\text{g g}_{\text{TDW}}^{-1}) = 100 \cdot \frac{DW_{\text{Root}} \, (\text{g})}{TDW \, (\text{g})} \tag{6}$$

$$RLF_{\text{Depot}} \, (\text{cm}_{\text{Depot}} \, \text{cm}_{\text{TRL}}^{-1}) = 100 \cdot \frac{RL_{\text{Depot}} \, (\text{cm})}{TRL \, (\text{cm})} \tag{7}$$

2.3.5 Pot-experiments in nutrient-poor substrate

Nutrient-poor substrate was chosen as growing medium for soybeans because it was very poor in P but rich in K, and the water holding capacity, infiltration time and water retention after incubation under ash application were more similar to those of $Oxisol_1$ soil than the same values in sand. Around 1.25 L of nutrient-poor substrate, i.e. 1150 g, were homogeneously blended with necessary amounts of each fertilizer in a closed measuring cup (3 L) by shaking. Five replicates were harvested at the time of transplant in fertilization treatments for K and/ or P (K_0 and P_0) analyses. These were considered as first harvests and allowed the calculation of P and K uptakes from the point of fertilizer application.

2.3.5.1 Bagasse ash as fertilizer for soybeans (dose-response, Exp. 3)

A dose-response experiment with bagasse ash was carried out to investigate the response of soybeans to bagasse ash as PK-fertilizer, and to compare the fertilization effects of this fertilizer to the effect of conventional fertilizer on plants. The analyses of BA_{NG1} ash (Table 1) as PK-fertilizer for soybeans were conducted between August and September 2016 and assessed six dosages of ash. These were equivalent to 0, 4.8, 14.5, 29, 43.5 and 58 t ha^{-1} based on a tillage depth of 20 cm (Table 3); and supplied around 0/ 0, 10/ 32, 30/ 96, 60/ 195, 90/ 289 and 120/ 385 mg P/ K kg^{-1} nutrient-poor substrate, respectively (Table 3). The addition of 30 mg P (from TSP) and 225 mg K kg^{-1} (from K_2SO_4) to a nutrient-poor substrate will hereafter be referred to as conventional fertilizer, and was considered as the optimal fertilizer for this experiment (Table 3). According to recommendations made by Bayerische Landesanstalt für Landwirtschaft (2020), no N fertilizer was applied, as the plants were inoculated with *B. japonicum*, which fixes N from atmospheric N_2. The relative humidity, temperatures and total irradiance were 52/ 66% (day/ night), 25/ 20 °C (day/ night) and 7892 mmol m^{-2}. Each fertilizer treatment contained five replicates, which were harvested 44 d after fertilizer application.

Table 3: Fertilization treatments for dose-response experiment with bagasse ash from sugarcane factory Usina Nova Gália Ltda. (BA_{NG1}), Brazil. The levels of pH were measured after harvesting the plants. Conventional fertilization received phosphorus (P) and potassium (K) from triple-superphosphate (TSP) and K_2SO_4. The values were rounded to one decimal place.

BA_{NG1} ash (t ha^{-1})	0	4.8	14.5	29	43.5	58	Conv. fertilizer
BA_{NG1} ash (g kg^{-1})		2.6	7.9	13.2	23.7	31.6	
P (mg kg^{-1})		10.0	30.0	60.0	90.0	120.0	30.0
K (mg kg^{-1})		32.1	96.3	194.5	288.8	385.1	224.6
pH	6.2					6.2	6.1

Leaf areas were captured with the "ScreenHouse" platform 6, 12, 19 and 26 d after fertilizer application. After 26 d growth, the plants did not fit in the "ScreenHouse" chamber, which was the reason for stopping the non-invasive measurements. Total plant dry masses, biomass partitioning to nodulated roots, uptakes of P and K and total N in final harvest were considered. Additionally, N concentration in nodulated roots was measured and is presented as the concentration of N in mmol gram^{-1} root DW. Furthermore, total numbers of nodules per one gram of dry root biomass are included. After harvesting the plants, the pH levels were measured in control treatments and in those nutrient-poor substrates fertilized with 58 t BA_{NG1} ash ha^{-1} (Table 3).

2.3.5.2 Bagasse ash from controlled combustion as fertilizer for soybeans (Exp. 4)

Bagasse ash from controlled combustion, i.e. cBP ash (Table 1), was tested as PK-fertilizer for soybeans over the period from September to November 2016. Soybeans received cBP ash in amounts equivalent to 0, 22.8 and 54.7 t ha^{-1} at a tillage depth of 20 cm (Table 4). The cBP ash supplied 0/ 0, 30/ 161 and 72/ 387 mg P/ K kg^{-1} nutrient-poor substrate,

respectively (Table 4). The conventional fertilizer treatment received 30 mg P and 387 mg K kg^{-1} nutrient-poor substrate in the form of TSP and K_2SO_4 (Table 4). Phosphorus and K concentrations were matched to treatments with 12.4 g and 29.8 g cBP ash kg^{-1} nutrient-poor substrate, respectively (Table 4). The relative humidity, temperatures and total irradiance were 52/ 64% (day/ night), 24/ 19 °C (day/ night) and 4201 mmol m^{-2}. Each fertilization treatment contained five replicates, which were harvested 58 d after the application of fertilizer. Longer growing periods, as compared to BA_{NG1} ash-fertilized soybeans, were due to slower growth. This slower growth, may be explained by the difference in total light irradiance.

Table 4: Treatments with combusted bagasse pellets (cBP) ash as phosphorus (P) and potassium (K) fertilizer for soybeans. Conventional fertilization received P and K from triple-superphosphate (TSP) and K_2SO_4. The pH levels were measured after harvesting the plants. The values were rounded to one decimal place.

cBP ash (t ha^{-1})	0	22.8	54.7	Conv. fertilizer
cBP ash (g kg^{-1})		12.4	29.8	
P (mg kg^{-1})		30.0	72.0	30.0
K (mg kg^{-1})		161.2	386.8	386.8
pH	6.4		6.2	6.1

Leaf areas were captured with the "ScreenHouse" platform 7, 14, 28, 35, 42, 49 and 56 d after fertilization beginning. Total plant dry masses, biomass partitioning to nodulated roots and uptakes of P and K were considered. The pH levels were measured in control treatments and in nutrient-poor substrate fertilized with 54.7 t cBP ash ha^{-1} after harvesting the plants (Table 4).

2.3.5.3 Effect of fertigation on phosphorus uptake from bagasse ash (Exp. 5)

Over the period from August to September 2017, this experiment aimed to investigate the effect of up to 134.9 t cBP ash ha^{-1} as P-fertilizer for soybeans and the effect of additional nutrient supply on P uptake from the ash. Based on a tillage depth of 20 cm, equivalent amounts of 0, 57.5 and 134.9 t ha^{-1} of cBP ash were homogeneously incorporated into nutrient-poor substrate (Table 5). The fertilizers with cBP ash contained around 0/ 995, 73/ 488 and 170/ 995 mg P/ K kg^{-1} nutrient-poor substrate, respectively (Table 5). Conventional fertilizer treatment received around 73 mg P and 995 mg K kg^{-1} nutrient-poor substrate from TSP and K_2SO_4 (Table 5). To reduce the dependency on N-fixation from atmospheric N_2, all soybeans received around 19 mg N kg^{-1} in the form of ammonium nitrate. The total number of replicates was six, and one half of the replicates received additional nutrients excluding P from the application of 30 ml of modified 1/3 half strength of Hoagland's solution weekly (Table S2). The relative humidity was 52/ 67% (day/ night), the temperature 25/ 20 °C (day/ night) and the total irradiance was 6427 mmol m^{-2}. The harvest was carried out 35 d after the application of fertilizer.

Total plant dry masses and biomass partitioning to nodulated roots were considered. Soybeans receiving P and K from 57.5 t cBP ash ha^{-1} and from the first harvest were

Table 5: Treatments with up to 134.9 t of combusted bagasse pellets (cBP) ash ha^{-1} as phosphorus (P) and potassium (K) fertilizer for soybeans. Conventional fertilization received P and K from triple-superphosphate (TSP) and K_2SO_4. All fertilizer treatments received nitrogen (N) from ammonium nitrate. The pH levels were measured after harvesting the plants. The values were rounded to one decimal place.

cBP ash (t ha^{-1})	0	57.5	134.9	Conv. fertilisation
cBP ash (g kg^{-1})	0	31.3	73.3	
P (mg kg^{-1})		72.6	170.4	72.6
K (mg kg^{-1})	994.8	487.5	994.8	994.8
N (mg kg^{-1})	19.4	19.4	19.4	19.4
pH	6.1		6.2	5.8

only analysed for P, whereas the biomasses of no-P control soybeans, 134.9 t ha^{-1} of cBP ash and conventionally fertilized plants were analysed for N, P, K, S, Mg , B, Ca, Cu, Fe, Mn, Si, Zn, Na, Cl and Al. Subsequently, the concentrations of listed minerals were calculated. After harvesting the plants, the pH levels were measured in control treatments and in nutrient-poor substrates fertilized with 134.9 t cBP ash ha^{-1} (Table 5).

2.3.5.4 Effect of co-processing bagasse pellets with chicken manure on availability of P from the ashes to soybeans (Exp.6)

This experiment focussed on the effects of co-gasification and co-combustion of bagasse pellets with chicken manure on the availability of P from the ashes to soybeans. Over the period from April to June 2017, the soybeans received 0 and 54 mg P kg^{-1} nutrient-poor substrate in forms of gBP, gBP/ CM$_{85:15}$ and cBP/ CM$_{80:20}$ ashes (Table 1). Based on "dose-response" experiment (Figure 10), 54 mg P kg^{-1} nutrient-poor substrate was below the optimal P-fertilizer in the form of bagasse ash, which allowed variations in biomass accumulations and uptakes of P. The ash treatments contained around 13 g of gBP, 3 g of gBP/ CM$_{85:15}$ and 2 g of cBP/ CM$_{80:20}$ ashes kg^{-1} nutrient-poor substrate, respectively (Table 6). The concentration of K was adjusted to 244 mg kg^{-1} nutrient-poor substrate with K_2SO_4, so that all fertilizations contained the same amounts of K (Table 6). Additionally, 23 mg of N kg^{-1} in a nutrient-poor substrate was added in the form of ammonium nitrate to reduce the dependency on N fixation from the atmospheric N_2 (Table 6). The relative humidity was 47/ 62% (day/ night), temperatures 25/ 19 °C (day/ night) and the total irradiance was, 8828 mmol m^{-2}. Each fertilizer treatment contained ten replicates.

Over course of the growth, the leaf areas were captured using the "ScreenHouse" platform every week starting 5 d after the application of fertilizer. Five soybeans were harvested at the time of fertilizer application, and 5 replicates were harvested both 27 d and 42 d after fertilizer application. Total dry weights of soybeans and biomass partitioning to nodulated roots from last harvest, as well as the uptakes of P from both harvests are presented and discussed. The pH levels in substrates from the final harvest were measured and are presented in Table 6.

Table 6: Ashes from gasification of bagasse pellets (gBP), and co-gasification and co-combustion of bagasse pellets and chicken manure in ratios of 85:15 (gBP/ $CM_{85:15}$) and 80:20 by dry weights (cBP/ $CM_{80:20}$), respectively, as phosphorus (P) fertilizer for soybeans. Potassium (K) and nitrogen (N) were adjusted by K_2SO_4 and ammonium nitrate. The pH levels were measured after harvesting the plants. The values were rounded to one decimal place.

Ashes	No P	gBP	gBP/ $CM_{85:15}$	cBP/ $CM_{80:20}$
Dosages (t ha^{-1})	0.0	24.2	5.8	4.1
Dosages (g kg^{-1})	0.0	13.1	3.2	2.2
P (mg kg^{-1})	0.0	54.0	54.0	54.0
K (mg kg^{-1})	244.0	244.0	244.0	244.0
N (mg kg^{-1})	23.0	23.0	23.0	23.0
pH	6.2	6.0	6.2	6.0

2.3.6 Pot experiments in acidic Oxisol soil

The pH level may influence the availability of P (Schachtman et al., 1998, Fageria and Baligar, 2008, Sanz-Saez et al., 2017) from bagasse and bagasse-based ashes to the plants. Subsequently, the analyses were performed in Oxisol soils with various acidities (Table 2). Pre-selection and pre-cultivation of soybeans was the same as described for soybeans growing in nutrient-poor substrates (2.3.5). Between 1 and 1.15 L of Oxisol soil, i.e. 1300 to 1500 g, were homogeneously blended with necessary amounts of fertilizers in a closed measuring cup (3 L) by shaking. Five pre-cultivated plantlets were harvested at the time of fertilizer application and used for calculation of P uptake (2.3.5).

2.3.6.1 Effects of co-combustion bagasse with nutrient rich residues on the availability of P from bagasse-based ashes to soybeans (Exp. 7)

Over the period from August to September 2017, the effects of co-combustion of bagasse pellets with chicken manure and with sewage sludge ashes were investigated on the availability of P from the ashes to soybeans in 1500 g of acidic $Oxisol_2$ soil (Table 2). Soybeans received 261.9 mg P kg^{-1} acidic $Oxisol_2$ soil in the form of cBP, cBP/ $CM_{80:20}$ and cBP/ $SSL_{83:17}$ ashes (Table 7). Two no-P controls with pH levels 4.5 and 6 (limed with calciumcarbonat, Manna) were included. In all fertilizer treatments, the K concentrations were equilibrated to that of 1.5 g kg^{-1} $Oxisol_2$ soil supplied by cBP ash. Additionally, all fertilizer treatments received 15.6 mg N kg^{-1} $Oxisol_2$ soil in the form of ammonium nitrate, which reduced the dependency on N from atmospheric N_2-fixation. The relative humidity was 52/ 65% (day/ night), temperatures 25/ 20 °C (day/ night) and total irradiance was 7140 mmol m^{-2}. Each fertilizer treatment contained four replicates.

Over course of the growth, the leaf areas were captured using the "ScreenHouse" platform every week starting 6 d after the application of fertilizer. Total plant dry masses, biomass partitioning to nodulated roots and uptake of P were considered. The pH levels were measured in $Oxisol_2$ soil after 42 d growth (Table 7).

Table 7: Ashes from combustion of bagasse pellets (cBP), and co-combustion of bagasse pellets and sewage sludge and chicken manure in ratios of 83:17 (cBP/ $SSL_{83:17}$) and 80:20 by dry weights (cBP/ $CM_{80:20}$), respectively, as phosphorus (P) fertilizer for soybeans in Oxisol soil. Potassium (K) and nitrogen (N) were adjusted by K_2SO_4 and ammonium nitrate. No-P controls with different pH levels were included. The pH levels were measured in Oxisol soils after harvesting the plants. The values were rounded to one decimal place

Ashes	No P (pH 4.5)	No P (pH 6)	cBP	cBP/ $SSL_{83:17}$	cBP/ $CM_{80:20}$
Dosages (t ha^{-1})	0	0	54.2	4.5	5.3
Dosages (g kg^{-1})	0	0	29.4	2.4	2.9
P (mg kg^{-1})	0	0	261.9	261.9	261.9
K (g kg^{-1})	1.5	1.5	1.5	1.5	1.5
N (mg kg^{-1})	15.6	15.6	15.6	15.6	15.6
pH	4.5	6.1	5.0	4.6	6.0

2.3.6.2 Availability of P from bagasse/ chicken manure ashes and its thermochemical products for soybeans compared to P from TSP (dose-response, Exp. 8)

The "dose-response" experiment was conducted from July to August 2018 and aimed to investigate the availability of P from co-combusted bagasse pellets/ chicken manure ashes and the thermochemical products for soybeans. Acidic $Oxisol_2$ soil was limed to a pH of 5.3, because of usual practise in Oxisol soils. Around 0, 100, 180, 262 and 340 mg P kg^{-1} $Oxisol_2$ soil in the forms of cBP, cBP/ $CM_{80:20}$ and cBP/ $CM_{60:40}$ ashes and cBP/ $CM_{60:40}$+Na and cBP/ $CM_{60:40}$+Na/K thermochemical products were homogeneously incorporated in 1300 g soil (Table 8). A positive control treatment received the same dosages of P as provided by ashes but in the form of TSP (Table 8). In all fertilizer treatments, the K concentrations was equilibrated to 754 mg kg^{-1} $Oxisol_2$ soil supplied by cBP/ $CM_{60:40}$+Na/K (Table 8). Additionally, all fertilizer treatments received 323 mg K kg^{-1} Oxisol soil in the form of K_2SO_4 to exclude any K deficiency when using cBP/ $CM_{60:40}$+Na/K. This resulted in 1.1 g K kg^{-1} $Oxisol_2$ soil (Table 8). Furthermore, all fertilizer treatments received 18 mg N kg^{-1} Oxisol soil in the form of ammonium nitrate (Table 8). The relative humidity was 42/ 59% (day/ night), temperatures were 24/ 17 °C (day/ night) and total irradiance was 4048 mmol m^{-2}. Each fertilization treatment contained four replicates, which were harvested 22 d after fertilizer application.

Total dry biomass yields, biomass partitioning to the nodulated roots and uptakes of P from the beginning of the fertilization to the end of growth are presented and discussed. After harvesting the plants, the pH levels were measured in no-P control treatment, and in $Oxisol_2$ soil, which received around 100 and 340 mg P kg^{-1} Oxisol soil in the forms of cBP/ $CM_{80:20}$ and cBP/ $CM_{60:40}$ ashes, cBP/ $CM_{60:40}$+Na and cBP/ $CM_{60:40}$+Na/ K thermochemical products, and TSP (Table 8).

Table 8: Co-combusted bagasse pellets and chicken manure ashes and its thermochemical products as phosphorus (P) fertilizer for soybeans in Oxisol soil. Bagasse pellets and chicken manure were co-combusted in a ratios of 80:20 (cBP/ $CM_{80:20}$) and 60:40 by dry weights (cBP/ $CM_{60:40}$). cBP/ $CM_{60:40}$ ash was thermochemically treated with Na_2SO_4 (cBP/ $CM_{60:40}+Na$) and Na_2SO_4/ K_2SO_4 (cBP/ $CM_{60:40}+Na/K$). Control fertilizations without any phosphorus (no P) and triple-superphosphate (TSP) were included. Potassium (K) and nitrogen (N) were adjusted by K_2SO_4 and ammonium nitrate. The pH levels were measured in Oxisol soils after harvesting the plants. The values were rounded to one decimal place.

Fertilizer	No P	cBP/ $CM_{80:20}$	cBP/ $CM_{60:40}$	cBP/ $CM_{60:40}$ +Na	cBP/ $CM_{60:40}$ +Na/K	TSP
0 P (mg kg^{-1})	0					
pH	5.3					
101 P (mg kg^{-1})		4.1	2.5	2.5	2.7	
pH		6.0	6.0	6.0	6.0	5.5
180 P (mg kg^{-1})		7.4	4.4	4.4	4.9	
262 P (mg kg^{-1})		10.7	6.4	6.4	7.1	
340 P (mg kg^{-1})		13.9	8.3	8.3	9.2	
pH		7.1	7.1	7.1	7.0	5.5
K (g kg^{-1})	1.1	1.1	1.1	1.1	1.1	1.1
N (mg kg^{-1})	18.0	18.0	18.0	18.0	18.0	18.0

2.3.6.3 Bagasse ash as an additive of conventional P-fertilizer (Exp. 9)

Over the period from August to September 2017, cBP ash was tested as an additive to with the use of TSP fertilizer alone. Soybeans received 102 mg P kg^{-1} Oxisol soil in the form of a cBP ash and TSP blend (55% of total P from TSP, cBP/ $TSP_{45P:55P}$) or 114 mg P kg^{-1} in Oxisol soil from TSP (Table 9). The dosage of P supplied by cBP/ $TSP_{45P:55P}$ blend and TSP was lower than needed for optimal growth (Figure 18) and gave the soybeans the range required for divergent growth. Potassium was adjusted to 1.5 g kg^{-1} of acidic $Oxisol_2$ soil and all $Oxisol_2$ soil fertilizer treatments received 15.6 mg N kg^{-1} in the form of ammonium nitrate (Table 9), which reduced the dependency on N from atmospheric N_2-fixation. The relative humidity was 52/ 65% (day/ night), temperatures were 25/ 20 °C (day/ night) and total irradiance was 7141 mmol m^{-2}. Each treatment contained five replicates, which were harvested 41 d after fertilizer application.

Table 9: Triple-superphosphate (TSP) and its blend with combusted bagasse pellets (cBP) ash (cBP/ $TSP_{45P:55P}$) as P fertilizer for soybeans in Oxisol soil. Potassium (K) and nitrogen (N) were adjusted by K_2SO_4 and ammonium nitrate. The pH levels in Oxisol soils were measured after harvesting the plants. The values were rounded to one decimal place.

Treatments	No P	cBP/ $TSP_{45P:55P}$	TSP
Ash dosages (t ha^{-1})	0	54.5	
Ash dosages (g kg^{-1})	0	29.4	
P (mg kg^{-1})	0	102 (55% of total P from TSP)	114.0
K (g kg^{-1})	1.5	1.5	1.5
N (mg kg^{-1})	15.6	15.6	15.6
pH	4.5	4.6	4.5

Over course of the growth, the leaf areas were captured weekly using the "ScreenHouse" platform, starting 7 d after fertilizer application. Total dry weights of soybeans, which included shoots and root organs, biomass partitioning to nodulated roots, uptakes of P by soybeans and the total content of P in the soil are presented and discussed. The pH levels in Oxisol soils were measured after harvesting the plants (Table 9).

2.3.6.4 Bagasse ash-based fertilizer product for soybeans (Exp. 11)

Over the period from March to May 2018, pelletized and granulated bagasse-based products were tested as fertilizer for soybeans.

The treatments included no fertilizer and granulated and pelletized fertilizer products, which were produced from bagasse ash and chicken manure in a ratio of 90:10 by dry weight. The fertilizer products supplied 218, 100 and 524 mg N, P and K kg^{-1} to Oxisol$_2$ soil, respectively. The no-fertilization control did not receive any N, P and K. The relative humidity was 48/ 61% (day/ night), temperatures were 24/ 18 °C (day/ night) and total irradiance was 8683 mmol m^{-2}. Each fertilization treatment contained five replicates, which were harvested 59 d after fertilizer application.

Total dry biomasses of soybeans and biomass partitioning to nodulated roots were considered and discussed.

2.3.7 Measurements

2.3.7.1 Non-invasive measurements

In experiments 2.3.5.1, 2.3.5.2, 2.3.5.4, 2.3.6.1 and 2.3.6.3, the shoot area of soybean plants was captured in the "ScreenHouse" platform every week, which allowed to investigate the shoot growth dynamics. The measurements were based on Nakhforoosh et al. (2016) and Scharr et al. (2017). In brief, four sides, i.e. 0°, 90°, 180° and 270°, of soybeans were imaged (Point Gray Grasshopper2, 5MP color camera, by FLIR Integrated Imaging Solutions Inc., Richmond, British Columbia, Canada) from a 45° angle and the sum of the four pictures represented the projected shoot area of soybeans. Final images were taken one to two days before the last harvest. Data from the final measurements allowed the calculation of calibration curves based on the correlation (e.g. Figure 5) between projected and true leaf areas, which were measured after harvesting the plants (Li- 3100, Li-cor, Nebraska, USA). Thus, non-invasive shoot area measurements allowed an estimation of the leaf areas of soybeans over the course of growth.

2.3.7.2 Harvest

The plants were harvested and all organs were separated, i.e. leaflets, stem inc. petioles, pods, roots, and nodules. Separation of roots and nodules depended on the experiment.
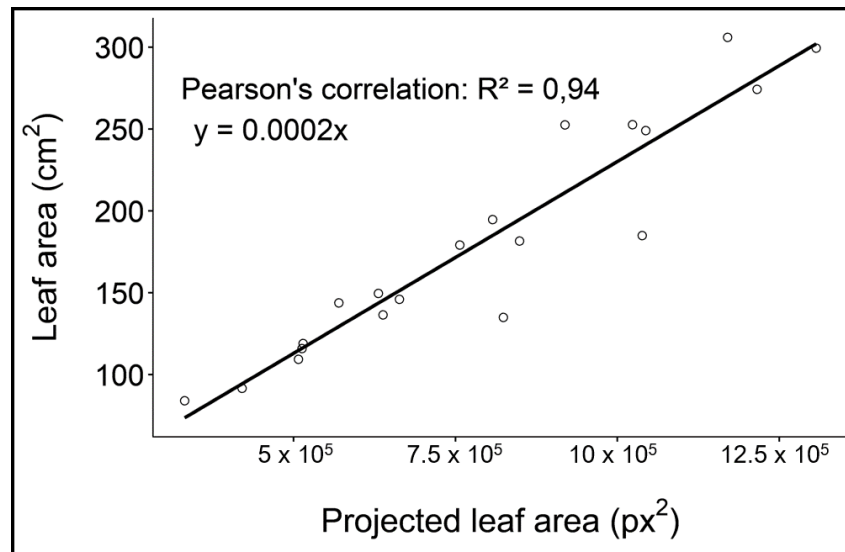
Figure 5: Correlation of projected shoot areas measured in the "ScreenHouse" platform from a 45° angle imaged 56 d after fertilizer application and the true leaf area of soybeans (exp. 2.3.5.2) measured 58 d after fertilizer application (Li- 3100, Li-cor, Nebraska, USA).

The leaflet areas were measured (Li- 3100, Li-cor, Nebraska, USA) and the organ samples were dried at 65 °C to constant weight (TR 1050, Nabertherm GmbH, Lilienthal, Germany). Afterwards, the dry weights were measured (PG503-S, Mettler Toledo GmbH, Gießen, Germany). Taking dry weights of individual organs allowed the analysis of both the total dry weights of plants and the biomass partitioning in response to different forms of P-fertilizer and dosages. However, of particular interest was the variation in biomass partitioning to the roots, which is represented by root mass fraction (RMF, Eq. 6), as it may be related to acquisition of limited P (Vance et al., 2003, Schachtman et al., 1998, Gutierrez-Boem and Thomas, 1999, Zogli et al., 2017). Prior to washing the roots, 250 g of nutrient-poor substrates and soils were sampled and dried at 30 °C to constant weight. These were used for chemical analyses.

2.3.7.3 Chemical analyses of soybean biomasses, uptakes of P and K

After measuring dry weights, all organs from the same plant were either ground collectively (MM 400, Retsch GmbH, Haan, Germany) or ground separately for chemical analysis, e.g. 2.3.5.1. The analyses were conducted by ZEA-3 (FZJ, Jülich, Germany). In Brief, fifty grams of biomass were digested with 2 ml of HNO_3 and 1 ml of H_2O_2 in triplicate in a microwave (Mars 5, Prg. Pflanzen160 X-Press, Kamp-Lintfort, Germany). Afterwards, the samples were diluted with ultra-pure water (Milli-Q Reference, Merck, Darmstadt, Germany) to a total volume of 14 ml. Before measurement in ICP-OES (iCAP 6500, Fisher Scientific, Schwerte, Germany), subsamples were diluted to a ratio of 1:20 and were measured for P in all experiments (with the exception of 2.3.6.4), for K in experiments 2.3.5.1, 2.3.5.2 and 2.3.5.3, as well as S, Ca, Mg, Fe, Mn, Zn, Cu, B,

Mo, Co, Ni, Na, Si and Al in experiment 2.3.5.3. The standard deviations of triplicates ranged from 5 to 10%. Nitrogen was measured in an elementar Vario El Cube (CHN mode, Elementar Analysensysteme GmbH, Langenselbold, Germany) by combusting 2.50 mg of root biomass (2.3.5.1) and total plant (2.3.5.3). Nitrogen was measured using a thermal conductivity detector. To measure Cl⁻, around 1 g of biomass sample (2.3.5.3) was incubated in 25 ml ultra-pure water (Milli-Q Reference, Merck, Darmstadt, Germany) in the shaker for 24 h, centrifuged and filtered. The supernatants were used to measure Cl⁻ (AS14 (column), 1 mM sodium bicarbonate + 8 mM sodium carbonate (ICS-3000, Fischer Scientific, Life Technologies GmbH, Darmstadt, Germany).

The estimations of P and K uptakes were based on the calculations reported by Thind et al. (2017). The method was modified by inclusion of second harvest, as the fertilizer application started with pre-cultivated soybeans, which were harvested at the time of plantlet transplantation (n=5). The uptakes of P and K were based on total amounts of minerals in soybean biomass, including all shoot and root organs (Eq. 8 to Eq. 10). Relative agronomic effectiveness (RAE) was calculated according to Frazão et al. (2019) as described in Eq. 11 and Eq. 12, which are based on the uptakes of P and K from ash, TSP and K_2SO_4 and unfertilized rooting media.

$$\Delta P \, (\text{mmol}) = P_{\text{final or intermadiate harvest}} \, (\text{mmol}) - P_{\text{first harvest}} \, (\text{mmol}) \tag{8}$$

$$\Delta P \, (\text{mmol}) = P_{\text{final harvest}} \, (\text{mmol}) - P_{\text{intermadiate harvest}} \, (\text{mmol}) \tag{9}$$

$$\Delta K \, (\text{mmol}) = K_{\text{final harvest}} \, (\text{mmol}) - K_{\text{first harvest}} \, (\text{mmol}) \tag{10}$$

$$RAE \, (\% \text{ P from TSP}) = 100 \cdot \frac{P_{\text{ash}} \, (\text{mmol}) - P_{\text{No P control}} \, (\text{mmol})}{P_{\text{TSP}} \, (\text{mmol}) - P_{\text{No P control}} \, (\text{mmol})} \tag{11}$$

$$RAE \, (\% \text{ K from } K_2SO_4) = 100 \cdot \frac{K_{\text{ash}} \, (\text{mmol}) - K_{\text{No K control}} \, (\text{mmol})}{K_{K_2SO_4} \, (\text{mmol}) - K_{\text{No K control}} \, (\text{mmol})} \tag{12}$$

2.3.7.4 Chemical analyses of the substrate and soil samples

Elementary analyses of nutrient-poor substrate and Oxisol soils were conducted by ZEA-3 (FZJ, Jülich, Germany). In brief, 50 mg of the soil sample and 0.25 g of lithium borate were digested at 1000 °C (muffle furnace) for 30 min. The melted phase was diluted in 30 ml HCl (5%) and filled to 50 ml with ultra-pure water (Milli-Q Reference, Merck, Darmstadt, Germany). Afterwards, the subsamples were diluted to a ratio of 1:20 with ultra-pure water (Milli-Q Reference, Merck, Darmstadt, Germany) and measured

in ICP-OES for P (2.3.3 and 2.3.6.3) or additionally for K, Si, Ca, Mg, Zn, Al, Fe, Mn, Cu, Ni and Na (2.3.3; iCAP 6500, Fisher Scientific, Schwerte, Germany). Nitrogen was measured in an elementar Vario El Cube (CHN mode, Elementar Analysensysteme GmbH, Langenselbold, Germany) by combustion of around 65-100 mg of the sample. Nitrogen was measured by a thermal conductivity detector.

2.3.7.5 pH measurements

The pH levels were measured in ashes (Table 1), rooting media (Table 2 and 2.3.3.1), in nutrient-poor substrates and soil samples after harvesting the plants (Table 3 to Table 9) and in cattle manure compost (Table S1). The ratio of ash and rooting media to 0.01 M CaCl$_2$ was 1:2.5 (w/v), whereas the ratio of cattle manure compost to 0.01 M CaCl$_2$ was 1:5 (w/v), as cattle manure compost absorbed 25 ml of 0.01 M CaCl$_2$. The samples were incubated at 250 rpm (TH15, Incubator Hood, Edmund Bühler GmbH, Bodelshausen, Germany) overnight and were either centrifuged at 4000 g for 5 min (Allegra 25R centrifuge, Bleckman Coulter, Indianapolis, United States) or left for 1-2 h for setting prior measuring the pH levels (SevenGo Duo, Mettler Toledo GmbH, Gießen, Germany).

2.3.7.6 Electric conductivity

Preparation of samples for measurements of electric conductivity (EC) was the same as reported for measurements of pH levels, though ultra-pure water (Milli-Q Reference, Merck, Darmstadt, Germany) was used instead of 0.01 M CaCl$_2$. Electric conductivity was measured with a SevenGo Duo (SevenGo Duo, Mettler Toledo GmbH, Gießen, Germany).

2.4 Soybean field experiment in Goiânia, Brazil (Exp. 10)

Based on experiments under controlled greenhouse conditions, a field experiment was conducted at Brazilian Federal University of Goiás (**U**niversidade **F**ederal de **G**oiás, UFG, Goiânia, Brazil, 16°35'27.76"S, 49°17'18.43" W). The experiment was conducted over the period from December 2017 to April 2018, which aimed to investigate bagasse ash and its blends with TSP and cattle manure compost as P-fertilizer for soybeans (*Glycine max* (L.) *Merr.* var. BRS 7470IPRO) under field conditions.

2.4.1 Growing conditions

The plants grew at 13/ 11 h light/ dark conditions and received in total 12.55 mol m^{-2} photosynthetically active radiance (PAR). The maximum and minimum air temperatures, humidity and precipitations were 34.2/ 16.4 °C, 52/ 81.5% and 0/ 53 mm, respectively. Environmental data over the course of the experiment are depicted in Figure 6 A-D.
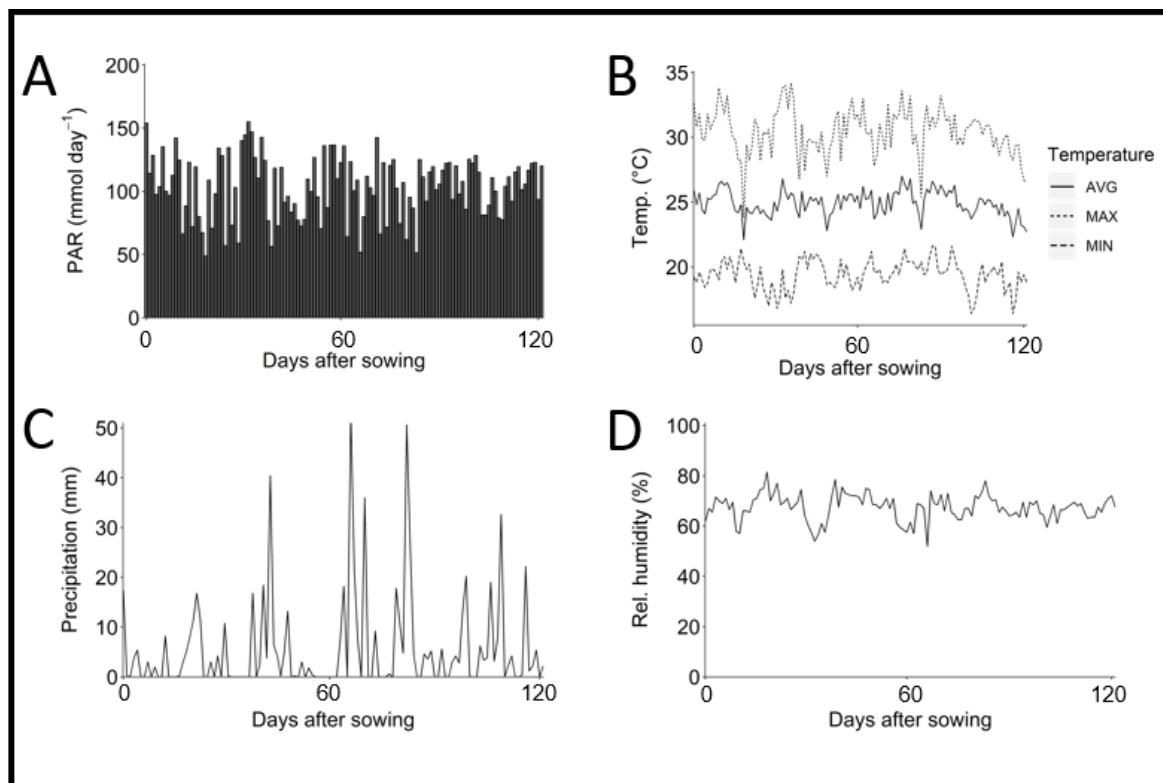
Figure 6: Environmental data over the course of the field experiment (Universidade Federal de Goiás, Goiânia, Brazil, 16°35'27.76"S, 49°17'18.43" W). (A) photosynthetic active radiance (PAR), (B) air temperature, (C) precipitation , (D) relative air humidity.

2.4.2 Background information to *Glycine max* (L.) *Merr.* var. BRS 7470IPRO

Soybean *Glycine max* (L.) *Merr.* var. BRS 7470IPRO was assigned to maturity group 7.4 and was reported to complete the life cycle 110 d after sowing (EMBRAPA Soja, 2017). This variety characteristically exhibits multiple resistances against glyphosate, varying worms, bacterial pustule, frogeye leaf spots, rod cancer, oidium and Meloidogyne javanica root-knot nematodes (EMBRAPA Soja, 2017). Soybean grains were inoculated with *Bradyrhizobium japonicum* (GRAP NOD, Agrocete, Ponta Grossa, Brazil) prior to sowing. Soybeans were sown in lines that were 45 cm apart. The density of plants was approximately 271000 soybeans ha^{-1}, i.e. 11 plants m^{-1}.

2.4.3 Soil and field preparation

Oxisol soil from the field contained around 0.05% P and 0.08% K (Table 2). Furthermore, 2.31, 2.00 and 63.00 mg dm^{-3} of P, Na and K were extractable with a Mehlich-I solution (data not shown). The solubilities of Ca, Mg and Al were measured in 1 M KCl and were 1.33, 0.52 and 0 cmolc dm^{-3}, respectively (data not shown). The solubility of H and Al was measured in calcium acetate and was 2.3 cmolc dm^{-3} (data not shown). The pH

level measured in 0.01 M $CaCl_2$ was 5.0. The soil contained 38.8% clay, 16.8% slit and 44.5% sand. The analyses were conducted by Dr. Marcia Cristina da Silva (Laboratório de análise de sementes (LASEM), Goiânia, Brazil).

According to local agronomical practise, the field area was treated with herbicides and limed (Lamartine Nogueira, UFG, Goiânia, Brazil) before planting the soybeans. Active ingredients were glyphospate isopropylamine salt (Glizmax) and flumioxazin (Flumizin 500). These were sprayed on the field area one month before planting soybeans. No tillage of the soil was practised and furrows (approx. 9 cm depth) for fertilizers were prepared (PST Plus, Marchesan Tatu, Matão, Brazil, MF 4299, Massey Ferguson, AGCO, Duluth, United States).

In a block design, the field area was subdivided in four parcels (86.25 m x 4.05 m), which each contained 10 furrows. The distance between parcels was 2.25 m. Each parcel contained 11 plots (2.25 m x 2.25 m) with 6 furrows (Figure 7). Each plot contained a different P-fertilizer formula. The distance between the plots, was 0.9 m (Figure 7). This area did not contain any fertilizer. After distributing the fertilizers, the furrows were closed and new furrows (approx. 4 cm depth) next to the fertilizers were prepared.

2.4.4 Fertilization treatments

All fertilizers were supplied on P_2O_5 dosage-basis. While bagasse ash (BA_{NG2}) and its blends with cattle manure compost (CMC) supplied 40, 80 and 160 kg P_2O_5 ha^{-1}, blends of BA_{NG2} with TSP supplied 32, 65 and 130 kg P_2O_5 ha^{-1} (Table 10). Controls with CMC and TSP contained 80 and 40 kg P_2O_5 ha^{-1}, respectively (Table 10). Potassium and nitrogen amounts were adjusted to highest dosages supplied by 160 kg P_2O_5 ha^{-1} in the form of BA_{NG2}/ CMC, i.e. 293 kg K_2O and 210 kg N ha^{-1} (Table 10). Additionally, all soybeans growing within fertilized areas (Figure 7, blue area) received 60 kg K_2O ha^{-1}, which resulted in a total K_2O supply of 352 kg K_2O ha^{-1} (Table 10). Soybeans growing around fertilized areas (Figure 7, i.e. grey area) did not receive any fertilizer.

2.4.5 Chemical treatments of soybeans

According to local agronomical practise in Brazil, the soybean seeds were covered with insecticide (Cruise 350 FS, active ingredient: thiamethoxam) and fungicide (Apron RFC, active ingredients: fludioxonil and Metalaxil-M) prior to sowing. Furthermore, herbicide (Glyphotal TR) was applied 29 d after sowing, and fungi- and insecticides were applied 46 d (Aproach Prima, Unizeb 800 WP, Ampligo), 74 d (Nativo, Unizeb 800 WP, Rapel, Assist) and 91 d (Aproach Prima, Unizeb 800 WP, Cruise 700WS, Assist) after sowing. The active ingredients of Glyphotal TR, Aproach Prima, Unizeb 800 WP, Ampligo, Nativo, Rapel, Assist, Cruiser 700WS were glyphosate isopropylamine salt, cyproconazole and pycoxistrobine, mancozeb, λ-cyhalothrin and chlorantraniliprole, tebuconazole and trifloxystrobin, acephate, mineral oil, thiamethoxam, respectively. In a stage between beginning (R7) and full maturity (R8) of soybeans, i.e. 117 d after sowing, the soybeans
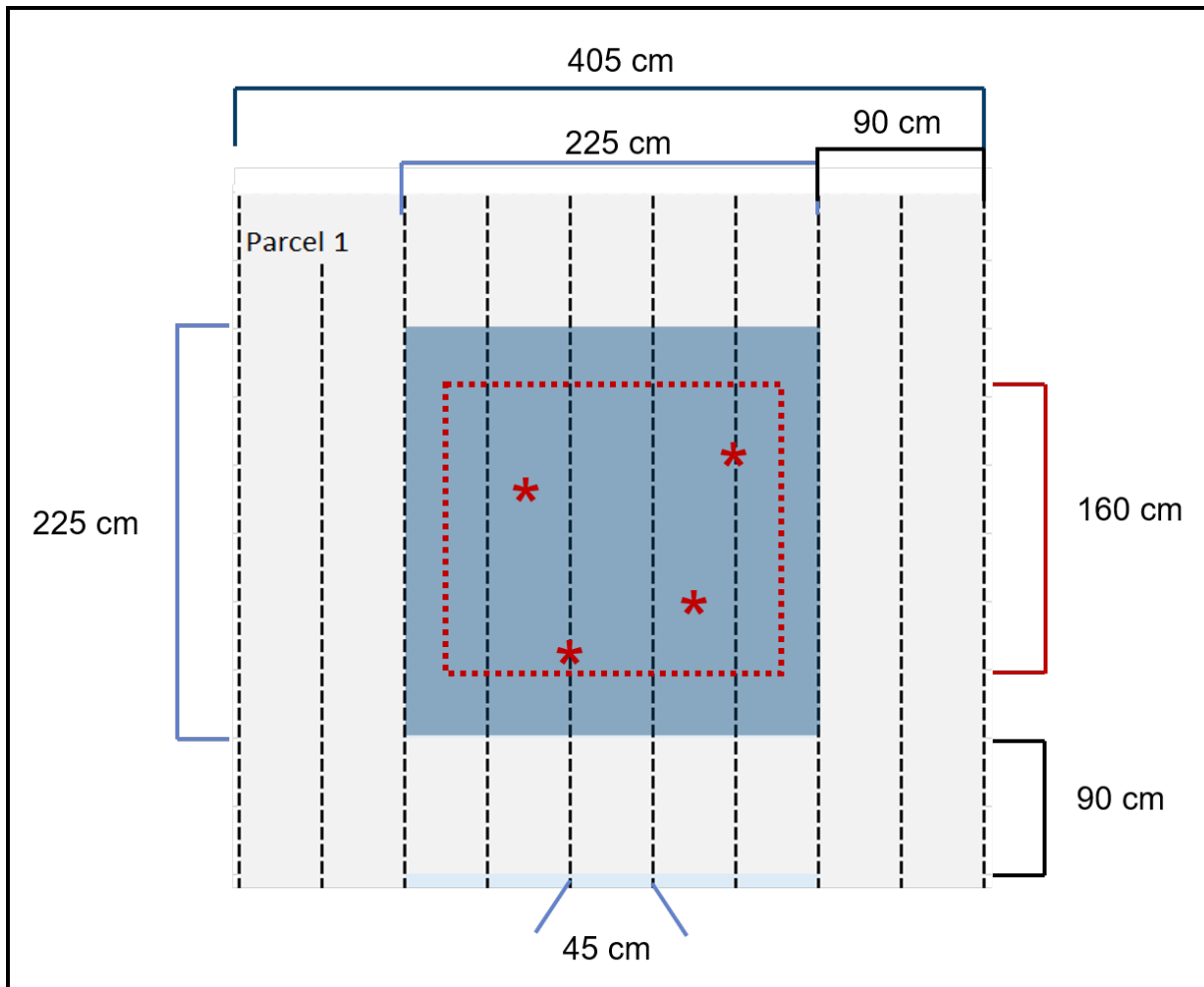
Figure 7: Plot illustration from the field experiment. Ten broken lines in black indicate the lines of sown soybeans. The distance between the furrows was 45 cm. Only the six furrows within the blue area received P from 40, 80 and 160 kg P_2O_5 ha^{-1} from bagasse ash (BA_{NG2}) and its blend with cattle manure compost (50% of total P sourced from BA_{NG2}), 80 kg P_2O_5 ha^{-1} from cattle manure compost and 40 kg P_2O_5 ha^{-1} from triple-superphosphate (TSP). Blends of BA_{NG2} and TSP supplied 32, 65 and 130 kg P_2O_5 ha^{-1} (70% of total P sourced from BA_{NG2}). The furrows containing P-fertilizer received N and K equivalent to 210 kg and 352 kg ha^{-1}, respectively. The outer two furrows and the furrows within 90 cm between fertilized plots did not receive any N, P or K fertilizer. Only soybeans growing along the four inner lines within the 160 cm central square were harvested (area marked with broken red lines). Two soil samples from the fertilized furrows and two samples from between fertilized furrows (red asterisks) were taken 14, 65 and 119 d after sowing.

Table 10: Field fertilizer treatments at Universidade Federal de Goiás (UFG, Goiânia, Brazil). Bagasse ash from sugarcane factory Usina Nova Gália Ltda. (BA_{NG2}), cattle manure compost (CMC), and triple superphosphate (TSP) were tested as P-fertilizers for soybeans. Blends of BA_{NG2}/ CMC received 50% of total P_2O_5 (phosphorus pentoxide) from BA_{NG2}, whereas the blend of BA_{NG2}/ TSP contained 70% of total P_2O_5 from BA_{NG2}. Nitrogen (N) and potassium oxide (K_2O) were adjusted by urea and Na_2SO_4.

P_2O_5 dosages	Fertilizer dosages	BA_{NG2}	BA_{NG2}/ $CMC_{50P:50P}$	TSP	CMC	BA_{NG2}/ $TSP_{70P:30P}$
32 kg ha^{-1}	Fertilizer doses (ha^{-1})					21.7 kg/ 2.5 t
	K_2O (kg ha^{-1})					352
	N (kg ha^{-1})					210
40 kg ha^{-1}	Fertilizer doses (ha^{-1})	4.5 t	2.2 t/ 1.6 t	87.7 kg		
	K_2O (kg ha^{-1})	352	352	352		
	N (kg ha^{-1})	210	210	210		
65 kg ha^{-1}	Fertilizer doses (ha^{-1})					43.5 kg/ 5.0 t
	K_2O (kg ha^{-1})					352
	N (kg ha^{-1})					210
80 kg ha^{-1}	Fertilizer doses (ha^{-1})	9 t	4.5 t/ 2.8 t		5.7 t	
	K_2O (kg ha^{-1})	352	352		352	
	N (kg ha^{-1})	210	210		210	
130 kg ha^{-1}	Fertilizer doses (ha^{-1})					87.7 kg/ 10.1 t
	K_2O (kg ha^{-1})					352
	N (kg ha^{-1})					210
160 kg ha^{-1}	Fertilizer doses (ha^{-1})	18 t	9.0 t/ 5.7 t			
	K_2O (kg ha^{-1})	352	352			
	N (kg ha^{-1})	210	210			

were treated with paraquat (Gramaxone), which enhanced the maturation. This is also a common practise in Brazil if the deadline for planting the next crop is approaching.

2.4.6 Soil sampling and analyses

Two soil samples were taken (0-20 cm depth) from fertilizer-containing furrows and two samples from between the furrows, 14, 65 and 119 d after sowing. All four samples from the same plot and sampling date were combined and dried at air temperature until they reached a constant weight. The pH levels and P extracted in Mehlich-I were measured from homogeneously mixed samples and only from treatments of interest based on yield analyses. These were 80 kg P_2O_5 ha^{-1} in the form of BA_{NG2}, CMC and its blend (40 kg P_2O_5 ha^{-1} derived from BA_{NG2}), 160 kg P_2O_5 ha^{-1} in the form of BA_{NG2}, 40 kg P_2O_5 ha^{-1} in the form of TSP and 65 kg P_2O_5 ha^{-1} in the form of BA_{NG2}/ $TSP_{70P:30P}$ from all three sampling dates. The pH levels were measured in 0.01 M $CaCl_2$ at a ratio of 1:2.5 (v/w). Extraction of P was performed in Mehlich-I solution. The measurements were conducted by Hannes Herzel (BAM, Berlin, Germany).

2.4.7 Harvest of the grain yields

The outer lines of soybeans, which contained fertilizers, were not harvested because of smaller plant sizes and probable border effects. Subsequently, only lines of plants from the area of interest, i.e. the inner part of the plot within the 160 cm square, red box from

Figure 7, were harvested by hand. From each plot, pods of 10 randomly chosen plants were removed and assessed. The numbers of filled pods, empty pods, total pods and seeds were recorded. Pods of remaining plants were also removed. After drying at 65 °C for 5 d, the grains were culled (LT-20 DEMO, HALDRUP GmbH, Ilshofen, Germany) and the collective weight of 1000 grains from each treatment was recorded (AX4202, Sartorius Lab Instruments GmbH & Co. KG, Goettingen, Germany).

The estimation of grain yield was based on averaged (AVG) number of seeds plant^{-1}, dry weight (DW) of seeds and planting density of soybeans ha^{-1} (Eq. 13).

$$Grain\,yield\,(\text{t ha}^{-1}) = 271000\,(\text{t ha}^{-1}) \cdot \frac{AVG_{\text{No. seeds/ plant}} \cdot DW_{1000\,\text{seed}}\,(\text{kg})}{10 \cdot 10^5} \qquad (13)$$

2.4.8 Removal of phosphorus from field

The grains were milled using a ball mill (MM 400, Retsch GmbH, Haan, Germany). Phosphorus was measured as described in 2.3.7.3. Phosphorus recovery from the field was considered as the total amount of P measured in the grains (Eq. 14), as roots, straw and husks remain on the field during harvest. The calculation of relative P effectiveness (RPE) was based on Telse et al. (2017), which is the removal of P by the grain yield relative to P in grains of soybeans fertilized with TSP (P_{TSP}; Eq. 15).

$$P_{\text{Recovery}}\,(\text{kg ha}^{-1}) = 1000 \cdot \frac{Grain\,yield\,(\text{t ha}^{-1}) \cdot P_{\text{grains}}\,(\text{wt\%})}{100} \qquad (14)$$

$$RPE\,(\%\ \text{of TSP}) = 100 \cdot \frac{P_{\text{Fertilizer}}\,(\text{kg ha}^{-1})}{P_{\text{TSP}}\,(\text{kg ha}^{-1})} \qquad (15)$$

2.5 Statistical analyses

The randomization and statistical analyses were performed using statistical software RStudio, version 1.2.1355 (R: free software environment for statistical computing and graphics (2019), https://www.r-project.org/). Randomization of plants was based on R package agricolae. Data were compared with one- or two-way analyses of variance (ANOVA). A Tukey's HSD posthoc test was applied to data from all greenhouse experiments and Duncan's multiple range test was applied to data from field experiments when ANOVAs were statistically significant (p <0.05, used R package agricolae). Analyses of variance with p-values around 0.2 were considered as trends. ANOVAs of all data are summarized in Tables S6 to S17. Data were calculated as arithmetic means ± standard error of means of replicates. The figures were generated using the R package ggplot2.

2.6 Summary of technical equipment and software programs

Table 11: List of software programs

Programs, packages, templates	Developer
Root image analyses	PaintRhizo, FZJ, Jülich, Germany
ImageJ 1.46r	NIH, https://imagej.nih.gov/ij/
RStudio, version 1.2.1355	www.R-Project.org
R package: agricolae	Felipe de Mendiburu
R package: ggplot	H, Chang W, Henry L, Pedersen TL, Takahashi K, Wilke C, Woo K, Yutani H, Dunnington D, Rstudio
LaTeX, TEX Maker 5.0.4	http://www.xm1math.net/texmaker/
Template for dissertation	based on Dr. Torsten Richter, https://www.tortools.de/doku.php/latex:vorlagen

Table 12: List of technical equipment

Type	Equipment
Allegra 25R centrifuge, Bleckman Coulter, Indianapolis, United States	Centrifuge
ArtFuel-Gasifier	Circulating fluidized bed gasifier
ATIKA 322050, Altrad Lescha Atika GmbH, Burgau, Germany	Concrete mixer
AX4202, Sartorius Lab Instruments GmbH & Co. KG, Goettingen, Germany	Balance
Berkili Siebmaster 1, SM1E, 18 x 8 mm sieve, HOWA parts, Warstein, Germany	Soil sieving machine
Bruker 600-MHz spectrometer	NMR
Canon EOS 450D, EFS-5 18-55 mm	Photo camera
Canon EOS 70D, EF 14 mm	Photo camera
D8 Advance, Bruker AXS, Germany	XRD
Elementar Vario El Cube, Elementar Analysensysteme GmbH, Langenselbold, Germany	CHNOS Elemental Analysis
ES 300-E Roxor, FREYMATIC AG, Domat/Ems, Switzerland	Soil shredder
HDG Compact 100 (100 kW thermal output), HDG Bavaria GmbH, Massing, Germany	Furnace
iCAP 6500, Fisher Scientific, Schwerte, Germany	ICP-OES
ICS-3000, Fischer Scientific, Life Technologies GmbH, Darmstadt, Germany	Ion chromotography system
Li- 3100, Li-cor, Nebraska, USA	Leaf area scanner
LT-20 DEMO, HALDRUP GmbH, Ilshofen, Germany	Thresher and cleaner
Mars 5, Prg. Pflanzen160 X-Press, Kamp-Lintfort, Germany	Microwave
MF 4299, Massey Ferguson, AGCO, Duluth, United States	Tractor
Milli-Q Reference, Merck, Darmstadt, Germany	Ultra-pure water
MM 400, Retsch GmbH, Haan, Germany	Milling machine
PG503-S, Mettler Toledo GmbH, Gießen, Germany	Balance
PP120B, qteck GmbH, Germany	Pelletizer
PST Plus, Marchesan Tatu, Matão, Brazil	Plough/ sowing equipment
R02, Maschinenfabrik Gustav Eirich GmbH & Co KG, Germany	Mixer
Retsch Z 1, Retsch GmbH, Haan, Germany	Milling machine
ScreenHouse platform, FZJ, Jülich, Germany	Imaging chamber
SevenGo Duo, Mettler Toledo GmbH, Gießen, Germany	Portable pH/ EC Meter
Sodium vapour lamps (SON-T AGRO 400, Philips	Artificial light
TH15, Incubator Hood, Edmund Bühler GmbH, Bodelshausen, Germany	Incubator
TR 1050, Nabertherm GmbH, Lilienthal, Germany	Oven

3 Results

3.1 Selection of growing medium

Prior to starting experiments on plants in a soil context, the effect of bagasse ash on certain physical and chemical attributes of Oxisol soil, sand and nutrient-poor substrate, were determined. The analyses aimed to find a substrate for highly controlled experiments under greenhouse conditions, as the chemical compositions of Oxisol soil available at IBG-2 varied and was rich in Fe and Al (Table 2), which may decrease the availability of P to soybeans. A substrate, which was very poor in phosphorus (P) and whose water household, pH levels and electric conductivity would be least modified by bagasse ash was needed. Both sand and nutrient-poor substrate contained less P than Oxisol soil (Table 2). Five dosages of bagasse ash (BA_{SP}) were homogeneously incorporated into the rooting media resulting in 0, 2.5, 7.5, 15, and 22.5 g BA_{SP} L^{-1} of medium.

In Oxisol soil the water infiltration time (WIT) and water retention after incubation (WRaI) were not significantly affected by increasing the dosage of bagasse ash, while water holding capacity (WHC) significantly decreased, and pH level and electric conductivity (EC) significantly increased starting from 2.5 g BA_{SP} L^{-1} Oxisol soil, i.e. 5 t ha^{-1} (Table 13).

In sand, the WHC and WRaI significantly decreased, whereas the WIT, pH level and EC significantly increased with increasing dosages of bagasse ash (Table 13). In contrast to Oxisol soil, none of measured factors were significantly affected by high dosages of BA_{SP} ash in a nutrient-poor substrate environment (Table 13).

Mineral analyses (Table 2) showed that the nutrient-poor substrate contained approximately four times less of total P than Oxisol soil. Subsequently, experiments aiming at detailing P availability from bagasse and bagasse-based ashes to soybeans were conducted on a nutrient-poor substrate under highly controlled greenhouse conditions. To allow also for a "From Lab to Field" approach aimed at identifying bagasse ash fertilizer potential under realistic scenarios, pot experiments under controlled conditions using Oxisol soil were also conducted. This allowed the comparison between a highly controlled substrate to less controlled soil environment, and was followed by field experiments at Brazilian Federal University of Goiás (UFG, Goiânia, Brazil). To conclude the "From Lab to Field" approach, pot experiments using Oxisol soil were run in the greenhouse again, testing bagasse ash-based fertilizer products for soybeans.

Table 13: Effects of homogeneously incorporated bagasse ash from unknown sugarcane factory in São Paulo state (BA_{SP}), Brazil, on rooting media (sand, nutrient-poor substrate and Oxisol soil) based on ash dosage. The effects are represented by water holding capacity (WHC), water infiltration time (WIT), water retention after incubation (WRaI) at room temperature (sand) or at 60 °C to enhance the drying of nutrient-poor substrate and Oxisol1 soil, pH levels and electric conductivity (EC). WRaI in sand, Oxisol soil and nutrient-poor substrate was measured 9, 4 and 5 d after incubation beginning and is relative to not dried state (T_0). Values labelled with the same letter are not significantly different within the same treatment rows (Tukey's test, p $\leq$0.05, n =5).

Growing medium	Ash dose (t ha^{-1})	WHC (wt%)	WIT (sec. L^{-1})	WRaI (% of T_0)	pH	EC (S m^{-1})
Sand	0	25.64 ^{a}	94.36 ^{c}	1.58 ^{ab}	6.94 ^{d}	0.011 ^{e}
	5	25.30 ^{ab}	90.72 ^{c}	2.11 ^{a}	7.31 ^{c}	0.014 ^{d}
	15	24.70 ^{bc}	105.12 ^{c}	0.76 ^{bc}	7.85 ^{bc}	0.019 ^{c}
	30	24.07 ^{cd}	142.48 ^{b}	0.25 ^{c}	8.26 ^{ab}	0.024 ^{b}
	45	23.90 ^{d}	183.32 ^{a}	0.08 ^{c}	8.31 ^{a}	0.03 ^{a}
Oxisol soil	0	54.13 ^{a}	14243.20 $^{n.s.}$	0.26 $^{n.s.}$	5.79 ^{d}	0.037 ^{c}
	5	49.43 ^{b}	14562.80 $^{n.s.}$	0.17 $^{n.s.}$	5.86 ^{c}	0.037 ^{c}
	15	50.24 ^{b}	13762.80 $^{n.s.}$	0.23 $^{n.s.}$	6.01 ^{bc}	0.039 ^{bc}
	30	49.36 ^{b}	19035.20 $^{n.s.}$	0.17 $^{n.s.}$	6.20 ^{ab}	0.043 ^{ab}
	45	47.75 ^{b}	16983.20 $^{n.s.}$	0.29 $^{n.s.}$	6.50 ^{a}	0.048 ^{a}
Nutrient poor substrte	0	53.86 $^{n.s.}$	3250.00 $^{n.s.}$	0.10 $^{n.s.}$	6.19 $^{n.s.}$	0.009 $^{n.s.}$
	5	55.18 $^{n.s.}$	3360.40 $^{n.s.}$	0.13 $^{n.s.}$		
	15	54.42 $^{n.s.}$	3648.93 $^{n.s.}$	0.12 $^{n.s.}$		
	30	54.50 $^{n.s.}$	4013.60 $^{n.s.}$	0.13 $^{n.s.}$		
	45	54.11 $^{n.s.}$	3716.93 $^{n.s.}$	0.11 $^{n.s.}$	6.22 $^{n.s.}$	0.010 $^{n.s.}$

3.2 Bagasse ash toxicity analyses

Prior to starting the analyses of bagasse ashes as fertilizer for soybeans, the toxicity effects of bagasse ashes on germination, radicle elongation and root development were tested.

3.2.1 Bagasse ash does not inhibit germination and radicle elongation of cress seeds

To identify potential plant toxicity of two bagasse ashes from Brazilian sugarcane factories located in Goiás (BA_G) and São Paulo (BA_{SP}) states, garden cress (*Lepidium sativum*) seeds were germinated on 0, 7.5, 15 and 22.5 mg BA_{SP} and BA_G ml^{-1} ultra-pure water. Germination rate, radicle elongations and germination indices were evaluated.

Germination of garden cress seeds increased by 34% (BA_{SP}) and 27% (BA_G) compared to the control treatment (no ash; p $\leq$0.1, Table 14). Radicle lengths of seedlings from both ash-germinated treatments were significantly increased compared to the control treatment. Radicle length of seedlings growing on 15 mg BA_{SP} and BA_G ml^{-1} ultra-pure water was significantly longer than both other treatments. At 22.5 mg BA_{SP} and BA_G ml^{-1} ultra-pure water, radicle elongation was significantly inhibited (Table 14). The radicle lengths

of seedlings germinating on BA_{SP} and BA_G did not statistically differ (p $\leq$0.1, Table 14).

The germination indices (GI), which are expressed as germination rates and radicle lengths relative to the control treatment, tended to increase about 3-fold following BA_{SP} and BA_G treatment as compared to the control treatment (p $\leq$0.15, Table 14). According to Zucconi et al. (1981), GI higher than 80% are considered to be indicative of no phytotoxicity.

Table 14: Cress toxicity test as an indicator for seed germination rate (GR), radicle elongation (RE) and germination index (GI), which is expressed as germination rate and radicle length relative to the control treatment. Treatments contained 0 to 22.5 mg ash from sugarcane factories located in in Goiás (BA_G) and São Paulo (BA_{SP}) states ml^{-1} ultra-pure water. Values labelled with the same letter are not significantly different at p $\leq$0.05 (Tukey's test, p $\leq$0.05, n=3).

Treatments	GR (%)	RE (mm)	GI (%)
Control	42.86 [n.s.]	2.77 [b]	100 [n.s.]
BA_G	70.16 [n.s.]	4.84 [a]	286.44 [n.s.]
BA_{SP}	76.83 [n.s.]	5.04 [a]	326.09 [n.s.]

Dosage: RE (mm) 0 mg ash ml^{-1}	7.5 mg ash ml^{-1}	15 mg ash ml^{-1}	22.5 mg ash ml^{-1}
2.77 [c]	5.01 [ab]	5.46 [a]	4.31 [b]

3.2.2 Soybean roots are not repelled by bagasse ash

To identify potential evasive behaviour of soybean roots towards bagasse ash, a rhizoton system was set up. Here, around 215 mg P (in 88g cBP ash) was deposited in a single depot within the otherwise nutrient-poor substrate. The depot was approximately 10 cm below and 10 cm to the side of the pre-cultivated soybean plantlet. Based on pre-experiments in rhizotron system and higher availability of P from TSP than from bagasse ash, 92 mg P $plant^{-1}$ in the form of TSP were also included as a nutrient depot and served as a positive control treatment. Plants without P-fertilization were used as negative control treatment. Roots growths of soybeans captured 13 and 20 d after fertilizer application are presented, whereas the others were ignored because of missing statistical supports even after 20 d growth.

Approximate behaviour of emerging soybean roots towards the P-containing ash depot was observed 4 to 6 days after fertilizer application (data not shown). As early as 13 d after fertilizer application, soybean roots receiving P from bagasse ash and TSP tended to proliferate in, around and above the depot area (Figure 8 A, p $\leq$0.2, Table S3). Nonetheless, even after 21 days, the trends were not statistically supported (p $\leq$0.2, Table S3). Additionally, the soybeans receiving P from bagasse ash increased the branching of roots in lower part of the rhizotron (Figure 8 B). Furthermore, total dry matter and biomass partitioning to the roots did not statistically differ after 21 d of growth (Table S3).

Without any P-fertilization soybeans roots tended to increase branching mainly in the lower part of the rhizotrons (Figure 8 B). Compared to control plants, plants receiving P
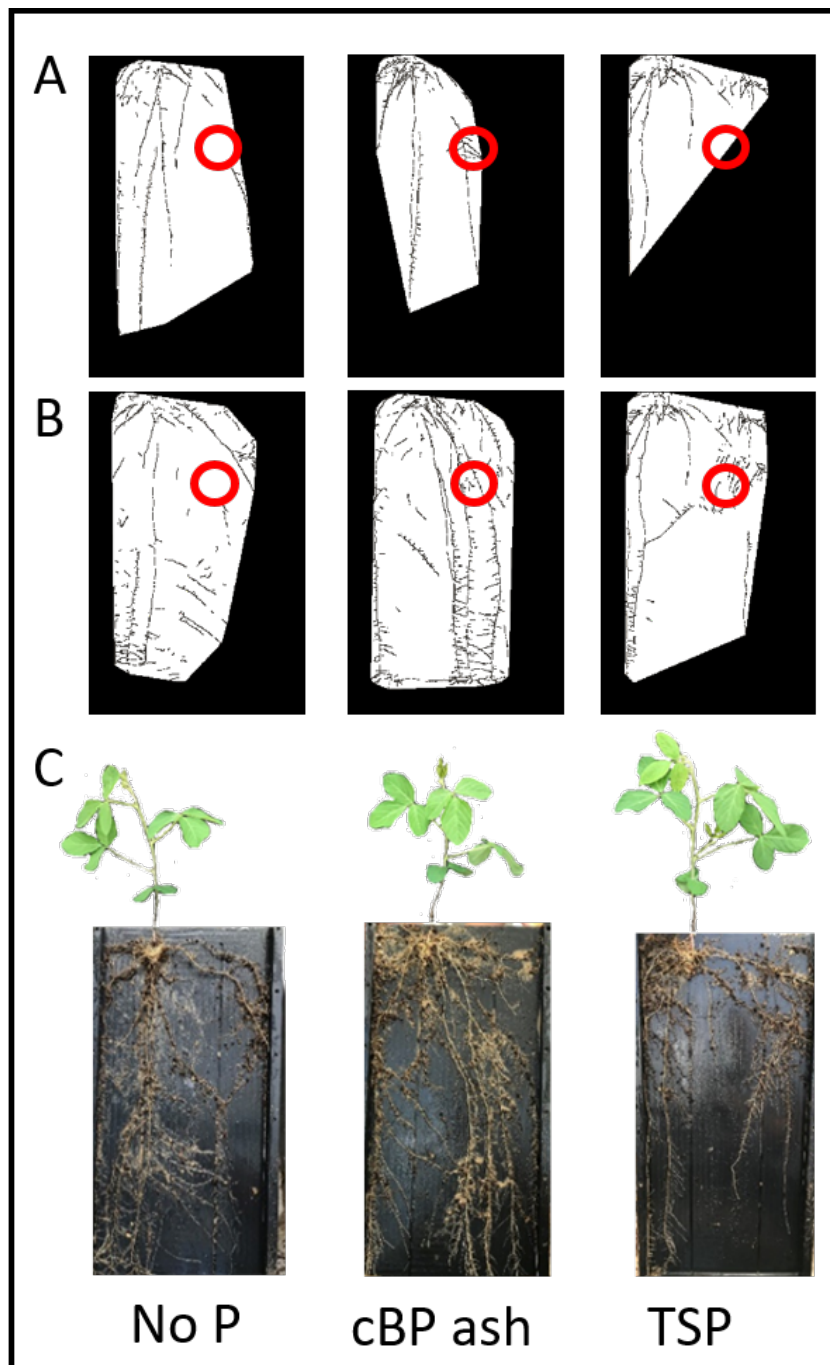
Figure 8: Response of soybean roots to localized phosphorus (P) supply. The fertilizer depot (red circle) received 0, 215 and 92 mg P from combusted bagasse pellets (cBP) ash (88 g) and triple superphosphate (TSP). (A) Root architectures after 13 d growth, (B) root architectures after 21 d growth, and (C) shoot and root architectures after 21 d growth (imaged during destructive analyses).

from TSP had 10% of total root length localized in and around the P-fertilizer while in cBP ash had 12% (p ≤0.2, Table S3).

3.3 Pot experiments in a nutrient-poor substrate

Analyses of bagasse and co-processed bagasse/ chicken manure ashes were performed by ^{31}P NMR spectroscopy, XRD and sequential extraction of P to identify P-forms (used here as generic term for P-species, crystalline P compounds and interactions with other ions, such as Ca, Mg, Fe and Al, detected by ^{31}P NMR spectroscopy, XRD and sequential extraction, respectively). Finally, P availability to soybeans was tested in a nutrient-poor substrate environment.

3.3.1 Availability of P from bagasse ash produced at Brazilian sugarcane factory

Chemical composition, P-species and solubility were analysed in bagasse ash obtained from a Brazilian sugarcane factory (Usina Nova Gália Ltda., Paraúna, Brazil), further named BA_{NG1} ash. This was followed by soybean growth. The aim of a greenhouse pot experiment was to determine the effects of bagasse ash as a plant fertilizer, using ash as it is produced at sugarcane factories in Brazil under typical conditions of around 1050 °C.

3.3.1.1 Chemical composition of BA_{NG1} ash, P forms and solubility fractions

Although the combustion temperature in the boiler at Usina Nova Gália Ltda. was approximately 1050 °C (based on information from Usina Nova Gália Ltda.), BA_{NG1} ash still contained visible bagasse fibres (Figure 9 A) and 5 wt% C (Table 1) indicating low combustion efficiency. The ash still contained 0.38 wt% P, 1.21 wt% K, 1.26 wt% Ca, 0.46 wt% Mg, 0.10 wt% Na, 5.38 wt% Fe and 3.86 wt% Al (Table 1). In total 56% of total P from BA_{NG1} ash was soluble in citric acid (Table 1).

While all P was expected to be converted into crystalline P compounds after thermal processing, the high carbon content in BA_{NG1} ash called for ^{31}P NMR spectroscopy analyses (Vestergren et al., 2012), as P in organic forms might also occur. The BA_{NG1} ash contained shifts at around 5.7, 6 to 3 and -4.7 ^{31}P ppm^{-1} (Figure 9 B), indicating the presence of orthophosphates, pyrophosphates and varying orthophosphate monoesters, respectively (Cade-Menun, 2005). Orthophosphate monoesters are of organic origin whereas ortho- and pyrophosphates are mineral compounds. Out of all P extractable in 0.25 M NaOH + 50 mM Na$_2$EDTA and detected by ^{31}P NMR spectroscopy, around 98% occurred as orthophosphate making all other P-species to minor compounds (Figure 9 B). XRD analyses of crystalline P compounds remained inconclusive (Table 15) due to high background intensities of amorphous compounds.

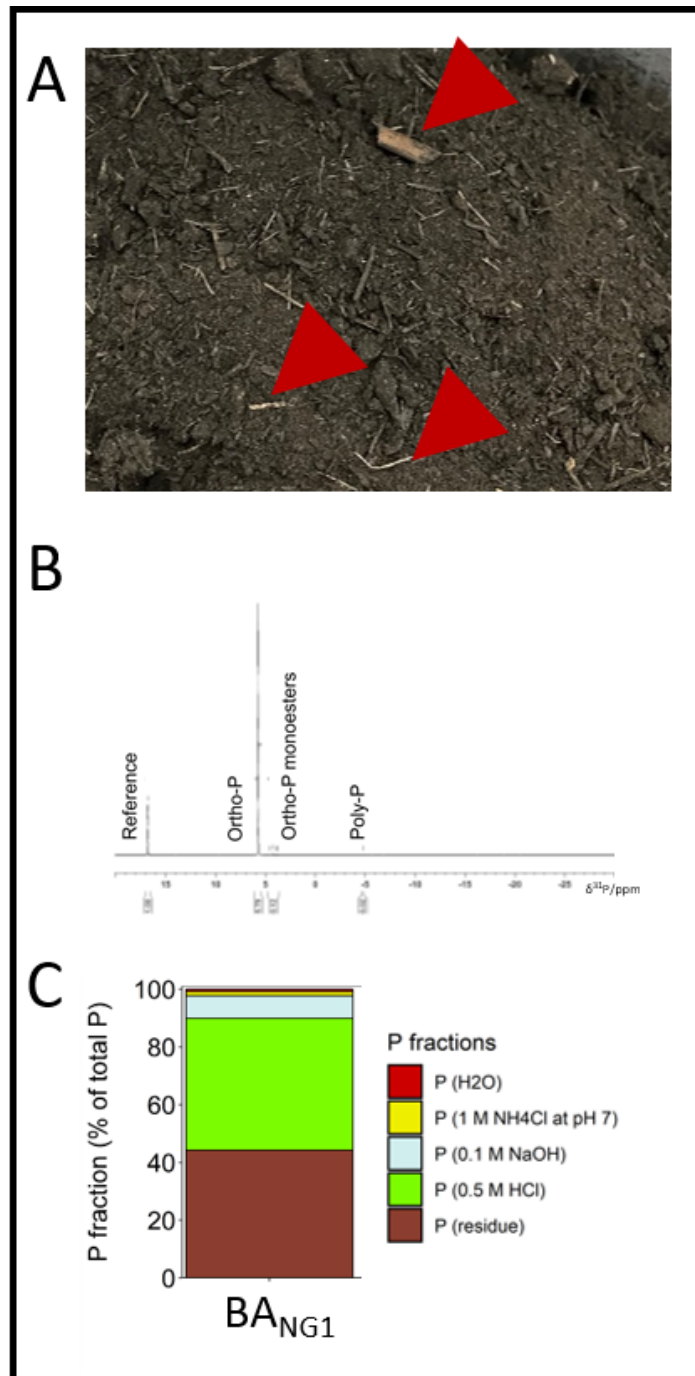**Figure 9:** Bagasse ash from a Brazilian sugarcane factory Usina Nova Gália Ltda. (BA$_{NG1}$; A). Red arrows point at bagasse fibres, which remained after combustion. (B) Phosphorus (P)-species determined by ^{31}P NMR spectroscopy (the spectra in ^{31}P NMR were referred to methylenediphosphonic acid at 16.8 ppm), and (C) P-fractions extracted in water, 1 M NH$_4$Cl at pH 7, 0.1 M NaOH and in 0.5 M HCl.

Table 15: Crystalline compounds identified in (1) bagasse ash from sugarcane factory Usina Nova Gália Ltda. (BA_{NG1}), (2) combusted bagasse pellets (cBP) ash, (3) gasified bagasse pellets (gBP) ash, (4) in an ash from co-gasification of bagasse pellets and chicken manure in a ratio of 85:15 by dry weight (gBP/ $CM_{85:15}$), (5 and 6) in ashes from co-combustion of bagasse pellets and chicken manure in ratios of 80:20 (cBP/ $CM_{80:20}$) and 60:40 by dry weights (cBP/ $CM_{60:40}$), and (7) in an ash from co-combustion of bagasse pellets and sewage sludge in a ratio of 83:17 by dry weight (cBP/ $SSL_{83:17}$), as well as (8 and 9) in products from post-thermochemical treatments of cBP/ $CM_{60:40}$ and Na_2SO_4 (cBP/ $CM_{60:40}+Na$) and Na_2SO_4/ K_2SO_4 (cBP/ $CM_{60:40}+Na/K$). Two undefined P compounds were soluble in citric acid (CA) and formic acid (FA). Powder X-ray diffraction analyses were conducted and interpreted by Hannes Herzel, BAM, Berlin, Germany.

No.	Ashes	Whitlockite ≈ $Ca_9Fe(PO_4)_7$	P compound soluble in CA	P compound soluble in FA	$Ca_2Na_xK_{1-x}(PO_4)_2$	$CaNaPO_4$	evtl. $FePO_4$	Quartz SiO_2	Christobalite SiO_2	Hemetite Fe_2O_3	Magnetite Fe_3O_4	$K_4CaSi_3O_9$	$KAlSiO_4$	Calcite $CaCO_3$
1	BA_{NG1} ash						X	X		X				
2	cBP ash						X	X		X				
3	gBP ash							X			X			
4	gBP/ $CM_{85:15}$	X						X			X	X		X
5	cBP/ $CM_{80:20}$		X	X	X			X	X				X	X
6	cBP/ $SSL_{83:17}$	X						X			X	X		
7	cBP/ $CM_{60:40}$		X	X	X									
8	cBP/ $CM_{60:40}+Na$					X								
9	cBP/ $CM_{60:40}+Na/K$					X								

Sequential extraction of P allowed the acquisition of five fractions of P (Qian et al., 2009), which were soluble in water (P_{H2O}), 1 M NH_4Cl at pH 7 (P_{NH4Cl}), 0.1 M NaOH (P_{NaOH}) and in 0.5 M HCl (P_{HCl}). Phosphorus that was not soluble in any of extraction solution was referred to residual fraction ($P_{residual}$). Both P_{H2O} and P_{NH4Cl} were the minor fractions in BA_{NG1} ash and were below 1% of total P, respectively (Figure 9 C). While the fraction of P_{NaOH} was 8% of total P, the fractions of P_{HCl} and $P_{residual}$ dominated in BA_{NG1} ash and were 46 and 44% of total P (Figure 9 C).

3.3.1.2 Response of soybeans to increasing dosages of BA_{NG1} ash

Pre-cultivated soybean plantlets were grown on a nutrient-poor substrate, which contained six dosages of BA_{NG1} ash (0 to 31.6 g kg^{-1} nutrient-poor substrate), for 44 days. This allowed the evaluation of the effects of BA_{NG1} ash and to determine the dosage of ash needed to show an effect comparable to that of conventional fertilizer (TSP/ K_2SO_4).

Compared to unfertilized control plants, ash dosages delivering as little as 10 mg P/ 32 mg K kg^{-1} nutrient-poor substrate resulted in significantly increased total plant dry biomass from shoot and root organs, and tended to decrease the biomass partitioning to the roots (Figure 10 C and D). The most significant increase of biomass accumulation
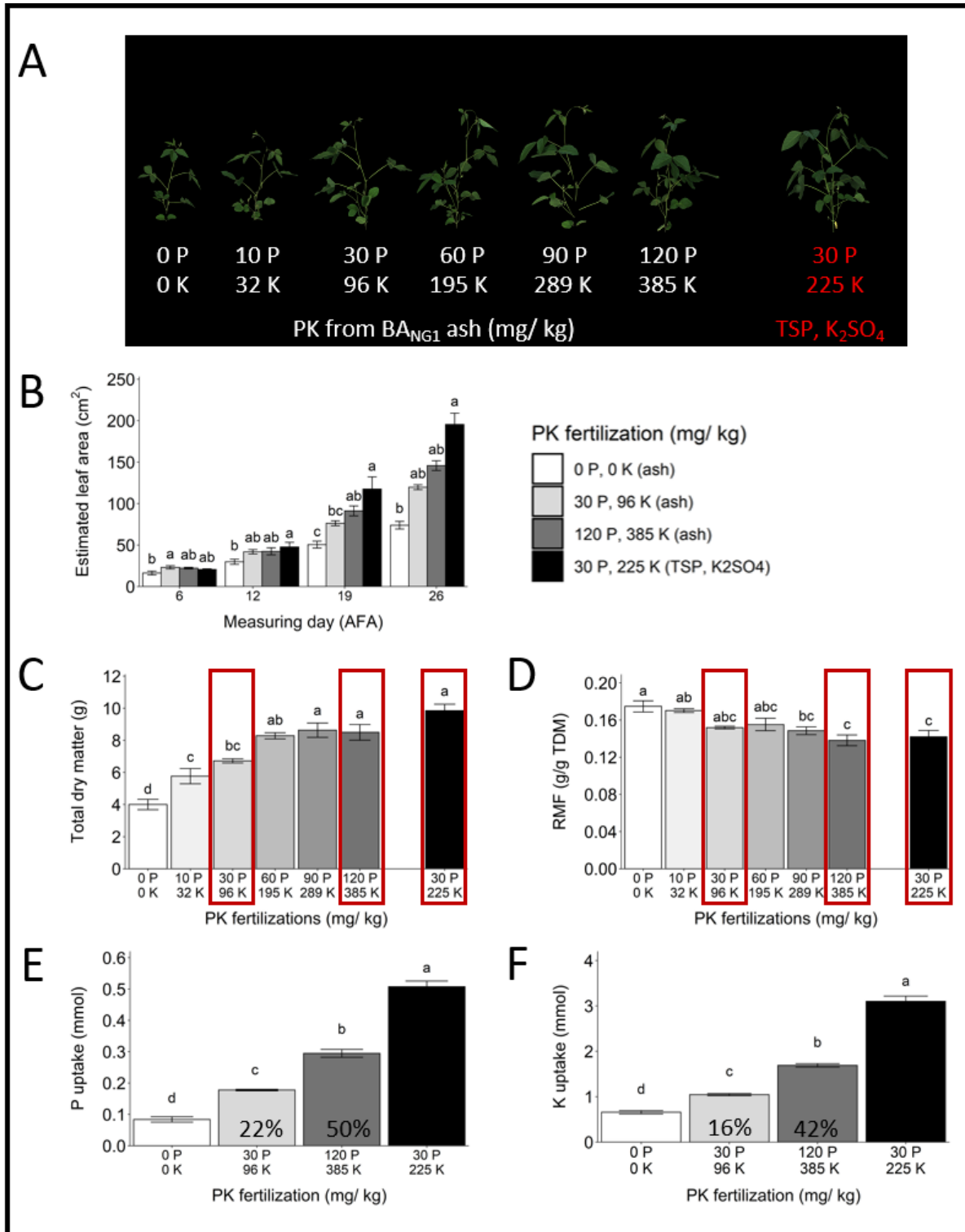
Figure 10: Effect of increasing bagasse ash dosages on soybean morphology and phosphorus (P) and potassium (K) uptakes after 44 d growth. (A) Phenotypic differences of soybeans, (B) estimated leaf areas between 6 and 26 d after fertilizer application (AFA), (C) total dry matters (TDM) from shoot and root organs, (D) biomass partitioning to the roots represented by root mass fractions (RMF), and uptakes of (E) P and (F) K during 44 d growth. Red label in A indicates plants fertilized with P and K from triple-superphosphate (TSP) and K_2SO_4. The values within the bars (E and F) represent relative agronomic effectiveness compared to P and K from TSP and K_2SO_4. The error bars represent standard errors of the means. Identical letters indicate no statistical differences (Tukey's test, p $\leq$0.05, n = 5).

was observed after fertilizer with around 90 mg P/ 290 mg K kg^{-1} substrate was added; further increasing the ash dosage did not promote biomass yield but did significantly decrease the biomass partitioning to the roots (Figure 10 C and D). Soybeans receiving 30 mg P/ 96 mg K kg^{-1} nutrient-poor substrate from BA$_{NG1}$ ash significantly increased their leaf areas as compared to unfertilized plants after only 6 d of growth, whereas plants receiving 30 mg P and 225 mg K kg^{-1} nutrient-poor substrate from conventional fertilizers, i.e. TSP and K$_2$SO$_4$, needed additional 6 d (Figure 10 B). Starting 12 d after fertilizer application, the leaf areas of soybeans fertilized with 30 mg P/ 96 mg K kg^{-1} nutrient-poor substrate and 120 mg P/ 385 mg K kg^{-1} nutrient-poor substrate in the form of BA$_{NG1}$ ash did not statistically differ from unfertilized plants, although they tended to follow the trends shown by plants fertilized with conventional fertilizer (Figure 10 B). This showed that BA$_{NG1}$ ash fertilized soybeans were not able to compete with TSP/ K$_2$SO$_4$-fertilized soybeans after 26 d growing. However, shoot growth, total dry biomass and biomass partitioning to the roots in soybeans fertilized with 120 mg P/ 385 mg K kg^{-1} nutrient-poor substrate in the form of BA$_{NG1}$ ash and 30 mg P/ 225 mg K kg^{-1} nutrient-poor substrate from TSP/ K$_2$SO$_4$ did not statistically differ after 44 d growth (Figure 10 A-D). On the one hand, this indicates that fertilizer effects can vary with regards to the plant's growing period, while on the other hand, this shows that by increasing dosage of ash, soybeans can attain a comparable biomass to those treated with conventional fertilizer.

Over a growth period of 44 d, plants fertilized with 30 mg P and 96 mg K kg^{-1} nutrient-poor substrate in the form of BA$_{NG1}$ ash took up significantly less P$_{0-44}$ and P$_{0-44}$, than soybeans that received equal amounts of P from TSP (Figure 10 E and F). Phosphorus and K uptakes reached 22 % and 16 % of that of soybeans fertilized with TSP and K$_2$SO$_4$ (Figure 10 E and F), which was 8% and 14% of total P and K supplied by BA$_{NG1}$ ash.

The increase of the P and K dosages to 120 mg P and 385 mg K kg^{-1} nutrient-poor substrate in the form of BA$_{NG1}$ ash resulted in an increase in the accumulation of soybean total dry biomass, and a decrease of biomass partitioning to the roots. The uptakes of P$_{0-44}$ and K$_{0-44}$ remained significantly lower than those from TSP and K$_2$SO$_4$ (Figure 10 E and F), which limits the use of BA$_{NG1}$ ash as fertilizer for soybeans.

3.3.2 Availability of P from bagasse ash combusted under lab conditions

Bagasse ash from the Brazilian sugarcane factory was combusted at around 1050 °C, washed with water from the grid at the base of the boiler, separated from the water by gravity and subsequently stored in open environment. According to Demeyer et al. (2001), among other factors, combustion conditions, handling, and storage all affect the availability of P from biomass ashes to plants. Thus, ashes used in the following experiments were produced from combustion of bagasse pellets (called cBP = **c**ombusted **b**agasse **p**ellets) that were produced at the sugarcane factory Usina Nova Gália Ltda., Brazil, and stored under controlled conditions at FRAUNHOFER UMSICHT, Germany, after delivery. The

following section focuses on the resulting chemical composition of cBP ash, concentrating on P as a plant nutrient and its availability to soybeans in a nutrient-poor substrate environment.

3.3.2.1 Chemical composition of cBP ash, P forms and solubility fractions

Prior to combustion, bagasse pellets contained around 46 wt% C, 0.028 wt% P, 0.12 wt% K, 0.11 wt% Ca, 0.05 wt% Mg, 0.007 wt% Na, 0.37 wt% Fe and 0.27 wt% Al (more details see Table S1). After combustion of bagasse pellets at 400 to 800 °C for 45 min. ($\lambda =$ 2.2), the bagasse pellets ash (cBP ash) contained 0.62 wt% C, 0.24 wt% P, 1.3 wt% K, 0.93 wt% Ca, 0.52 wt% Mg, 0.04 wt% Na, 3.8 wt% Fe and 2.76 wt% Al (Table 1). In total 19% of total P from cBP ash was soluble in citric acid (Table 1). Visually, cBP ash contained no bagasse fibres but did contain coagulated/ melted ash particles larger 1 mm (Figure 11 A).

Shifts around 5.7 to 6.1 and -4 to -5 in ^{31}P NMR spectroscopy analyses indicated ortho- and pyrophospahtes (Figure 11 B). Orthophosphates dominated over pyrophosphates and accounted for 94% of all P detected by ^{31}P NMR spectroscopy (Figure 11 B). In regard to crystalline P compounds, XRD analyses remained inconclusive due to high background intensities of amorphous compounds.

Around 82% of total P was not soluble in any of the extraction solutions (Figure 11 C). While P_{HCl} was 11% of total P, P_{NaOH}, P_{NH4Cl} and P_{H2O} were minor fractions and were around 3%, 3%, and <1% of total P, respectively (Figure 11 C).

3.3.2.2 cBP ash as fertilizer for soybeans

Soybeans receiving 30 mg P/ 161 mg K kg^{-1} nutrient-poor substrate from cBP ash needed 42 d to significantly increase the leaf area compared to unfertilized plants (Figure 12 B). The leaf area of 30 mg P/ 161 mg K kg^{-1} nutrient-poor substrate fertilized plants after 42 d growth was comparable to conventionally fertilized plants (30 mg P and 387 mg K kg^{-1} nutrient-poor substrate) after only 28 d growth (Figure 12 B). Increasing the amount of ash P and K to 72 mg P/ 387 mg K kg^{-1} nutrient-poor substrate resulted in the significant increase in leaf area, also being observed at 28 d. This increase was irrespective of provision of K, as similar amounts from cBP ash and TSP did not result in same leaf areas (Figure 12 B).

Soybeans fertilized with cBP ash containing 30 mg P/ 161 mg K kg^{-1} nutrient-poor substrate accumulated significantly less dry biomass and tended to partition more biomass to the roots than conventionally fertilized soybeans ($P \leq 0.1$, Figure 12 C and D). The uptakes of P_{0-58} and K_{0-58} followed the trends of biomass accumulation and were 11% and 8% of the amounts provided by TSP and K_2SO_4 (Figure 12 E and F), which was 5% of the total P and K supplied by cBP ash, respectively. Although soybeans fertilized with 170 mg P/ 995 mg of K kg^{-1} from cBP ash accumulated a biomass comparable to that of conventionally fertilized plants, the uptakes of P_{0-35} and K_{0-35} remained significantly lower
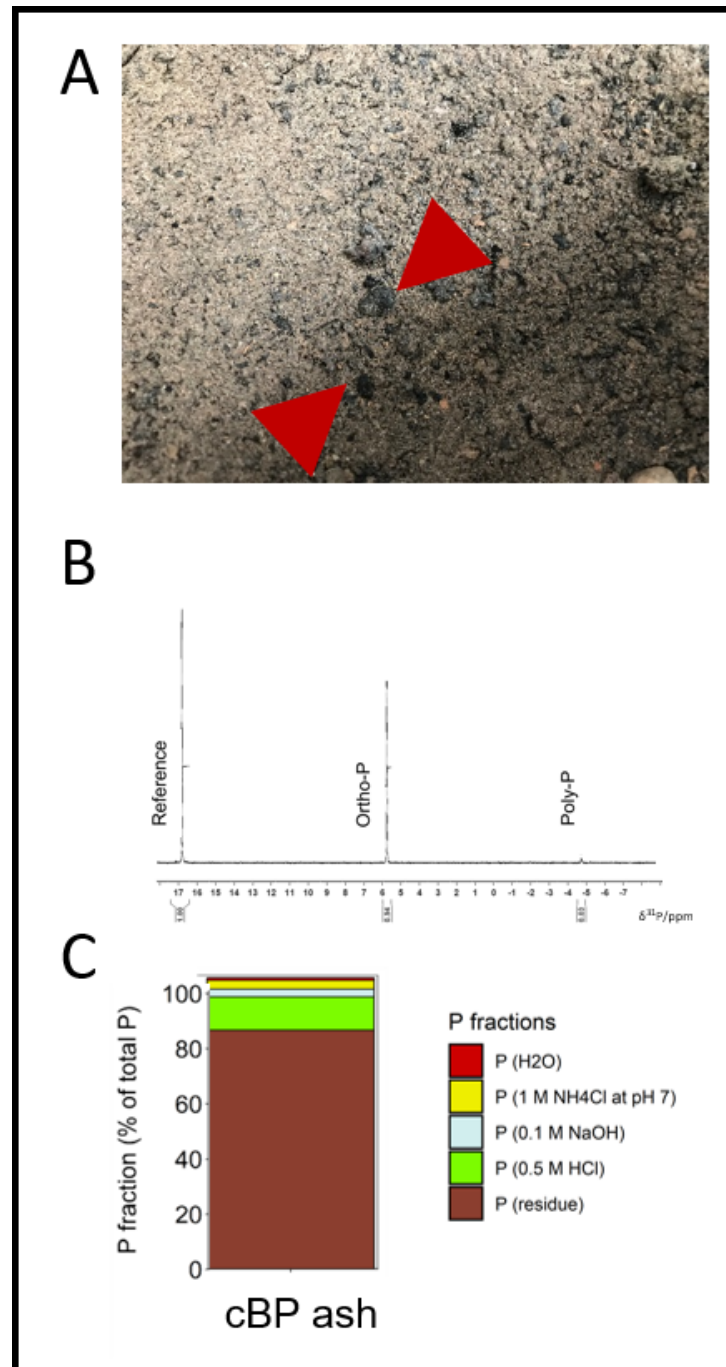
Figure 11: Ash from combustion of bagasse pellets (cBP) under lab conditions at 400-800 °C for 45 min (A). Red arrows point at coagulated/ melted ash particles, which were formed during combustion. (B) Phosphorus (P)-species determined by ^{31}P NMR spectroscopy. The spectra in ^{31}P NMR were referred to methylenediphosphonic acid at 16.8 ppm. (C) P-fractions extracted in water, 1 M NH$_4$Cl at pH 7, 0.1 M NaOH and in 0.5 M HCl.
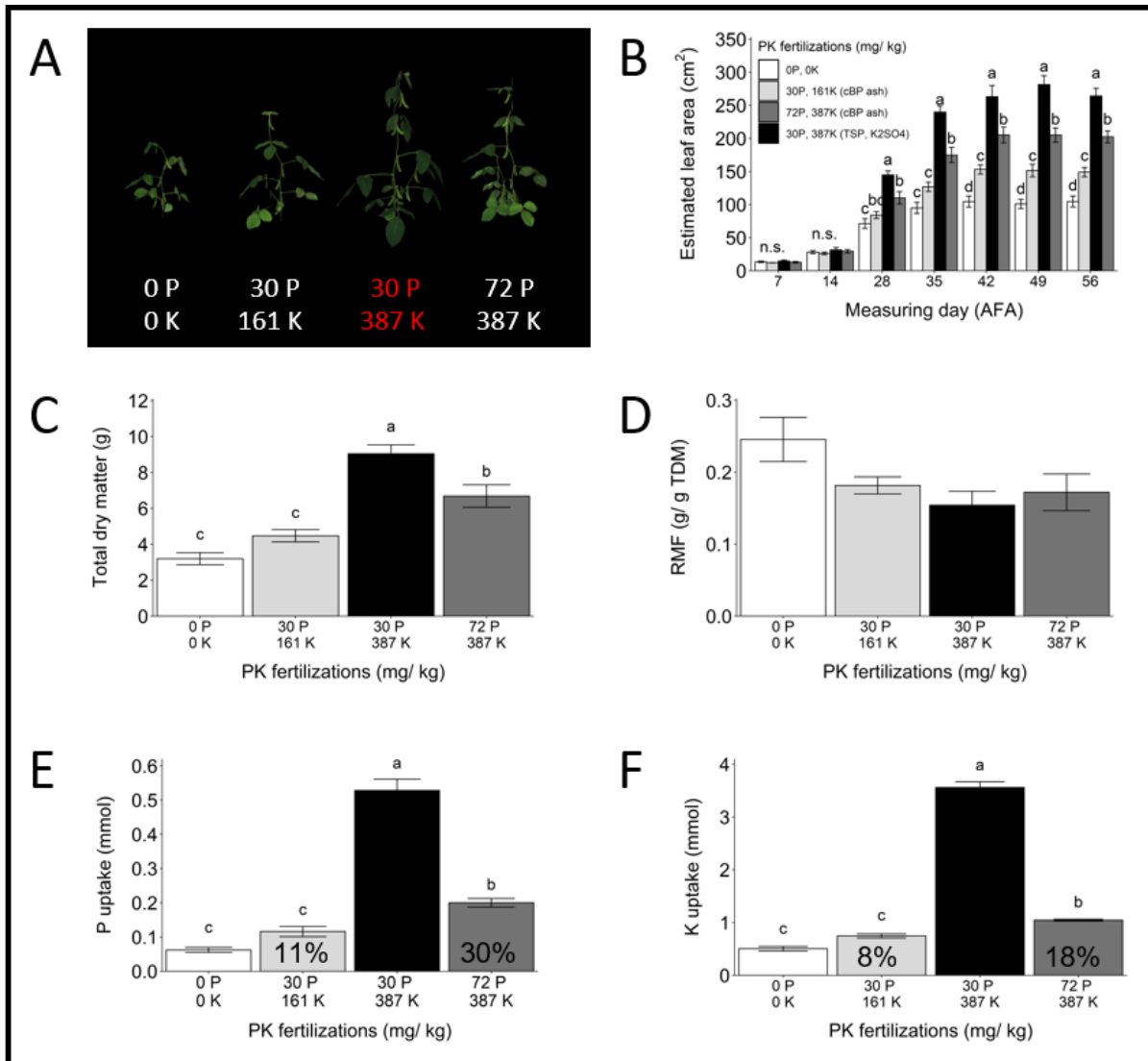
Figure 12: Effect of bagasse pellets (cBP) ash on soybean morphology and phosphorus (P) and potassium (K) uptakes after 58 d growth. (A) Phenotypic differences of soybeans, (B) estimated leaf areas between 7 and 56 d after fertilizer application (AFA), (C) total dry matters (TDM) of soybeans from shoot and root organs, (D) biomass partitioning to the roots represented by root mass fractions (RMF; $p \leq 0.1$), and uptakes of (E) P and (F) K during 58 d growth. Red label in A indicates fertilization with P and K from triple-superphosphate (TSP) and potassium sulphate (K_2SO_4). The values within the bars (E and F) represent relative agronomic effectiveness compared to P and K from TSP and K_2SO_4. The error bars indicate standard errors of the means. Identical letters indicate no statistical differences (Tukey's test, $p \leq 0.05$, n = 5).

(Table S4). Compared to unfertilized soybeans, biomass accumulation and uptake of P_{0-58} and K_{0-58} from 30 mg P/ 161 mg K kg^{-1} nutrient-poor substrate in the form of cBP ash were not statistically different but tended to increase, whereas the biomass partitioning to the roots tended to decreased (Figure 12 C-F).

An increase of P and K dosages to 72 mg P/ 387 mg K kg^{-1} nutrient-poor substrate in the form of cBP ash did not resulted in same amounts of total dry biomass and uptakes of P_{0-58} and K_{0-58} as compared to conventionally fertilized plants. Both total dry biomass and the uptakes of P_{0-58} and K_{0-58} were significantly lower in these plants as compared to conventionally fertilized plants and higher as compared to 30 mg P/ 161 mg K kg^{-1} nutrient-poor substrate cBP ash-fertilized plants (Figure 12 C-F). This indicates that K fertilization is not responsible for the limited fertilizer effect of cBP ash. Based on the fertigation of soybeans that received 73 mg P/ 488 mg K or 170 mg P/ 995 mg K kg^{-1} nutrient-poor substrate from cBP ash with all nutrients except P, additional supporting evidence was provided that P availability is responsible for the observed growth responses. No significant increase in total dry biomass accumulation of soybeans or uptake of P_{0-35} from cBP ash was observed, indicating sufficient concentrations of other minerals in the nutrient-poor substrates available to soybeans (Table S4). While additional fertigation of positive control plants significantly increased the uptake of K_{0-35}, it did not increase the uptake of K from cBP ash (Table S4), which indicated a sufficient supply of K by the ash.

3.3.2.3 Availability of minerals from cBP ash to soybeans

Soybeans fertilized with large amounts of BA_{NG1} and cBP ashes were able to accumulate comparable amounts of total dry biomass as conventionally fertilized plants even though much higher amounts of P and K were needed, the uptakes of P and K remained significantly lower (Figure 10, Figure 12 and Table S4). According to Müller-Stöver et al. (2018) fertilization of plants with large amounts of ash may imbalance the plant nutrition, because supplying minerals in excess may affect absorption and accumulation of other nutrients (Fageria, 2001). The interactions of minerals are measured by the level of growth response and the concentrations of nutrients in the biomass of plants (Fageria, 2001). Thus, to identify the role of nutrient imbalance on P and K uptakes, biomasses of soybeans receiving 0 and 170 mg P/ 995 mg K kg^{-1} nutrient-poor substrate from cBP ash (73 g cBP ash kg^{-1} nutrient-poor substrate) and 73 mg P/ 995 mg K kg^{-1} nutrient-poor substrate in the form of TSP and K_2SO_4 and partially fertigated with all nutrient excluding P (Table S4) were analysed for the concentrations of N, P, K, S, Mg, B, Ca, Cu, Fe, Mn, Si, Zn, Na, Cl and Al in soybean plants.

In the biomass of soybeans fertilized with cBP ash, the concentrations of Ca, Mg, Na and B were significantly higher by 1.3, 1.4, 1.4 and 1.8 times than those in the biomass of conventionally fertilized plants (Table 16). Compared to no-P control soybeans, which did not receive any P-fertilizer, the concentrations of Ca and Mg in cBP ash fertilized soybeans were significantly higher by 1.6 and 1.8 times. The concentrations of Na and B were not statistically different but did, however, tend to increase in cBP ash fertilized soybeans

Table 16: Concentrations of minerals in the biomass of soybeans fertilized with 0 and 73 g cBP ash kg^{-1} substrate and luxury supply of P (72.6 mg kg^{-1}) in the form of triple-superphosphate (TSP) after 35 d growth. One half of replicates received additional nutrients but no P every week. The data present averaged values from fertigated and not fertigated plants (treatment effect). Cu, Ni, Co, and Mo were below measuring limits. Numbers without letters were not statistically supported for pairwise comparisons. Identical letters indicate no statistical difference within the same mineral rows (Tukey's test, p $\leq$0.05, n = 6).

Elements	Abbrev.	Units	No P	73 g cBP ash kg^{-1}	Conv. fertilization
Nitrogen	N	mmol g^{-1} TDM	0.64 [b]	0.78 [b]	1.41 [a]
Phosphorus	P	mmol g^{-1} TDM	0.02 [b]	0.03 [b]	0.19 [a]
Potassium	K	mmol g^{-1} TDM	0.34 [b]	0.24 [c]	0.74 [a]
Sulphur	S	mmol g^{-1} TDM	0.06 [b]	0.04 [c]	0.10 [a]
Calcium	Ca	mmol g^{-1} TDM	0.16 [c]	0.26 [a]	0.23 [b]
Magnesium	Mg	mmol g^{-1} TDM	0.08 [c]	0.14 [a]	0.10 [b]
Boron	B	μmol g^{-1} TDM	0.87 [ab]	1.17 [a]	0.64 [b]
Zinc	Zn	μmol g^{-1} TDM	0.52 [a]	0.33 [b]	0.45 [a]
Manganese	Mn	μmol g^{-1} TDM	0.62 [b]	0.83 [ab]	0.96 [a]
Aluminium	Al	μmol g^{-1} TDM	4.34 [n.s.]	2.08 [n.s.]	1.63 [n.s.]
Iron	Fe	μmol g^{-1} TDM	2.62 [n.s.]	2.28 [n.s.]	2.73 [n.s.]
Sodium	Na	μmol g^{-1} TDM	3.38 [a]	3.52 [a]	2.50 [b]
Chlorine	Cl	mmol g^{-1} TDM	0.10 [n.s.]	0.03 [n.s.]	0.03 [n.s.]
Silicon	Si	μmol g-1 TDM	18.98 [n.s.]	22.94 [n.s.]	20.06 [n.s.]
Copper	Cu	wt%	< 0.001	< 0.001	< 0.001
Nickel	Ni	wt%	< 0.001	< 0.001	< 0.001
Cobalt	Co	wt%	< 0.001	< 0.001	< 0.001
Molybdenum	Mo	wt%	< 0.001	< 0.001	< 0.001

(Table 16). In contrast, the concentrations of N, P, K, S and Zn in soybeans fertilized with cBP ash were significantly lower by 1.8, 6.3, 3.1, 2.5 and 1.4, respectively, as compared to conventionally fertilized plants (Table 16). Compared to negative control soybeans, only the concentration of P and N did not statistically differ, whereas the concentrations of K, S and Zn significantly decreased by 1.4, 1.5 and 1.6 times, respectively (Table 16).

Following additional nutrient supply, the concentration of Zn significantly increased by 1.5 times in biomass of cBP ash fertilized soybeans, whereas the concentration of Na significantly decreased by 1.5 times (Table 17). Contrary to this, concentrations of Zn and Na did not statistically differ between fertigated and not fertigated soybeans, which received P and K from TSP and K$_2$SO$_4$ (Table 17). All other interactions were not statistically supported for pairwise comparisons (Table S5).

Table 17: Effect of fertigation with all nutrients but no P on Na and Zn accumulations. Identical letters indicate no statistical differences between different fertilization treatments within the same mineral and fertigation rows (Tukey's test, P $\leq$0.05, n = 3).

Elements	Abbrev.	Units	Fertigation	No P	73 g cBP ash kg^{-1}	Conv. Fertilization
Sodium	Na	μmol g^{-1} TDM	No	3.38 [ab]	4.18 [a]	2.6 [bc]
Sodium	Na	μmol g^{-1} TDM	Yes	3.38 [ab]	2.86 [bc]	2.41 [c]
Zinc	Zn	μmol g^{-1} TDM	No	0.43 [b]	0.26 [c]	0.44 [b]
Zinc	Zn	μmol g^{-1} TDM	Yes	0.6 [a]	0.4 [b]	0.46 [b]

While Cu, Ni, Co and Mo were below measuring limits, there were no statistical differences

between the concentrations of Al, Fe Cl and Si in differently fertilized soybeans (Table 16). In regard to Mn, the concentration did not statistically differ compared to either the negative control or to conventionally fertilized soybeans (Table 16).

3.3.3 Effect of bagasse and chicken manure co-combustion on solubility and availability of P from ashes to soybeans

Based on previous experiments, the availability of P from bagasse ashes and its subsequent effect on soybeans was significantly lower than that of TSP. While the increase in the dosage of P from bagasse ash was sufficient for the production of comparable total dry biomass, it interfered with nutrient uptake, so that the uptake of P remained significantly lower than from TSP. The effects of biomass feedstock on ash properties and the availability of P from the resulting ash were reported in previous studies (Demeyer et al., 2001, Schiemenz and Eichler-Löbermann, 2010). To identify the effects of feedstock modification and processing conditions on P content and availability, bagasse pellets were either processed by gasification only (gBP), by co-gasification with chicken manure (CM) in a ratio of 85:15 by dry weight (gBP/ $CM_{85:15}$) and by co-combustion with CM in a ratio of 80:20 by dry weight (cBP/ $CM_{80:20}$). Processing of ash was performed by project partners from FRAUNHOFER UMSICHT and CUTEC, Germany.

3.3.3.1 Chemical compositions of bagasse and bagasse/ chicken manure based ashes, P-forms and solubility fractions

Visually, gBP ash and gBP/ $CM_{85:15}$ ash did not differ and the particles were ≤ 250 μm in both ashes, whereas cBP/ $CM_{80:20}$ ash contained larger ash particles of ≥ 350 μm (Figure 13 A). The concentrations of P, Na, K, Ca and Mg substantially increased whereas the concentrations of Fe and Al decreased due to co-processing treatments (Table 1). In total 86%, 92% and 92% of total P from gBP, gBP/ $CM_{85:15}$ and cBP/ $CM_{80:20}$ ashes were soluble in citric acid (Table 1).

In gBP ash no crystalline P compounds were detected in XRD because of high background intensities of amorphous compounds, whereas gBP/ $CM_{85:15}$ ash contained whitlockite and cBP/ $CM_{80:20}$ contained $CaNa_xK_{1-x}(PO_4)_2$ and two undefined crystalline P compounds, which were soluble in formic and/ or citric acids, respectively (Table 15).

While gBP contained the largest fraction of insoluble P, i.e. 44%, and 54% of total P soluble in HCl, the fractions of P_{HCl} and $P_{residual}$ were 70% and 24% of total P in both chicken manure-based ashes (Figure 13 B). The fractions of P_{NaOH} and P_{NH4Cl} were 2% and 1% of total P in gBP ash, and ≤ 1% and 3% of total P in gBP/ $CM_{85:15}$ and cBP/ $CM_{80:20}$ ashes (Figure 13 B). Independent of the ash, the fractions of P_{H2O} were below 1% of total P (Figure 13 B). Although, the gBP and gBP/ $CM_{85:15}$ ashes contained around 55 wt% and 40 wt% carbon, none of analysed ashes contained organically bound P in detectable amounts (Figure S1).
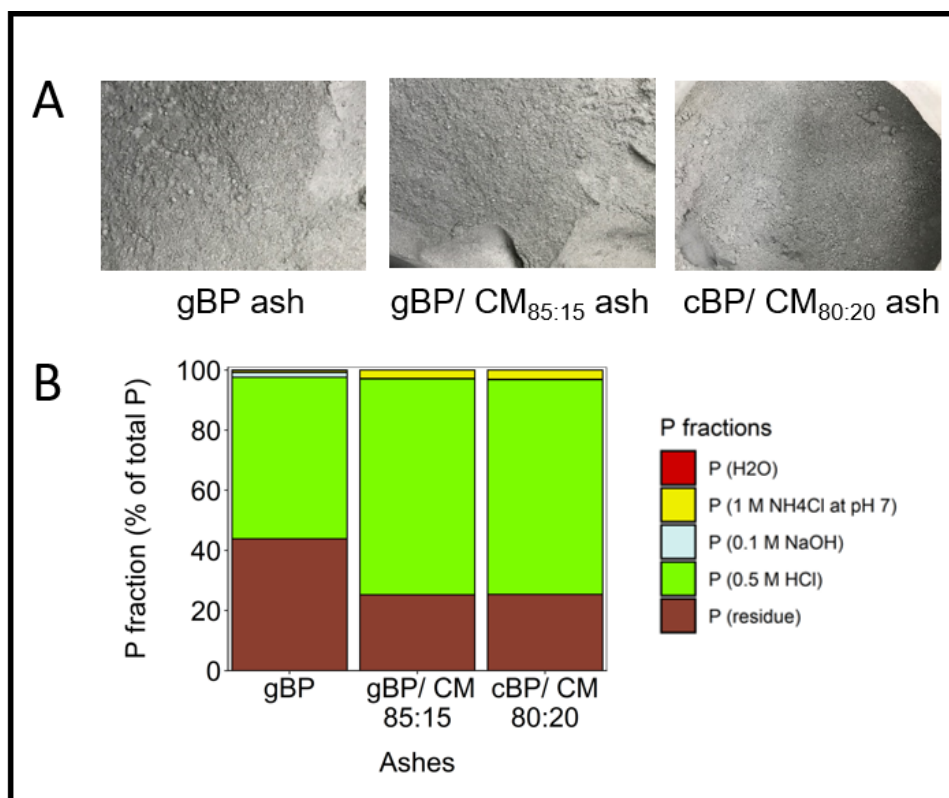
Figure 13: Visualisation of ashes and solubility of phosphorus. (A) Ashes from gasification of bagasse pellets (gBP) and co-gasification of bagasse pellets and chicken manure in a ratio of 85:15 by dry weight (gBP/ $CM_{85:15}$) as well as from co-combustion of bagasse pellets and chicken manure in a ratio of 80:20 by dry weight (cBP/ $CM_{80:20}$). (B) Solubility of phosphorus (P) in various extraction solutions based on sequential extraction.

3.3.3.2 Availability of P from bagasse and bagasse/ chicken manure-based ashes to soybeans

Soybeans receiving P from any of the co-processed ashes containing CM (i.e. gBP/ $CM_{85:15}$ and cBP/ $CM_{80:20}$) accumulated significantly larger total dry biomass compared to gBP ash and no-P control plants (Figure 14 C). This was also reflected by shoot growths and estimated leaf areas (Figure 14 A and B). Biomass partitioning to the roots did not statistically differ between gBP, gBP/ $CM_{85:15}$ and cBP/ $CM_{80:20}$ ash-fertilized soybeans but tended to decrease due to P supply from cBP/ $CM_{80:20}$ ash (Figure 14 D). Compared to no-P control plants, any supply of P significantly decreased the biomass partitioning to the roots (Figure 14 D).

The uptake of P from co-combusted ash (cBP/ $CM_{80:20}$) was significantly higher during the first period of growth (0-27 days), indicating more readily available P-forms in cBP/ $CM_{80:20}$ ash than in gBP and gBP/ $CM_{85:15}$ ashes (Figure 14 E). During the second period of growth (27-42 days), the uptakes of P did not statistically differ but plants took up significantly more P from cBP/ $CM_{80:20}$ ash than from gBP and gBP/ $CM_{85:15}$ ashes over the entire experiment (Figure 14 E). In total, 11%, 13% and 16% of total P supplied by
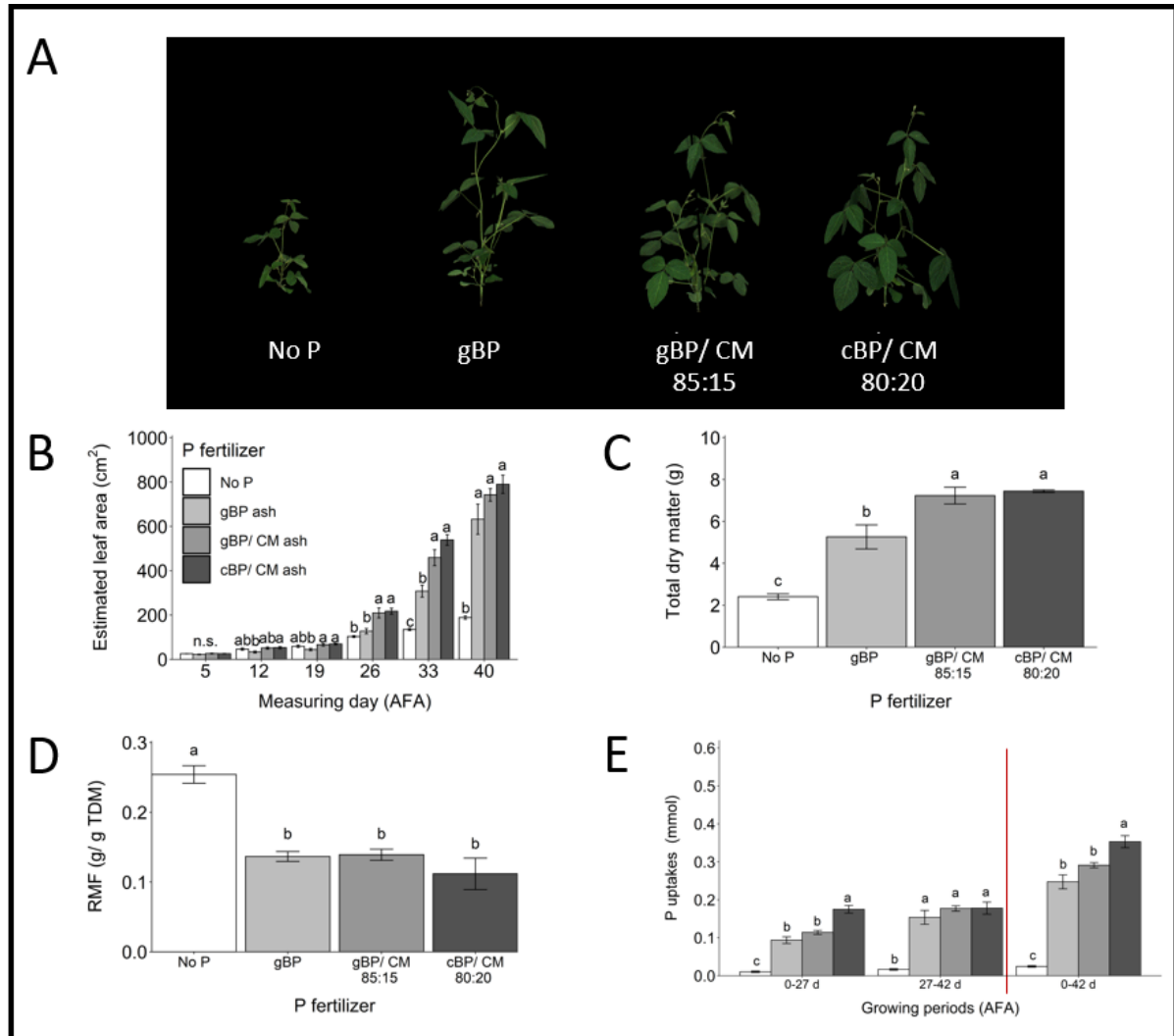
Figure 14: Ashes from gasification of bagasse pellets (gBP), and co-gasification and co-combustion of bagasse pellets and chicken manure in ratios of 85:15 (gBP/ $CM_{85:15}$) and 80:20 (cBP/ $CM_{80:20}$) by dry weights, respectively, as fertilizer for soybeans. (A) Effects on soybean shoot growths, (B) estimated leaf areas between 5 and 40 d after fertilizer application (AFA), (C) total dry matters (TDM) from shoot and root organs, (D) biomass partitioning to the roots represented by root mass fractions (RMF) and (E) uptakes of P between 0 and 27 d AFA, 27 and 42 d AFA and from 0 to 42 d AFA. The error bars indicate standard errors of the means. Identical letters indicate no statistical differences (Tukey's test, p $\leq$0.05, n = 5).

gBP, gBP/ $CM_{85:15}$ and cBP/ $CM_{80:20}$ ashes were taken up within 42 d growing (data not shown).

3.4 Pot experiments in an acidic Oxisol soil

3.4.1 Effect of co-combustion of bagasse with sewage sludge on the availability of P to soybeans

After detection of the positive effects of co-combustion on the availability of P from co-processed bagasse/ chicken manure ash to soybeans (Figure 14), further modification of the biomass in order to increase the P availability to soybeans was undertaken. As all municipal communities generate sewage sludge that may pose a problem for environment due to its disposal in the land outside of cities, its co-combustion with bagasse may have an added benefit of reducing this sewage sludge disposal problem. Subsequently, the availability of P from combusted bagasse pellets ash (cBP ash), and co-combusted bagasse pellets with chicken manure (cBP/ $CM_{80:20}$) and sewage sludge (83:17 by dry weight, cBP/ $SSL_{83:17}$) ashes (Table 1) were tested on soybeans in Oxisol soil under greenhouse conditions.

3.4.1.1 Chemical compositions of cBP/ $SSL_{83:17}$ ash, P-forms and solubility fractions

Visually, the sizes of ash particles varied and were larger in cBP/ $SSL_{83:17}$ ash than in cBP/ $CM_{80:20}$ ash (Figure 15 A). Co-combustions of bagasse with both SSL and CM highly increased the concentration of P, and, among other elements, of Ca and Mg (Table 1). While the co-combustion of BP with SSL also increased the concentrations of Al and Fe while decreasing K, the opposite occurred with the co-combustion of bagasse pellets with CM (Table 1). Subsequently, the cBP/ $CM_{80:20}$ ash contained larger fraction of alkali and alkaline earth metals, including K, Na, Ca and Mg, than cBP/ $SSL_{83:17}$ ash, i.e. in total 15 wt% vs. 5 wt% (Table 1). In total 19%, 92% and 92% of total P from cBP, cBP/ $SSL_{83:17}$ and cBP/ $CM_{80:20}$ ashes were soluble in citric acid (Table 1).

^{31}P NMR spectroscopy analyses allowed further P speciation. Thus, all ashes showed shifts at around 5.7 and -4.7 ^{31}P ppm[-1] (Figure 15 B), indicating the presence of ortho- and pyrophosphates (Cade-Menun, 2005). Additionally, cBP/ $SSL_{83:17}$ ash contained small shifts at around 14, 3, -4 and -19 ^{31}P ppm[-1] (Figure 15 B), indicating traces of aromatic phosphoric acid esters, orthophosphate monoesters, and polyphosphates, respectively (Cade-Menun, 2005).

As previously described, XRD analyses did not help to identify any P compounds in cBP ash (3.3.2.1), whereas three crystalline P compounds were identified in cBP/ $CM_{80:20}$ ash (Table 15). These were $CaNa_xK_{1-x}(PO_4)_2$ and two undefined crystalline P compounds

Figure 15: Ashes from combustion of bagasse pellets (cBP), and co-combustion of bagasse pellets and sewage sludge in a ratio of 83:17 (cBP/ $SSL_{83:17}$) and bagasse pellets and chicken manure in a ratio of 80:20 by dry weights (cBP/ $CM_{80:20}$). (A) Visualisation of the ashes, (B) phosphorus (P)-species determined by ^{31}P NMR, and (C) fractions of P extractable in water, 1 M NH_4Cl at pH 7, 0.1 M NaOH and in 0.5 M HCl. Red arrows point at coagulated particles, which were produced during combustion.

soluble in FA and/ or CA. In cBP/ $SSL_{83:17}$ ash only whitlockite ($Ca_9Fe(PO_4)_7$) was identified (Table 15).

Co-combustion of BP with SSL and CM reduced the fractions of insoluble P ($P_{residual}$) from 80% of total P in cBP ash to 30% and 25% in cBP/ $SSL_{83:17}$ and in cBP/ $CM_{80:20}$ ashes, respectively (Figure 15 C). This occurred, primarily, due to conversion of insoluble P into HCl soluble P, so that 60% and 70% of total P from cBP/ $SSL_{83:17}$ and cBP/ $CM_{80:20}$ ashes became soluble in 0.5 M HCl (Figure 15 C). Compared to cBP ash, co-combustion of BP with SSL decreased the fraction of 1 M NH_4Cl soluble P from 3% to less than 1% of total P and increased the fraction of 0.1 M NaOH soluble P from 3% to 7% of total P (Figure 15 C). While the fractions of P soluble in 1 M NH_4Cl soluble were comparable in cBP and cBP/ $CM_{80:20}$ ashes, co-combustion of BP with CM decreased the fraction of 0.1 M NaOH soluble P from 3% to ≤1 % of total P (Figure 15 C). Phosphorus soluble in water was the minor fraction in all ashes and was less than 1% of total P (Figure 15 C).

3.4.1.2 Co-combustion of bagasse with sewage sludge does not increase the availability of P

Pre-cultivated soybeans with similar morphology were transplanted in Oxisol soils containing no P-fertilizer application but differing in pH, i.e. around 4.5 and 6, and in soils containing 262 mg P kg^{-1} Oxisol in forms of cBP, cBP/ $SSL_{83:17}$ and cBP/ $CM_{80:20}$ ashes. The shoot area of soybeans was captured by taking images every week, which allowed the estimation of the increase in leaf area over the course of 40 d of growth.

Soybeans fertilized with cBP/ $CM_{80:20}$ ash significantly doubled the leaf area compared to all other fertilizer treatments within 6 d after fertilizer application (Figure 16 B). This trend remained over the course of 40 d of growth (Figure 16 B). Leaf areas of soybeans receiving P from cBP ash significantly increased compared to no-P control plants growing on pH 4.5 and 6 after 27 and 33 d growth, respectively. However, the leaf area of soybeans fertilized with cBP/ $SSL_{83:17}$ ash did not statistically differ compared to either no-P control plants or to cBP ash fertilized plants (Figure 16 B). Soybeans fertilized with cBP ash tended to have higher leaf areas compared to cBP/ $SSL_{83:17}$ ash fertilized plants (Figure 16 B). Trends of estimated leaf areas reflected shoot growth and total dry biomass accumulations of soybeans, i.e. total dry matters of soybeans receiving P from any ash significantly increased compared to no-P control plants (Figure 16 A and C). Soybeans receiving P from cBP/ $CM_{80:20}$ ash accumulated significantly higher dry biomass and took up significantly higher amounts of P, which was a 6-fold increase of P uptake relative to no-P control plants (Figure 16 C and E). Soybeans fertilized with cBP, cBP/ $SSL_{83:17}$ and cBP/ $CM_{80:20}$ ashes took up 0.6%, 0.7% and 1.3% of applied P, respectively. Only soybeans fertilized with cBP/ $SSL_{83:17}$ ash significantly decreased the biomass partitioning to the roots compared to no-P control plants growing on pH 6. It did not statistically differ compared to all other fertilizer treatments (Figure 16 D).
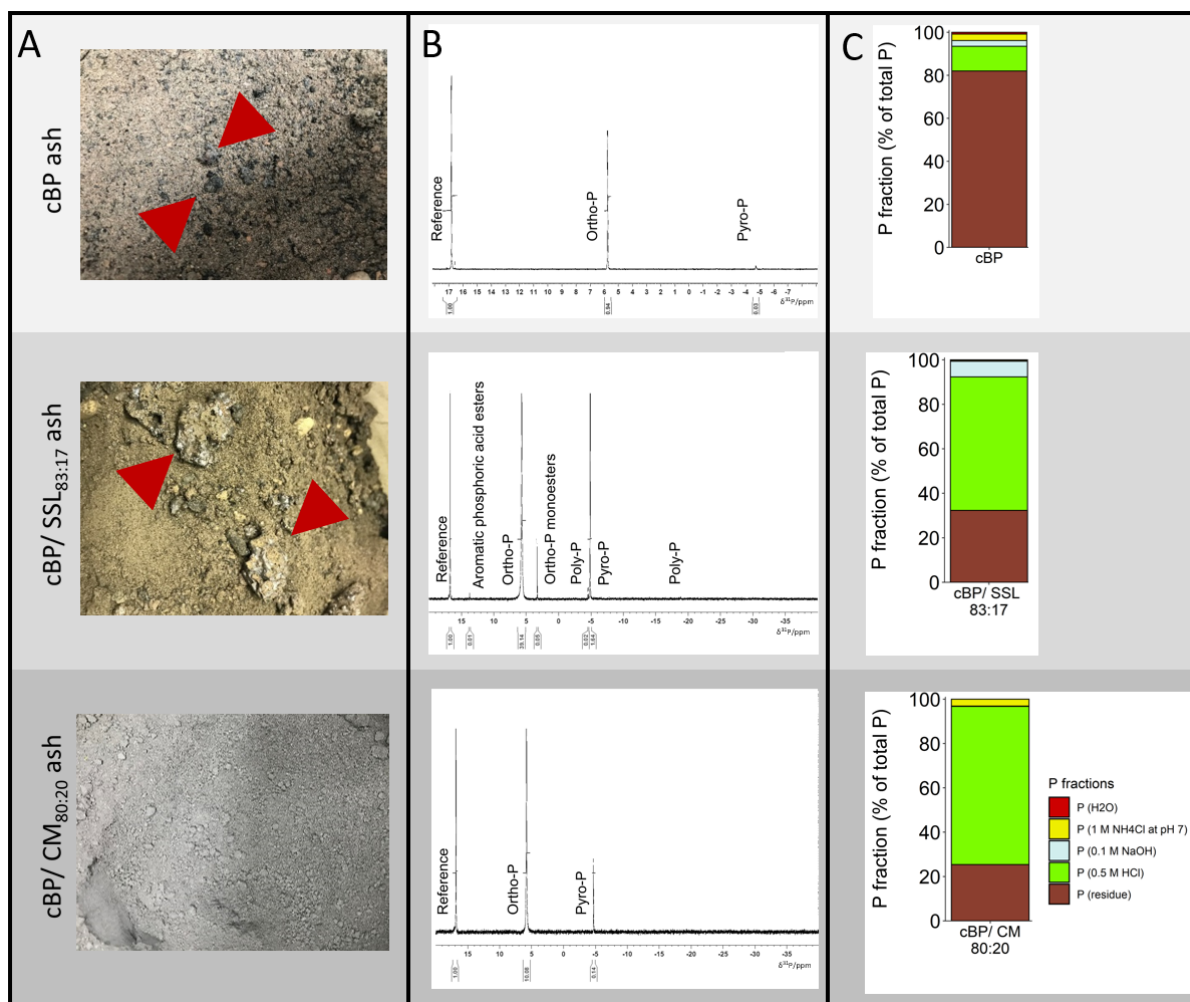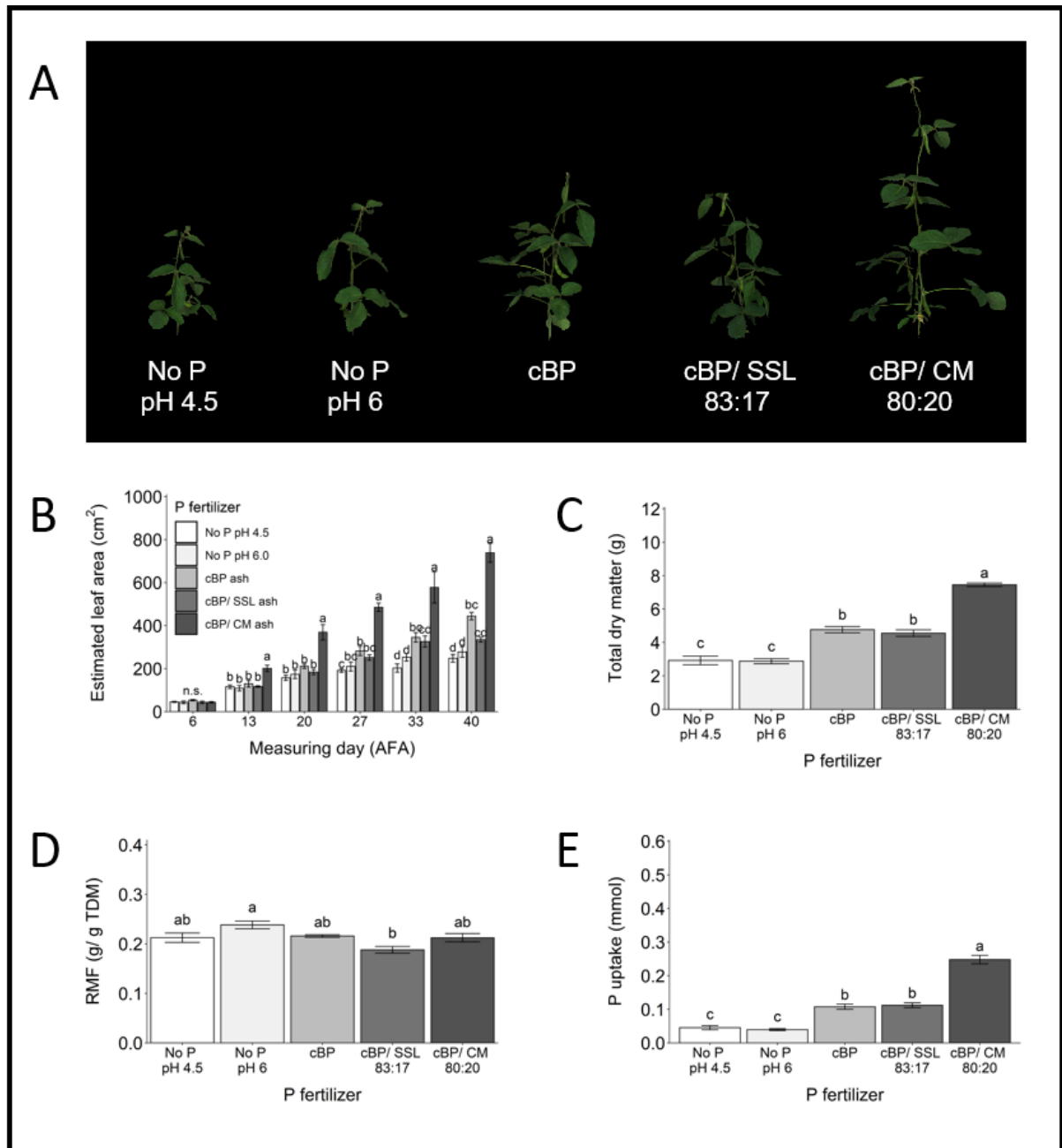
Figure 16: Ashes from combustion of bagasse pellets (cBP), and co-combustion of bagasse pellets and sewage sludge in a ratio of 83:17 (cBP/ $SSL_{83:17}$) and chicken manure in a ratio of 80:20 by dry weights (cBP/ $CM_{80:20}$) as phosphorus (P) fertilizer for soybean. (A) Soybeans shoot morphology, (B) estimated leaf areas between 6 to 40 d after fertilizer application (AFA), (C) total dry matters (TDM), (D) biomass partitioning to the roots represented by root mass fractions (RMF), and (E) uptakes of P over course of 42 d growth. The error bars indicate standard errors of the means. Identical letters indicate no statistical differences (Tukey's test, p $\leq$0.05, n = 4).

3.4.2 Effect of cBP/ CM ashes as P-fertilizer and the effects of thermochemical treatments on availability of P to soybeans in Oxisol soil

To test the relevance of chicken manure in bagasse-based ash on the availability of P to soybeans, two bagasse-based ashes with 20 wt% (cBP/ $CM_{80:20}$ see above) and 40 wt% chicken manure (cBP/ $CM_{60:40}$) were compared in a dose-response experiment containing 0 to 340 mg P kg^{-1} Oxisol soil. Additionally, the dose-response experiment included cBP/ $CM_{60:40}$ based thermophosphates and TSP treatments, which contained the same dosages of P as supplied by cBP/ $CM_{80:20}$ and cBP/ $CM_{60:40}$ ashes. As thermochemical treatments were reported to significantly increase the availability of P in sewage sludge ash-based products (Severin et al., 2014, Steckenmesser et al., 2017, Vogel et al., 2018), cBP/ $CM_{60:40}$ ash was thermochemically treated with Na_2SO_4 (cBP/ $CM_{60:40}$+Na) and Na_2SO_4/ K_2SO_4 (cBP/ $CM_{60:40}$+Na/K).

3.4.2.1 Characterisation of cBP/ CM based ashes and its thermochemical products

Characterization of P in cBP/ $CM_{80:20}$ and cBP/ $CM_{60:40}$ ashes, and in cBP/ $CM_{60:40}$+Na and cBP/ $CM_{60:40}$+Na/K thermochemical products were based on XRD and its solubility analyses in citric acid (CA), formic acid (FA) and neutral ammonium citrate (NAC). The analyses were conducted by Hannes Herzel (BAM, Berlin, Germany) and were partially published in Herzel et al. (2020).

The cBP/ $CM_{80:20}$ and cBP/ $CM_{60:40}$ ashes contained $CaNa_xK_{1-x}(PO_4)_2$ (x<0.5) and two undefined crystalline P compounds, which were soluble in FA and/ or CA (Table 15). Thermochemical treatments of cBP/ $CM_{60:40}$ with Na_2SO_4 and Na_2SO_4/ K_2SO_4 transformed all detected crystalline P compounds to calcium alkali phosphate, i.e. $CaNaPO_4$, as undefined P compounds and $CaNa_xK_{1-x}(PO_4)_2$ disappeared (Table 15).

Around 65% and 80% of total P from cBP/ $CM_{80:20}$ ash were soluble in NAC and CA, whereas it increased to 75% and 90% in cBP/ $CM_{60:40}$ ash (Figure 17). The thermochemical treatment of cBP/ $CM_{60:40}$ ash with Na_2SO_4 and Na_2SO_4/ K_2SO_4 increased the solubility of P to around 90% and 100%, respectively (Figure 17). Contrary to the extractions with NAC and CA, the solubility of P in FA remained around 80% of total P, independent of the ash and thermochemical treatments (Figure 17). As reference, 93%, 94% and 90% of total P from TSP were soluble in NAC, CA and FA (Figure 17).

3.4.2.2 cBP/ CM ashes and its thermochemical products cannot compete with TSP for availability of P

The fertilizer effects and the availability of P from cBP/ $CM_{80:20}$ and cBP/ $CM_{60:40}$ ashes and cBP/ $CM_{60:40}$+Na and cBP/ $CM_{60:40}$+Na/K thermochemical products and TSP were
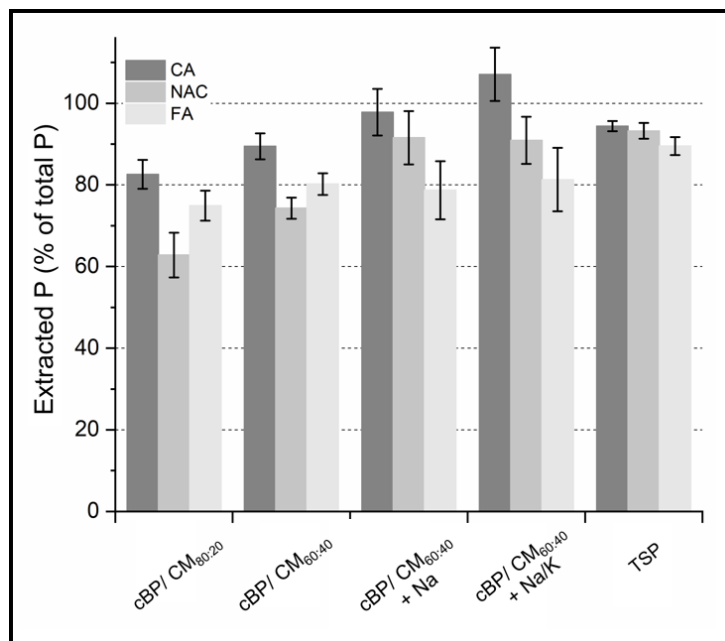
Figure 17: Solubility of P from ashes produced by co-combustion of bagasse pellets and chicken manure in ratios of 80:20 (cBP/ $CM_{80:20}$) and 60:40 (cBP/ $CM_{60:40}$) by dry weights, and products from thermochemical post-treatments of cBP/ $CM_{60:40}$ with Na_2SO_4 (cBP/ $CM_{60:40}$+Na) and Na_2SO_4/ K_2SO_4 (cBP/ $CM_{60:40}$+Na/K) as well as from triple-superphosphate (TSP) in citric acid (CA), formic acid (FA) and in neutral ammonium citrate (NAC). The analyses were performed by Hannes Herzel (BAM, Berlin, Germany).

evaluated in a "dose- response" experiment, which included 5 dosages of P between 0 and 340 mg P kg^{-1} Oxisol soil.

The growth of soybeans depended on the dosage and form of P applied to the plants. Compared to no-P control soybeans, only the fertilizer with around 100 mg P kg^{-1} Oxisol soil in the form of cBP/ $CM_{80:20}$ ash tended to increase the growth, while the fertilizers with 100 mg P kg^{-1} Oxisol soil in forms of cBP/ $CM_{60:40}$ ash, cBP/ $CM_{60:40}$+Na and cBP/ $CM_{60:40}$+Na/K thermochemical products and TSP significantly increased the total dry biomass accumulations of the plants (Figure 18 A). The dry biomass accumulations of soybeans fertilized with 100 mg P kg^{-1} Oxisol soil did not statistically differ between ash, thermochemical products and TSP fertilization treatments, although dry biomass tended to increase in the following series of treatments: TSP > cBP/ $CM_{60:40}$+Na > cBP/ $CM_{60:40}$+Na/K > cBP/ $CM_{60:40}$ and cBP/ $CM_{80:20}$ ashes (Figure 18 A). Soybeans receiving 340 mg P kg^{-1} Oxisol soil in the form of ash-based P-fertilizers tended to have a 1.3 times increase in the dry biomass compared to 100 mg P kg^{-1} Oxisol fertilized plants. Biomass accumulation of TSP fertilized plants significantly increased with increasing dosage of applied P, so that the total dry biomass almost doubled in soybeans receiving 340 mg P compared to 100 mg P kg^{-1} Oxisol soil fertilized soybeans (Figure 18 A). The total dry biomass accumulation of soybeans receiving P from cBP/ $CM_{80:20}$ and cBP/ $CM_{60:40}$ ashes and cBP/ $CM_{60:40}$+Na and cBP/ $CM_{60:40}$+Na/K thermochemical products did not statistically differ, although it tended to increase in the following
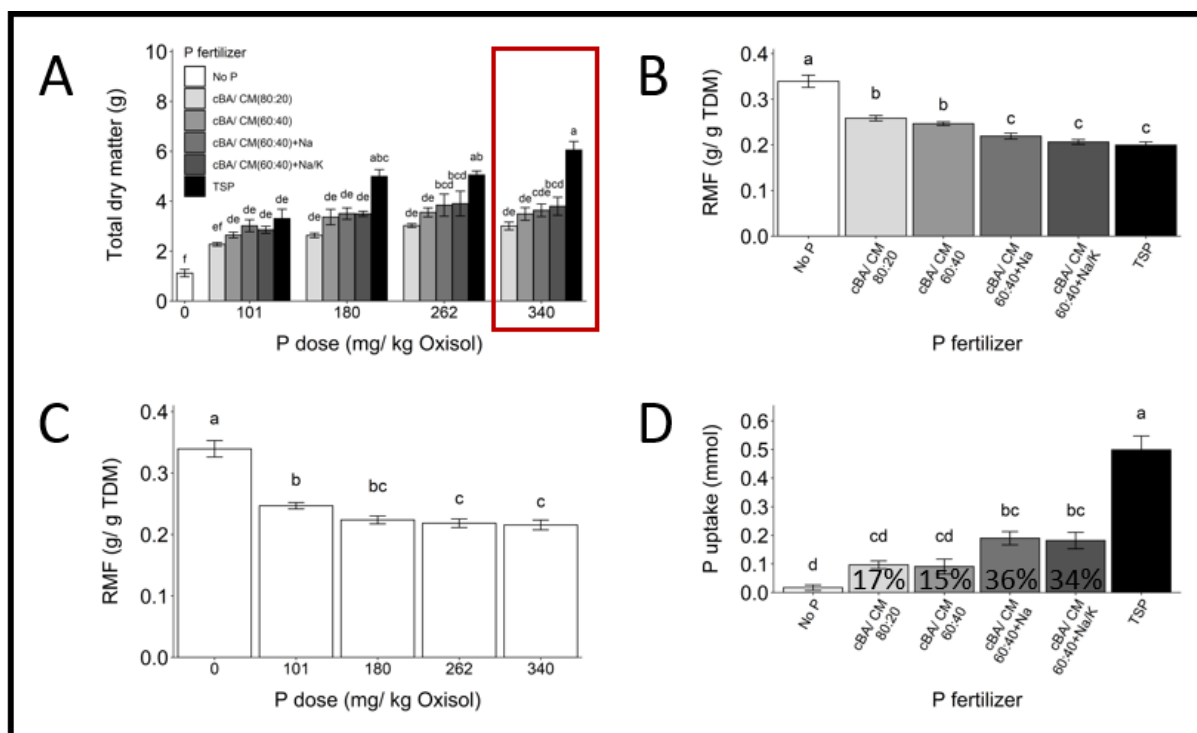
Figure 18: Dose-response of soybeans to two different bagasse pellets/ chicken manure ashes and their thermochemical products as phosphorus (P) fertilizer. Bagasse/ chicken manure ashes were co-combusted at ratios of 80:20 (cBP/ $CM_{80:20}$) and 60:40 (cBP/ $CM_{60:40}$) by dry weight. cBP/ $CM_{60:40}$ ash was thermochemically treated with Na_2SO_4 (cBP/ $CM_{60:40}$+Na) and Na_2SO_4/ K_2SO_4 (cBP/ $CM_{60:40}$+Na/K). (A) Total dry matter (TDM) of soybeans from shoot and root organs, (B) biomass partitioning to the roots represented by root mass fractions (RMF) by P-fertilization treatment, which included all dosages of P, (C) RMF by P dosage, which includes all P-fertilizer treatments, and (D) P uptakes from no P-fertilizer and fertilizers with 340 mg P kg^{-1} Oxisol soil during 22 d growth. In figure D, the values within the bars represent relative agronomic effectiveness compared to P from triple-superphosphate (TSP). The error bars indicate standard errors of the means. Identical letters present no significant differences (Tukey's test, P ≤0.05, n = 4).

series of treatments: 340 mg P kg^{-1} Oxisol soil in forms of cBP/ $CM_{60:40}$+Na/K > cBP/ $CM_{60:40}$+Na > cBP/ $CM_{60:40}$ and cBP/ $CM_{80:20}$ ashes (Figure 18 A).

Compared to unfertilized soybeans, partitioning of the biomasses to the roots significantly decreased in all soybeans receiving P in any form or dosage (Figure 18 B and C). While the partitioning of the biomass to the roots did not significantly differ between cBP/ $CM_{80:20}$ and cBP/ $CM_{60:40}$ ash-fertilized soybeans, both were significantly lower than in cBP/ $CM_{60:40}$+Na and cBP/ $CM_{60:40}$+Na/K thermochemical products and TSP fertilized plants (Figure 18 B). Biomass partitioning to the roots depended on the dosage of P supplied to the plants, so that, statistically, the lowest biomass partitioning to the roots occurred in soybeans receiving 262 and 340 mg P kg^{-1} Oxisol soil (Figure 18 C).

In agreement with total dry biomass, the highest significant uptake of P occurred from TSP (Figure 18 D). Soybeans fertilized with cBP/ $CM_{60:40}$+Na and cBP/ $CM_{60:40}$+Na/K

products doubled the uptakes of P compared to cBP/ $CM_{60:40}$ and cBP/ $CM_{80:20}$ ash-fertilized plants, albeit without any statistical support (Figure 18 D). The uptak of P from cBP/ $CM_{60:40}$ and cBP/ $CM_{80:20}$ ashes tended to increase by 5 times as compared to un-fertilized plants (Figure 18 D). The relative agronomic efficiencies of P from cBP/ $CM_{80:20}$ and cBP/ $CM_{60:40}$ ashes were around 15% and around 35% from cBP/ $CM_{60:40}$+Na and cBP/ $CM_{60:40}$+Na/K thermochemical products (Figure 18 D).

3.5 Bagasse ash as additive to save conventional P-fertilizer

Independent of the ash and rooting environment, the availability of P in bagasse ashes was significantly lower than from TSP (Figure 10, Figure 12 and Figure 18). Subsequently, the effects of bagasse ashes as additives of triple-superphosphate and organic fertilizer availability were tested under greenhouse and field conditions in Brazil following the approach "From Lab to Field".

3.5.1 Blend of cBP ash and TSP as fertilizer for soybeans under greenhouse conditions

Combusted bagasse pellets (cBP) ash, in combination with TSP (cBP/ $TSP_{45P:55P}$, 45% of total P sourced from cBP ash, i.e. 29.4 t cBP ash ha^{-1}), was tested on its fertilization effect and the availability of P to soybeans. Soybeans received around 100 mg P kg^{-1} Oxisol soil in the form of cBP/ $TSP_{45P:55P}$ and 115 mg P kg^{-1} Oxisol soil in the form of TSP, respectively. The fertilizers were homogeneously incorporated in acidic Oxisol soil (pH 4.5) and the seedlings grew for 41 d. The shoot area was captured using the "ScreenHouse" platform every week which allowed estimation of the leaf area over the course of growth.

A mere 14 d after fertilizer application, both cBP/ $TSP_{45P:55P}$ and TSP fertilized soybeans had significantly increased their leaf areas compared to no-P control plants and this increase continued through to the final harvest. This meant that, 41 d post-fertilizer application, the leaf areas were significantly 2 times greater than no-P control plants (Figure 19 A). The estimated leaf areas reflected total dry biomass accumulation (Figure 19 A and B). There were no statistically supported differences between soybeans receiving P from cBP/ $TSP_{45P:55P}$ and TSP (Figure 19 A and B). Contrary to estimated leaf areas and total dry biomasses, soybeans receiving P from cBP/ $TSP_{45P:55P}$ took up significantly more P than no-P control soybeans and significantly less than soybeans treated with TSP (Figure 19 C). The relative efficiency of P from cBP/ $TSP_{45P:55P}$ was 61% (Figure 19 C). Biomass partitioning to the roots did not statistically differ between differently fertilized plants (p $\leq$0.9, data not shown).

After harvesting the plants, the pH levels in the soils were measured and were around 4.5 in no-P, TSP and cBP/ $TSP_{45P:55P}$ fertilized Oxisol soils (Table 9). Soils receiving P from
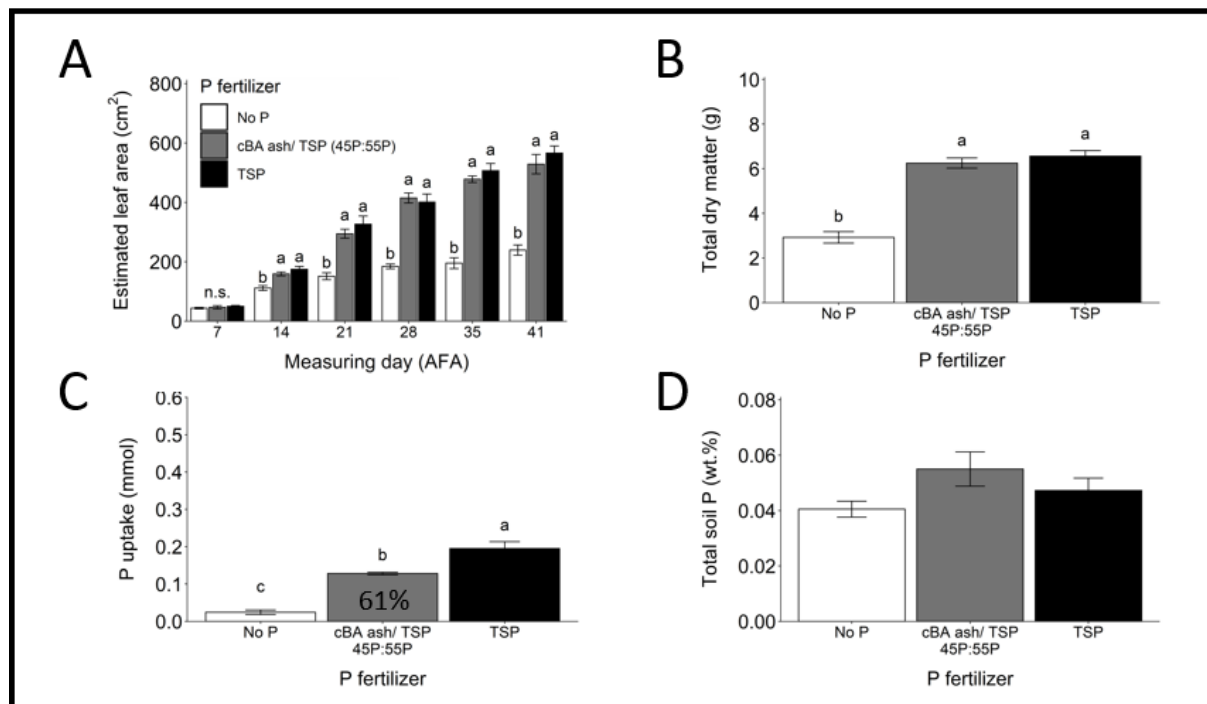
Figure 19: Blend of combusted bagasse pellets ash (cBP) and triple-superphosphate (TSP) at a ratio of 45 to 55 by P (cBP/ TSP$_{45P:55P}$) as fertilizer for soybeans under greenhouse conditions. (A) Estimated leaf areas between 7 and 41 d after fertilizer application (AFA), (B) total dry matter (TDM) of soybeans from shoot and root organs, (C) uptakes of P during 41 d growth, and (D) trend of total P in soil remaining after 41 d growth (p ≤0.15). In figure D, the value within the bar represents relative agronomic effectiveness compared to P from TSP. Biomass partitioning to the roots was not statistically supported and is not displayed in the figure (p ≤0.90). The error bars indicate standard errors of the means. Identical letters present no significant differences (Tukey's test, p ≤0.05, n = 4).

cBP/ TSP$_{45P:55P}$ and TSP tended to contain around 1.4 and 1.2 times more P compared to no-P control soils (p ≤ 0.15; Figure 19 D). Compared to the fertilization with TSP, soils receiving P from cBP/ TSP$_{45P:55P}$ tended to contain 1.2 folds more P (p ≤ 0.15; Figure 19 D).

3.5.2 "From Lab to Field": Bagasse ash as P-fertilizers under field conditions

Based on the greenhouse pot-experiment with cBP/ TSP$_{45P:55P}$ as P-fertilizer for soybeans, a field experiment under field conditions at Brazilian Federal University of Goiás (UFG, Goiânia, Brazil, 16°35'27.76"S 49°17'18.43"W), was conducted. In this experiment the effects of regular bagasse ash (BA$_{NG2}$, Table 1) and its blends with cattle manure compost (50% of P from BA$_{NG2}$,) and TSP (70% of P from BA$_{NG2}$) were tested as P-fertilizer for soybeans (BRS 7470IPRO). The dosages of supplied P are expressed on the basis of P$_2$O$_5$,

as it is the common unit for field fertilizations, and it ranged between 32 to 160 kg P_2O_5 ha^{-1} (Table 10).

The grain yields of soybeans fertilized with 40 to 160 kg P_2O_5 ha^{-1} in the form of BA_{NG2} tended to increase from 4.0 to 4.8 t ha^{-1} with the dosage of supplied P (Figure 20 A). While the yield of soybeans receiving 40 kg P_2O_5 ha^{-1} from BA_{NG2} tended to decrease 1.2 times as compared to the TSP fertilized plants receiving 40 kg P_2O_5 ha^{-1}, the increase of P_2O_5 dosage compensated the lower trend and resulted in comparable grain yields (Figure 20 A). Fertilization of soybeans with 32 kg P_2O_5 ha^{-1} from BA_{NG2}/ $TSP_{70P:30P}$ (70% of total P from BA_{NG2}) resulted in the same grain yield as plants receiving 40 kg P_2O_5 ha^{-1} from TSP, i.e. 4.6 t ha^{-1} (Figure 20A). Further increase of the dosage to 65 kg P_2O_5 ha^{-1} from BA_{NG2}/ $TSP_{70P:30P}$ further increased the yield, albeit without any statistical support, and then dropped with fertilization with 130 kg P_2O_5 (Figure 20 A). Although, the P_2O_5 dosage from cattle manure compost (CMC) was twice as high as from TSP, soybeans tended to show a 1.1 times decrease in grain yield (Figure 20 A). The blend of BA_{NG2} with CMC (50% of total P from BA_{NG2}) significantly increased or tended to increase the grain yield by 1.3 and 1.1 times compared to fertilizations with 80 and 40 kg P_2O_5 ha^{-1} in forms of CMC and TSP, respectively (Figure 20 A). Differences in the dry weight of 1000 grains were not statistically supported ($p \leq 0.75$), although they tended to be heavier in fertilizations with 80 kg P_2O_5 ha^{-1} from BA_{NG2}/ $CMC_{50P:50P}$ (Table 18).

Grain yields of soybeans fertilized with 40 kg P_2O_5 ha^{-1} from BA_{NG2} and TSP, 80 kg P_2O_5 ha^{-1} in the form of BA_{NG2}/ $CMC_{50P:50P}$, CMC and BA_{NG2}, 65 kg P_2O_5 ha^{-1} from BA_{NG2}/ $TSP_{70P:30P}$, and 160 kg P_2O_5 ha^{-1} from BA_{NG2} were analysed for P contents (Figure 20 A (red asterisks) and B). Total removal of P from the fertilizers by grain yields ranged between 17 and 26 kg ha^{-1} (Figure 20 B) and was significantly highest in plants receiving 80 kg P_2O_5 ha^{-1} from BA_{NG2}/ $CMC_{50P:50P}$ and 65 kg P_2O_5 ha^{-1} from BA_{NG2}/ $TSP_{70P:30P}$ followed by plants receiving 160 kg P_2O_5 ha^{-1} from BA_{NG2} and 40 kg P_2O_5 ha^{-1} from TSP (Figure 20 B). Soybeans fertilized with 40 kg P_2O_5 ha^{-1} from BA_{NG2} removed the least amount of P, which was 17 kg ha^{-1} (Figure 20 B). Soybean grain yields were highly correlated with the removal of P by soybean grains (R^2 0.96, Figure 20 C), which indicated the dependency of the yield increased due to the availability of P.

3.5.2.1 Soil analyses

Soils were sampled 14, 65 and 119 d after sowing, and only soils receiving 80 and 160 kg P_2O_5 ha^{-1} from BA_{NG2}, 80 kg P_2O_5 ha^{-1} from CMC and BA_{NG2}/ $CMC_{50P:50P}$, 40 kg P_2O_5 ha^{-1} from TSP and 65 kg P_2O_5 ha^{-1} from BA_{NG2}/ $TSP_{70P:30P}$ were analysed for pH levels (1:2.5, w/v, in 0.01 M $CaCl_2$) and quantity of P extractable in Mehlich-I solution (Table 18).

Independent of the fertilizer treatment, the pH levels ranged between 4.8 and 5.0 and were not significantly different (Table 18). This was probably due to the dilution effect of sampling the soils from the fertilizer furrow and in between furrows. This was also true for quantity of P extractable in Mehlich-I solution, although trends were observed ($p \leq 0.6$, Table 18). Thus, the extractability of P from Oxisol soil ranged between 8.8 and 4.4
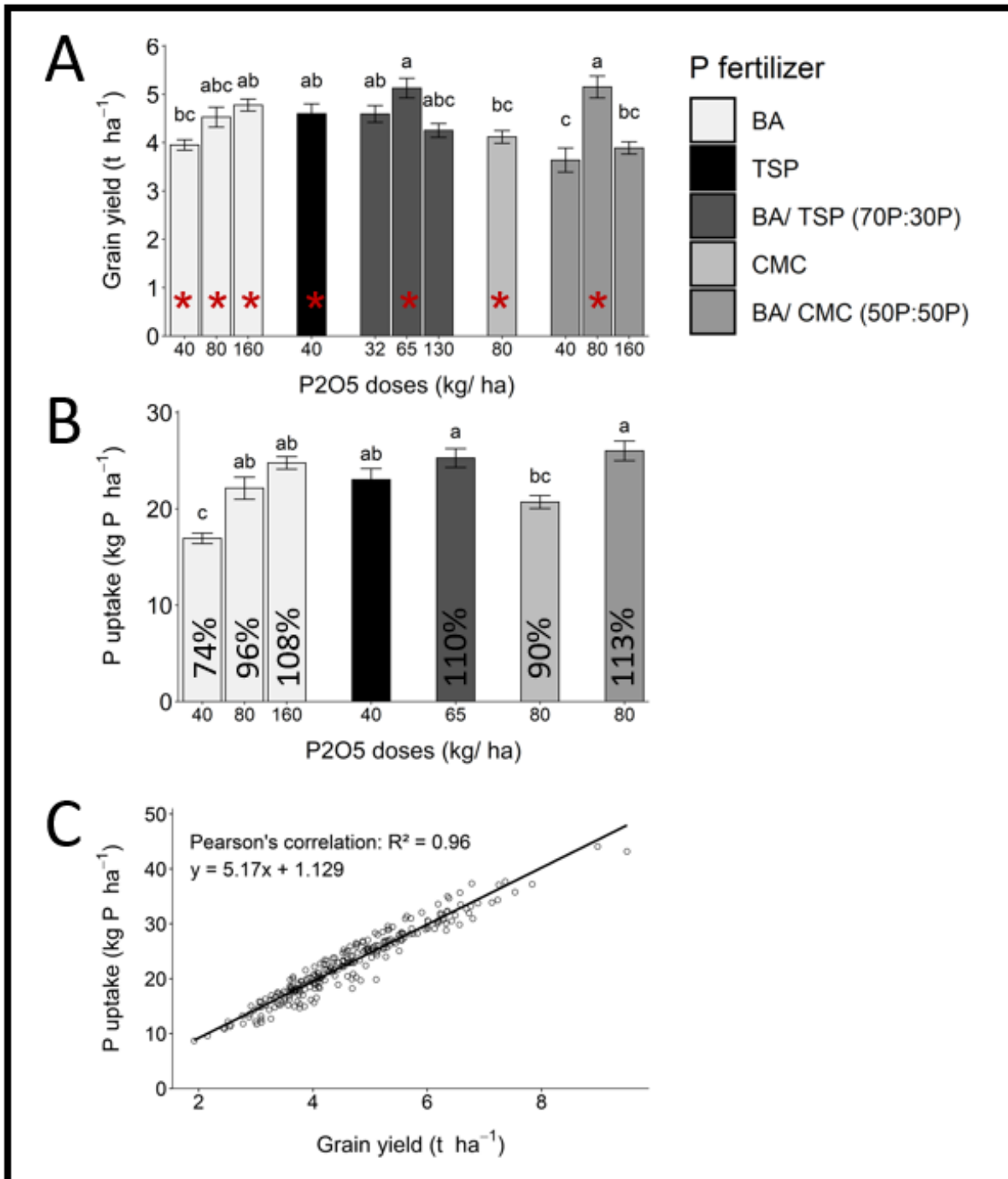
Figure 20: Soybean grain yield (A) and recovery of phosphorus (P) from the field (B). Soybeans received P_2O_5 (phosphorus pentoxide) from bagasse ash produced at sugarcane factory Usina Nova Gália Ltda. (BA_{NG2}), cattle manure compost (CMC), triple-superphosphate (TSP), the blends of BA_{NG2} and CMC (50% of P_2O_5 from BA_{NG2}), and the blends of BA_{NG2} and TSP (70% of P_2O_5 from BA_{NG2}). Red asterisks in figure A indicate the grains used for P uptake analyses. Values within the bars in figure B represent relative phosphorus effectiveness. The error bars indicate standard errors of the means. Identical letters present no significant differences. Ten soybeans from each plot were included in the calculation of grain yield and recovery of P resulting in total n = 40 (Tukey's test, p $\leq$ 0.05). (C) Correlation between grain yield and recovery of P (n = 280).

Table 18: Dry weight (DW) of 1000 grains, pH values and residual phosphorus (P) extractable in Mehlich-I solution. Soybeans received P_2O_5 (phosphorus pentoxide) from bagasse ash produced at sugarcane factory Usina Nova Gália Ltda. (BA_{NG2}), cattle manure compost (CMC), triple-superphosphate (TSP), the blends of BA_{NG2} and CMC (50% of P_2O_5 from BA_{NG2}), and the blends of BA_{NG2} and TSP (70% of P_2O_5 from BA_{NG2}). Sampling dates are expressed in days (d) after fertilizer application (AFA).

		DW 1000 grains (g)	$pH_{1:2.5}$ in 0.01 M $CaCl_2$			P Mehlich-I (mg L^{-1} Oxisol)		
	Sampling day (AFA)	121	14	65	119	14	65	119
	p-value	≤ 0.75	≤ 0.30	≤ 0.5	≤ 0.95	≤ 0.35	≤ 0.45	≤ 0.55
P_2O_5 dosages (kg ha^{-1})	Fertilizer							
40	BA_{NG2}	141.58						
80	BA_{NG2}	149.82	4.79	4.90	4.97	5.50	3.71	4.13
160	BA_{NG2}	148.69	4.87	5.04	4.90	6.48	3.48	3.80
40	TSP	146.15	4.96	4.85	4.84	5.65	4.36	3.98
32	BA_{NG2}/ $TSP_{70P:30P}$	142.17						
65	BA_{NG2}/ $TSP_{70P:30P}$	149.59	5.04	4.86	4.99	7.83	4.97	3.41
130	BA_{NG2}/ $TSP_{70P:30P}$	145.59						
80	CMC	146.21	4.82	4.85	4.89	4.35	5.46	3.12
40	BA_{NG2}/ $CMC_{50P:50P}$	148.48						
80	BA_{NG2}/ $CMC_{50P:50P}$	154.24	4.94	4.99	4.88	8.83	6.18	4.71
160	BA_{NG2}/ $CMC_{50P:50P}$	150.08						

mg L^{-1} Oxisol soil at the beginning and between 4.7 and 3.1 mg L^{-1} Oxisol soil to the end of the experiment (Table 18). While the extractability of P from 80 kg P_2O_5 ha^{-1} in the form of BA_{NG2}/ $CMC_{50P:50P}$ fertilized soil was greatest over the course of 119 d growth, the solubility of P from 80 kg P_2O_5 ha^{-1} from CMC was lowest at the beginning of the experiment, greatly increased 65 d after fertilizer application and dropped to its lowest solubility 119 d after fertilizer application (Table 18). The extractability of P from 65 kg P_2O_5 ha^{-1} BA_{NG2}/ $TSP_{70P:30P}$ fertilized Oxisol soil was second highest at the beginning of the experiment and second lowest at the end of the experiment (Table 18). Independent of the type of fertilizer, at the end of the experiment all soils still contained more extractable P than the soils before setting up the fertilizer treatments.

3.5.3 Bagasse based organic products as fertilizer for soybeans

Under field conditions in acidic Oxisol soil, bagasse ash-based organic fertilizer presented its capability to replace P from TSP for soybeans, although larger amounts of P_2O_5 would be needed. Subsequently, blends of combusted bagasse pellets (cBP) ash and chicken manure (CM) were granulated (Hannes Herzel, BAM, Berlin, Germany) and pelletized (Martin Meiller, FRAUNHOFER UMSICHT, Sulzbach-Rosenberg, Germany) to produce a bagasse ash-based organic fertilizer products for soybeans. The bagasse ash-based products were tested under greenhouse conditions, so that pre-cultivated soybeans received either no fertilizer (negative control) or around 218, 100 and 524 mg N, P and K kg^{-1} Oxisol soil. The plants grew for 59 d.

Compared to negative control soybeans, any supply of additional nutrients significantly increased the total dry biomass of plants (Figure 21 A). In regard to soybeans fertilized with granulated and pelletized cBP/ CM products, there was no significant difference between total dry matter of plants, although soybeans receiving nutrients from granulated cBP/ CM product tended to have higher biomass accumulation (Figure 21 A). Soybeans receiving nutrients from granulated and pelletized products did, however, tend to decrease the biomass partitioning to the root, but without any statistical support (Figure 21 B). Pelletized products consisted of cylindrical shape before fertilizer application, whereas granules decomposed partially after 59 d incubation in the soil (Figure 21 C).
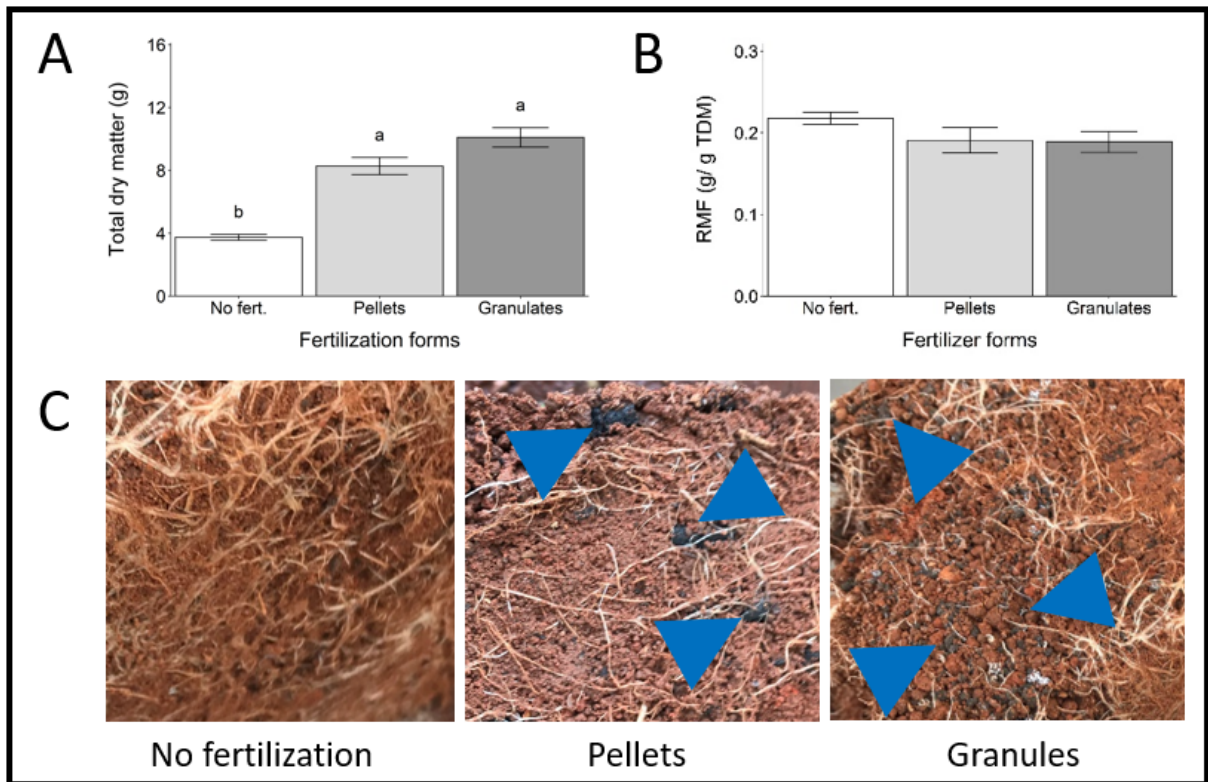
Figure 21: Effects of fertilizer products based on granulation and pelletization of bagasse pellets ash and chicken manure at ratios of 90:10 by dry weights. (A) Total dry matter (TDM) of soybeans from shoot and root organs, biomass partitioning to the roots represented by root mass fractions (RMF), and (C) fertilizer products (blue arrows) after harvesting the plants. The error bars indicate standard errors of the means. Identical letters present no significant differences (Tukey's test, p $\leq$0.05, n = 5). Data of RMF were not statistically supported for pair-wise comparisons (p=0.229).

4 Discussion

The experiments conducted in this dissertation demonstrated that bagasse and bagasse-based ashes increase the substrate and Oxisol soil fertility for soybeans, albeit the P-fertilization effects remained significantly lower than from triple-superphosphate (TSP). However, it was possible to reduce the consumption of P from TSP under greenhouse and/ or field conditions by using bagasse ash as additive to TSP and cattle manure compost as fertilizer for soybeans.

The fertilization effects of bagasse ash depended on the availability of P to soybeans, which was significantly lower than P from highly soluble TSP. Although total dry biomass accumulation of soybeans increased with increasing bagasse ash dosages, the uptakes remained significantly lower than from TSP. It was supposed that large amounts of ash as P-fertilizer supplied suboptimal proportions and quantities of minerals resulting in low efficiency of the fertilizer use by soybeans. Co-combustion and co-gasification of bagasse with nutrient rich residues increased the concentration of P in the ash and its availability to soybeans depending on co-processed biomass. Nonetheless, the availability of P from the ashes to soybeans remained significantly lower than from TSP and thermochemical treatments of co-combusted ash did not help. The yield of soybean grains and uptake of P increased due to additives of bagasse ash to TSP and cattle manure compost under greenhouse and/ or field conditions.

Thus, bagasse and bagasse-based ashes were not competitive with P from TSP, albeit blends of bagasse ash with TSP and organic fertilizers were able to reduce the consumption of mineral P under greenhouse and/ or field conditions. The effects of bagasse ash as PK- and P-fertilizer, effects of co-combustion and co-gasification of bagasse with nutrient-rich residues and thermochemical treatments on the availability of P as well as bagasse ash as additive to TSP and organic fertilizers are discussed in more detail following the approach "From Lab to Field".

4.1 Ashes as P-fertilizer for soybeans in nutrient-poor substrate

Four independent greenhouse pot experiment were conducted to evaluate the fertilization effects of bagasse ashes for soybeans growing in nutrient-poor substrate, and the availability of P from bagasse and co-processed bagasse/ chicken manure ashes to soybeans. Nutrient-poor substrate was used as growing medium, as it was poor in P, Fe and Al (Table 1), and the chemical and physical responses of the substrate were much lower than

of Oxisol soil (Table 13). Low concentrations of Al and Fe were needed to reduce the immobilization of P from fertilizers and to ensure optimal conditions for highly controlled experiments.

4.1.1 Sugarcane bagasse ash with poor availability of P as a fertilizer for soybeans

In pot experiments, the fertility of the substrate increased with increasing dosages of applied BA_{NG1} and cBP ashes (Figure 10, Figure 12 and Table S4). Positive fertilization effects of bagasse ashes were also reported for lettuce and cucumber (Lopez et al., 2009), corn (Pita et al., 2012), sugarcane (Akkajit et al., 2013), wheat (Thind et al., 2017, Gonfa et al., 2018), and bean and Chinese kale (Webber III et al., 2017) growing in different rooting media and soils. Results presented in this thesis suggest that the fertilization effects of both BA_{NG1} and cBP ashes were determined by the availability of P from ashes to soybeans.

The P-fertilization effects and P uptake from BA_{NG1} and cBP ashes by soybeans were significantly lower than from TSP providing equal amounts of P (Figure 10 B and E, Figure 12 C and E). The same trends were also reflected in the P extractability in citric acid (Table 1). Significantly lower P-fertilization effects and lower availability of P to soybeans were partially in agreement with previous studies investigating the availability of P from wood ash (Erich and Ohno, 1992), wheat straw and sewage sludge ashes (Müller-Stöver et al., 2018), and rape meal, rye straw and rye cereal ashes (Schiemenz and Eichler-Löbermann, 2010) to various crops. While Müller-Stöver et al. (2018) and Schiemenz and Eichler-Löbermann (2010) emphasized the relevance of biomass used for the production of ash, Schiemenz and Eichler-Löbermann (2010) additionally highlighted the relevance of the model crop. In the study of Schiemenz and Eichler-Löbermann (2010) plants took up the same amounts of P as the control plants fertilized with TSP depending on the crop and biomass ash. This effect was explained by plant specific adaptations to P deficient conditions and different mechanisms for mobilisation of poorly available P. Additionally, Schiemenz and Eichler-Löbermann (2010) discussed the effect of limited availability of other minerals on the uptake of P. Although the availability of K from BA_{NG1} and cBP ashes was significantly lower than from K_2SO_4 (Figure 10 F and Figure 12 F), it was not a limiting nutrient for soybean growth and the uptake of P. Fertilizations of soybeans with BA_{NG1} and cBP ashes supplied more K than P, i.e. P to K ratios 3:2 and 5.4:1 (Table 1), than recommended for soybeans growing in nutrient-poor soil, i.e. K to P ratio 1.3:1 (Bayerische Landesanstalt für Landwirtschaft, 2020). Additional supply of all nutrients, but no P, did not increase the uptake of K, N or P (Table S4 and Table S5), which excludes the deficiency effect of other nutrients on the uptake of P. All soybeans developed root nodules, which host N_2 fixing rhizobia (Babar et al., 2017), irrespective of treatment indicating ample N supply to the plants. No significant differences in nodule number per root mass or N concentration in roots were observed between unfertilized, ash treated and TSP fertilized plants (Figure S2). Thus, the results suggest that the fertilization effects of BA_{NG1} and cBP ashes were determined by the availability of P from

the ashes to soybeans, which depends, inter alia, on chemical composition of biomass feedstock, processing conditions and collection and the storage conditions of the ashes (Demeyer et al., 2001).

The combustion conditions were most decisive for the solubility and subsequently the availability of P from BA_{NG1} and cBP ashes to soybeans. Intensive fire conditions, indicated by low carbon content (Table 1) and coagulated particles in cBP ash (Figure 11 A), promote heterogeneous coagulation of P with basic cations (Qian et al., 2009), including Na, K, Zn, Fe and Ca (Tan and Lagerkvist, 2011, Freire et al., 2015), and formation of crystalline compounds (Thygesen et al., 2011). Subsequently, cBP ash contained 80% of total P insoluble in any extraction solution ($P_{residual}$, Figure 11 C), which is thus considered as not available to plants (Qian et al., 2009, Qian and Jiang, 2014). Contrary to cBP ash, BA_{NG1} ash contained less insoluble $P_{residual}$, i.e. 45% of total P (Figure 9 C), which includes insoluble inorganic and highly resistant organic P compounds (Qian et al., 2009). Although BA_{NG1} ash contained soluble orthophosphate monoesters, it was probably negligible for P-fertilization effects because of the low overall amounts demonstrated by ^{31}P NMR spectroscopy analyses (Figure 9 B).

Around 45% and 10% of total P from BA_{NG1} and cBP ashes were soluble in 0.5 M HCl (Figure 9 C and Figure 11 C), representing stable Ca- (Qian et al., 2009, Brod et al., 2015) and Mg-bound P (Qian et al., 2009). These complexes are relatively stable under alkaline pH levels (Kruse et al., 2015), so that P from Ca-/ and Mg complexes are not readily available to plants or may form poorly soluble compounds, such as apatite (Qian et al., 2009, Brod et al., 2015) or whitlockite (Brod et al., 2015). Formation of apatite occurs at temperatures above 700 °C and was reported to account for up to 80% of total P in manure ash (Tan and Lagerkvist, 2011). In cBP and BA_{NG1} ashes, the formation of apatite cannot be excluded, as the combustion temperatures reached up to 800 °C and 1050 °C, respectively (2.1). Soil acidity (Dorozhkin, 2012), rhizosphere acidification and root exudates, such as citric and malic acids (Hocking, 2001, Vance et al., 2003, Tawaraya et al., 2014), may influence the environment of mineral particles and increase their dissolution (Valsami-Jones et al., 1998, Dorozhkin, 2012). Furthermore, microorganisms solubilize varying P complexes in the soil (Hocking, 2001). Qin et al. (2011) reported that rhizobia solubilised P from Ca better than from Al, phytates and Fe in a rhizobia/ soybean system, which could have facilitated the dissolution of P from Ca and Mg in current study, as the soybean roots were inoculated with *B. japonicum.*

The fractions of P soluble in 0.1 M NaOH depended on the Al and Fe concentrations and the pH values in the ashes. Phosphorus soluble in 0.1 M NaOH is assumed to represent P complexed with Fe/Al hydroxides/oxides and/or elemental Fe and Al (Qian and Jiang, 2014, Brod et al., 2015), and as the concentrations of Al and Fe were higher in BA_{NG1} ash than in cBP ash (Table 1), the fraction of P_{NaOH} was also larger in BA_{NG1} ash (Figure 9 C and Figure 11 C). In addition, the lower pH level in BA_{NG1} ash than in cBP ash could have facilitated the sorption of P by Al and Fe (Murphy et al., 2006, Kruse et al., 2015). In general, many metal phosphates have low solubility. High Fe concentrations have been reported to decrease the availability of P from Fe-rich sewage sludge ashes (Smith et al., 2002, Müller-Stöver et al., 2018), which is in accordance with the current study comparing

the Fe concentrations, P solubilities in CA and availabilities from BA_{NG1} and cBP ashes for soybeans (Table 1, Figure 9 C, Figure 10 E, Figure 11 C and Figure 12 E).

Handling of bagasse ash after processing seemed to influence the amounts of P soluble in water and 1 M NH_4Cl in BA_{NG1} ash. Phosphorus soluble in water (P_{H2O}) was expected to be higher in BA_{NG1} ash than in cBP ash because of less intensive fire conditions, which was indicated by 5 wt% of carbon, unburned biomass fibrous and organic P-species in BA_{NG1} ash (Table 1, Figure 9 A and B, Figure 11 A and B). According to Beck and Unterberger (2006) and Tan and Lagerkvist (2011) low intensive fire conditions increase the availability of P from biomass ashes to plants, because P remains in unbound forms, such as P oxides and in the presence of water orthophosphoric acid (Beck and Unterberger, 2006). Nonetheless, the fractions of P_{H2O} did not largely differ between BA_{NG1} and cBP ashes (Figure 9 C and Figure 11 C). In both ashes, the fractions of P_{H2O} were below 1% of total P followed by around 3% of P extractable in 1 M NH_4Cl (Figure 9 C and Figure 11 C). The latter is considered to represent the labile P fraction (Qian et al., 2009, Qian et al., 2014, Brod et al., 2015), which can go into solution and get available to plants (Ebelhar, 2008). In BA_{NG1} ash, P soluble in water and 1 M NH_4Cl could have been lost during removal process of the ash from the boiler or underwent chemical reactions with other basic oxides, such as Ca and Mg, as the ash was washed in the removal water (2.1). It is currently not known which P-pools from cBP and BA_{NG1} ashes were accessed by soybeans in current study, but the uptake of more P than solubilized in water and 1 M NH_4Cl (Figure 9 C, Table 10 E, Figure 11 C and Figure 12 E) indicates the capability of soybeans to mobilize P from less soluble P-forms, such as P_{NaOH} and P_{HCl}. Probably, soybeans accessed P complexed with Ca, because legumes and rhizobia are known to acidify the rhizosphere (Haynes, 1983, Qin et al., 2011) facilitating the dissolution of Ca-bound P (Dorozhkin, 2012).

While increasing dosages of BA_{NG1} and cBP ashes incrementally increased the shoot growth and total dry biomass accumulations (Figure 10 A-C, Figure 12 A-C, Table S4), the uptakes of P and K remained significantly lower than from TSP and K_2SO_4 (Figure 10 E and F, Figure 12 E and F, Table S4). Large quantities of ashes were not toxic for garden cress seedlings and even attracted the roots of soybeans (Table 14, Figure 8). Large quantities of ash could have contributed to nutrient imbalances in the substrates relative to crop requirements as was suggested by Müller-Stöver et al. (2018). Imbalanced fertilization supplies suboptimal proportions and quantities of minerals resulting in low efficiency of the fertilizer (Mahajan and Gupta, 2009). The interactions between minerals may occur in the rhizosphere by formation of precipitates or complexes and competition for uptake or in the plant between ions with opposite and same charges (Fageria, 2001). Interactions can have positive (synergistic) or negative (antagonistic) effects on the uptake and utilisation of other minerals, or not interact at all (Fageria, 2001). Soybeans fertilized with cBP ash accumulated significantly less K and Zn than conventionally fertilized plants (Table 16). This could have occurred due to antagonistic interaction between Na, Mg and Ca with K and Zn in the rhizosphere, as nutrients with similar size, charge, geometry of coordination and electric configuration were reported to compete for uptake (Fageria, 2001, White, 2012). Zink regulates many enzymes and reactions (Broadley et al., 2012), so that the deficiency could have contributed to nutrient imbalance in soybeans. Although

Na may substitute the function of K at K-deficient condition (Broadley et al., 2012), high levels of Na were reported significantly to decrease the concentrations of K, P, Ca, Fe, and Cu in the biomass of soybeans (Weisany et al., 2014). In agreement with Hu and Schmidhalter (2005), additional nutrient supply of all nutrients beside of P significantly reduced the accumulation of Na, and increased the accumulation of Zn in the biomass of soybeans fertilized with cBP ash, whereas no significant effect on the uptake of K was detected (Table 17, Table S5) indicating more reasons for lower accumulation than only competition for uptake and interaction in the rhizosphere. The lower accumulation of K could have been due to significantly reduced accumulation of P (Table 16), because of its synergistic interaction (Armstrong, 1998). The availability of P could have been reduced due to precipitation with Ca and Mg (Vance et al., 2003) and/ or reduced by antagonistic interaction with other ions supplied in large amounts by the ash. According to Mahajan and Gupta (2009), even one mineral may imbalance the nutrition of the plant, which complicates the determination of mineral(s) responsible for nutrient imbalance in soybeans fertilized with large amount of ash. Solubility analyses of minerals in the ash and in the substrate as well as correlations of mineral accumulations in the biomass of soybeans could provide more information and could be done in future studies.

4.1.2 Co-combustion of bagasse with chicken manure increase the availability of P from ash to soybeans

According to Demeyer et al. (2001), Freire et al. (2015) and Schiemenz and Eichler-Löbermann (2010), the availability of P from biomass ashes to plants depends, inter alia, on biomass feedstock and processing conditions. Subsequently, bagasse pellets (BP) were co-combusted and co-gasified with chicken manure (CM) to increase the concentrations of P in the ash and the availability of P from the ash to soybeans. Higher concentration of P decreased the dosage of ash needed to meet soybeans P-requirement (Table 6).

Co-gasification and co-combustion of bagasse pellets with chicken manure increased the solubility and availability of P from the ashes to soybeans (Figure 13 B and Figure 14 B-E). This was reflected by significantly better growth of soybeans fertilized with gBP/ $CM_{85:15}$ and cBP/ $CM_{80:20}$ ashes compared to gBP ash fertilized plants starting 26 d after fertilization beginning and total dry matters after 41 d growth (Figure 14 B and C). Compared to ash from gasification (gBP), the increase of P availability increased due to chemical modification of the biomass, because the combustion conditions of gBP and gBP/ $CM_{85:15}$ ashes were comparable (2.1). While the concentrations of P, alkali and alkali earth metals increased due to the co-gasification of BP with CM in a ratio of 85:15 by dry weight, the concentration of Al tended to decrease and the concentration of Fe remained comparable to gBP ash (Table 1). This occurred due to dilution and enrichment effects with Al and Fe poor but Ca, K, Mg and Na rich chicken manure (Table S1). Although gBP, gBP/ $CM_{85:15}$ and cBP/ $CM_{80:20}$ ashes were gasified and combusted at comparable temperatures (2.1), gBP/ $CM_{85:15}$ and cBP/ $CM_{80:20}$ ashes contained more P_{NH4Cl} and P_{HCl} than gBP ash (Figure 13 B). Main drivers for increased solubility seemed to be higher concentrations of alkali and alkaline earth metals, i.e. Na

and K, and Ca and Mg, and increase of P concentration (Table 1), because crystalline P compounds such as whitlockite ($Ca_9M(PO_4)_7$, where M is most common Ca, Fe and/or Mg (Herzel et al, 2020) were formed in gBP/ $CM_{85:15}$ and $CaNa_xK_{1-x}(PO_4)_2$ and two undefined P compounds in cBP/ $CM_{80:20}$ ashes (Table 15). Higher fractions of soluble P in gBP/ $CM_{85:15}$ and cBP/ $CM_{80:20}$ ashes reflected significantly better growth of soybeans compared to gBP ash fertilized plants starting 26 d after fertilization beginning and total dry matters after 41 d growth (Figure 13 B and Figure 14 B and C). However, P from whitlockite is poorly available to soybeans (Herzel et al., 2020), which was reflected by not statistically different leaf areas, biomass partitioning to the roots and uptakes of P from gBP/ $CM_{85:15}$ ash compared to gBP ash fertilized plants (Figure 14 B, D and E). Nonetheless, inclining increases of shoot growth, leaf areas and uptakes of P were observed (Figure 14 B, D and E). Soybeans fertilized with both gBP and gBP/ $CM_{85:15}$ ashes were able to mobilize "inert P" represented by P_{HCl}, as the uptakes of P from gBP and gBP/ $CM_{85:15}$ ashes after 27 d growth were higher than supplied by P_{H2O}, P_{NH4Cl} and P_{NaOH} (Figure 13 B and Figure 14 E).

Processing conditions influence the availability of P from bagasse/ chicken manure ashes to soybeans. Although, the maximum processing temperatures, chemical compositions of the ashes and extractable fractions of P at varying pH levels from gBP/ $CM_{85:15}$ and cBP/ $CM_{80:20}$ ashes were comparable (2.1, Table 1, Figure 13 B), the solubility of P in citric acid was lower by 9% in cBP/ $CM_{80:20}$ ash than in gBP/ $CM_{85:15}$ ash (Table 1). Lower solubility of P in cBP/ $CM_{80:20}$ ash was not reflected by lower uptakes of P by soybeans, quite the contrary occurred and the soybeans took up significantly more P than from gBP/ $CM_{85:15}$ ash after 27 and 42 d growth (Figure 14 E). Better availability of P from cBP/ $CM_{80:20}$ ash than from gBP/ $CM_{85:15}$ ash was probably due to different crystalline P compounds (Table 15). While gBP/ $CM_{85:15}$ ash contained P in the form of whitlockite, which is poorly available to plants (Herzel et al., 2020), cBP/ CM ash contained $CaNa_xK_{1-x}(PO_4)_2$ and two undefined P compounds, which were soluble in formic and/ or citric acids (Table 15). All listed crystalline P compounds are Ca-based and were probably soluble in 0.5 M HCl, albeit P from whitlockite was probably less soluble than from $CaNa_xK_{1-x}(PO_4)_2$ (Herzel et al., 2020). Subsequently, $CaNa_xK_{1-x}(PO_4)_2$ could have contributed to significantly better availability of P for soybeans in the first 27 d of growth. The availability of P from undefined crystalline P compounds seemed to be comparable to the availability of P from whitlockite, since the uptakes of P from both ashes were comparable in the period from 27 to 42 d of growth (Figure 14 E). Referring to Boström et al. (2011), varying concentrations of the minerals in the biomass affect the ash transformation sequences and the properties of subsequent ash. Subsequently, the availability of $CaNa_xK_{1-x}(PO_4)_2$, which was also detected in poultry litter ash (Leng et al., 2019), and undefined crystalline P compounds in cBP/ $CM_{80:20}$ ash could have been due to bigger proportion of chicken manure in the ash. Furthermore, different crystalline P compounds in gBP/ $CM_{85:15}$ and cBP/ $CM_{80:20}$ ashes could be due to variations in processing temperatures during biomass processing, as Qian and Jiang (2014) emphasised the influence of temperature during thermal treatment on mineral structure more important than that of atmospheric conditions, whereas Herzel et al. (2020) reported formation of different crystalline P compounds depending on the processing method.

4.2 Bagasse-based ashes as P-fertilizer for soybeans in Oxisol soil

Following the "From Lab to Field" approach, further experiments were conducted in acidic Oxisol soil environment, which allowed to have more realistic soil environment as in Brazil where >60% of country area are covered by Oxisol soil (Schaefer et al., 2008). Sewage sludge was tested as biomass feedstocks for the production of bagasse-based ashes and thermochemical treatments on bagasse/ chicken manure ash were tested to increase the availability of P from the ashes to soybeans.

4.2.1 Co-combusted feedstock biomass affects the availability of P from bagasse-based ash to soybeans in Oxisol soil

Only co-combustion of bagasse with chicken manure increased the availability of P to soybeans. While the leaf areas of soybeans fertilized with cBP and cBP/ SSL$_{83:17}$ ashes did not statistically differ over course of 40 d growth, leaf areas of plants receiving P from cBP/ CM$_{80:20}$ ash significantly increased compared to no-P control and cBP and cBP/ SSL$_{83:17}$ ash fertilized plants already 13 d after fertilizer application and was significantly larger over course of the growth (Figure 16 B). Leaf areas reflected total dry biomasses of plants and uptakes of P (Figure 16 B, C and E). As already carved out in previous discussion (4.1), inter alia, the chemical composition of ashes determined the solubility of P in varying extraction solutions and the availability to soybeans. Compared to combusted bagasse pellets (cBP) and co-combusted bagasse pellets/ chicken manure ashes (cBP/ CM$_{80:20}$), the co-combustion of bagasse pellets (BP) with SSL in a ratio of 83 to 17 by dry weight (cBP/ SSL$_{83:17}$) increased not only the concentration of P but also the concentrations of Fe, Al, Cu and Zn in the ash (Table 1). The concentrations of most other minerals, which are relevant for the nutrition of plants, including Mg, Ca, K, and additionally Na decreased compared to cBP and cBP/ CM$_{80:20}$ ashes (Table 1). This was obviously driven by different chemical compositions of biomasses, i.e. bagasse pellets, sewage sludge and chicken manure (Table S1). The pH levels in cBP/ SSL$_{83:17}$, cBP and cBP/ CM$_{80:20}$ ashes followed the concentrations of alkali and alkaline earth metals in the ashes (Table 1). Only the fertilization with cBP/ CM$_{80:20}$ ash increased the pH level of Oxisol soil to around 6, whereas Oxisol soils fertilized with cBP and cBP/ SSL$_{83:17}$ ashes remained at around 4.5 (Table 7). Subsequently, a fixation of P with Fe and Al in cBP and cBP/ SSL$_{83:17}$ ashes and cBP and cBP/ SSL$_{83:17}$ ash fertilized Oxisol soils could have occurred, because the solubility and fixation of P depends on the pH level (Vance et al., 2003). Improved growth of soybeans and uptake of P from cBP/ CM$_{80:20}$ ash occurred not due mobilization of P from Fe-/ Al-bound P from Oxisol soil reserves due to pH increase, as the growth and uptakes of P by no-P control soybeans growing on pH 4.5 and 6 were comparable (Figure 16 E). In agreement with previous discussion

(4.1) the fractions of P soluble in 0.1 NaOH followed the concentrations of [Fe and Al] and was highest in cBP/ $SSL_{83:17}$ followed by cBP and cBP/ $CM_{80:20}$ ashes (Figure 15 B). Compared to cBP ash, cBP/ $SSL_{83:17}$ ash contained larger fraction of P soluble in 0.5 M HCl (Figure 15 B), which reflected the increase of P extractable with citric acid (Table 1) but not the availability of P to soybeans (Figure 16 E). Although, 60 to 70% of total P from cBP/ $SSL_{83:17}$ and cBP/ $CM_{80:20}$ were soluble in 0.5 M HCl (Figure 15 B), only the availability of P from cBP/ $CM_{80:20}$ ashes significantly increased compared to cBP ash (Figure 16 E). This was against the expectation, as acidic soil environment and exudates from soybeans and rhizobia were expected to facilitate the dissolution of Ca- and Mg-based phosphates (Owens et al., 2019). cBP/ $SSL_{83:17}$ and cBP/ $CM_{80:20}$ ashes contained different crystalline compounds determined by XRD analyses (Table 15). According to Herzel et al. (2020), P from whitlockite, which was also detected in cBP/ $SSL_{83:17}$ ash (Table 15), is less available to plants than P from $CaNa_xK_{1-x}(PO_4)_2$ and potentially from two undefined crystalline P compounds soluble in formic and/ or citric acids detected in cBP/ $CM_{80:20}$ ash. Availability of P from organic P pool, i.e. aromatic phosphoric acid esters, ortho-phosphate monoesters and potentially from polyphosphate (Figure 15 B), was negligible because of small amounts.

Next, the effects of cBP/ $CM_{80:20}$ ash was evaluated as P-fertilizer in a "dose-response" experiment, because the biomass partitioning to the roots of soybeans fertilized with cBP/ $CM_{80:20}$ ash still indicated nutrient deficiency (Figure 16 D). Compared to P from TSP, the availability of P from cBP/ $CM_{80:20}$ ash was significantly lower, albeit it depended on the dosage of P supplied to the plants. This was reflected by total dry biomass accumulations and partitioning of the biomass to the roots (Figure 18 A-C). Lower availability of P from cBP/ $CM_{80:20}$ ash than from TSP found support by the uptake of P from 340 mg P kg^{-1} Oxisol soil (Figure 18 D). The different availability of P from cBP/ $CM_{80:20}$ ash and TSP was probably driven not only by different crystalline P compounds i.e. two undefined P compounds and $CaNa_xK_{1-x}(PO_4)_2$ in cBP/ $CM_{80:20}$ ash (Table 15) vs. $Ca(H_2PO_4)_2 \cdot H_2O$ in TSP, but also by different pH levels in the Oxisol soils. These were around 5.5 and 6 to 7 in soils receiving P from TSP and cBP/ $CM_{80:20}$ ash, respectively (Table 8). Plants take P in the form of $H_2PO_4^-$ (Schachtman et al., 1998, Młodzińska and Zboińska, 2016, Hawkesford et al., 2012, Zogli et al., 2017), which occurs at pH levels between 5.0 and 6.0 (Schachtman et al., 1998). It may become immobile through adsorption processes with Fe and Al hydroxides at acidic conditions and precipitation with Ca and Mg carbonate at alkaline conditions (Vance et al., 2003). It is well known that more than 80% of supplied P becomes immobile and unavailable for plant uptake (Schachtman et al., 1998 and Sanz-Saez et al., 2017), which explains the low fertilization effects of P at low fertilized conditions and increased fertilization effects due to increasing dosages of P in the form of TSP (Figure 18 A and D). However, increased dosages of P in the form of cBP/ $CM_{80:20}$ ash hardly increased the total dry biomass of soybeans (Figure 18 A). This was probably due to transformation of $H_2PO_4^-$ into HPO_4^{2-}, which is less available to plants (Schachtman et al., 1998); and precipitation of $H_2PO_4^-$ with Ca and Mg carbonates due to the increase of pH level (Vance et al., 2003). Additionally, large amounts of cBP/ $CM_{80:20}$ ash could have reduced the uptake of P and the growth of soybeans due to suboptimal ratios and concentrations of minerals (see discussion 4.1.1).

In the same experiment, another fertilization treatment with cBP/ $CM_{60:40}$ ash was included. The larger fraction of chicken manure in bagasse based ash did not increase the availability of P from cBP/ CM ash to soybeans. Soybeans receiving P from cBP/ $CM_{60:40}$ ash behaved similar to plants fertilized with cBP/ $CM_{80:20}$ ash (Figure 18 A-D), which was in agreement with crystalline P compounds determined by powder XRD analyses, because the identified P compounds were the same (Table 15). However, total dry biomass of soybeans fertilized with cBP/ $CM_{60:40}$ ash tended to increase and the biomass partitioning to the roots tended to decrease compared to cBP/ $CM_{80:20}$ ash-fertilized plants (Figure 18 A and B), which indicated in agreement with extraction analyses of P in CA and NAC a better availability of P for soybeans (Figure 17).

Thermochemical treatments of sewage sludge ash with Na_2SO_4 were reported to increase the availability of P to maize growing in sandy soil (Severin et al., 2014), in nutrient-poor subsoil (Steckenmesser et al., 2017) and in sandy soil/ quartz sand and loamy sand/ quartz sand blends (Vogel et al., 2018). In current study, thermochemical treatments with Na_2SO_4/ and Na_2SO_4/ K_2SO_4 were applied to cBP/ $CM_{60:40}$ ash and successfully transformed 2 undefined crystalline P compounds and $Ca(Na,K)PO_4$ into $CaNaPO_4$ (Table 15). $CaNaPO_4$ was reported to be completely soluble in CA (Gunawardane and Glasser, 1979), in NAC (Herzel et al., 2016) and in FA (Kratz et al., 2019). In current study, thermochemical treatments of cBP/ $CM_{60:40}$ ash with Na_2SO_4 and Na_2SO_4/ K_2SO_4 increased the solubilities of P in NAC and CA from 75% and 90% in cBP/ $CM_{60:40}$ ash, to 90% and 100% in both cBP/ $CM_{60:40}$+Na and cBP/ $CM_{60:40}$+Na/K thermochemical products (Figure 17). However, the solubility of P in FA remained at around 80% in cBP/ $CM_{60:40}$ ash and its thermochemical products (Figure 17). The extraction mechanisms of CA, NAC and FA differ and allowed to identifying the needs for soybeans to solubilize the P from the fertilizer (Camps-Arbestain et al., 2017). Subsequently, it was possible to constitute, that a combination of soil acidification and exudation of chelating compounds was needed to solubilize P from cBP/ $CM_{60:40}$ ash and its thermochemical products, because CA is a weak acid but strong metal-complexing agent, and NAC acts as a complexing agent, whereas FA has low complexing ability and solubilizes P through pH decrease (Camps-Arbestain et al., 2017). The increased solubilities of P in CA and NAC reflexed the trends for P availability from cBP/ $CM_{60:40}$ ash and its thermochemical products to soybeans growing in Oxisol soils (Figure 17 and Figure 18). The trends indicated increased total dry biomass accumulations and uptakes of P due to the fertilizations with cBP/ $CM_{60:40}$+Na and cBP/ $CM_{60:40}$+Na/K products, and significant decreases of biomass partitioning to the roots compared to cBP/ $CM_{60:40}$ ash-fertilized plants (Figure 18 A and B). While thermochemical treatments of sewage sludge ash were reported significantly to increase the availability of P to maize (Severin et al., 2014, Steckenmesser et al., 2017 and Vogel et al., 2018), it was not the case for P from cBP/ $CM_{60:40}$ ash. This was probably due to different chemical compositions of cBP/ $CM_{60:40}$ ash compared to sewage sludge ash. Contrary to sewage sludge ash (Steckenmesser et al., 2017, Herzel et al., 2016, Vogel et al., 2018), cBP/ $CM_{60:40}$ ash was rich in alkali and alkaline earth metals but poor in Fe and Al (Table 1). This could have facilitated the formation of calcium alkali phosphates (Table 15) instead of whitlockite and other phosphate forms reported in sewage sludge ash (Steckenmesser et al., 2017), and detected in gBP/ $CM_{85:15}$ and cBP/

$SSL_{83:17}$ ashes (Table 15). Subsequently, the thermochemical treatment of cBP/ $CM_{60:40}$ ash was redundant, as P from both $CaNa_xK_{1-x}(PO_4)_2$ and $CaNaPO_4$ is considered to be available to plants (Herzel et al., 2020). The transformation of 2 undefined crystalline P compounds and other P-species from amorphous phase could have contributed to the increase of P solubility and its subsequent availability to soybeans. Although, the solubility of P in CA and NAC from cBP/ $CM_{60:40}$+Na and cBP/ $CM_{60:40}$+Na/K thermochemical products were at least as high as the solubilities of P from TSP (Figure 17), the fertilization effects, including uptakes of P, were significantly lower than from TSP (Figure 18 A and D). The lower P-fertilization effects and lower availability of P from thermochemical products than from TSP were also presented from sewage sludge ash-based thermochemical products (Steckenmesser et al., 2017). However, it was not discussed. This was probably due to (i) slow dissolution of Ca-based phosphates (Tan and Lagerkvist, 2011, Müller-Stöver et al., 2018, Vance et al., 2019), (ii) high level of soil pH as it was discussed for cBP/ $CM_{80:20}$ and cBP/ $CM_{60:40}$ ashes and/ or (iii) nutrient imbalance, which could have been even more severe than in cBP/ $CM_{60:40}$ ash-fertilized soils because of additional Na_2SO_4 supply for thermochemical treatments.

4.3 "From Lab to Field": Ash as additive to conventional fertilizer

Following the "From Lab to Field" approach, analyses of bagasse ash and bagasse ash as additive to conventional mineral and organic P-fertilizer were investigated under greenhouse and field conditions with the goal to reduce the dependency on rock-P based P-sources.

4.3.1 Bagasse ash as additive saved conventional P-fertilizer consumption

The lower availability of P from bagasse and bagasse-based ashes than from TSP observed under greenhouse conditions (Figure 16 E and Figure 18 D) was confirmed on the field (Figure 20 B), albeit the grain yields of soybeans receiving the same dosage of P in the forms of TSP and BA_{NG2} did not statistically differ but tended to decrease due to the fertilization with ash (Figure 20 A). The relative effectiveness of BA as P-fertilizer was 74% of TSP (Figure 20 B). The higher effectiveness of BA_{NG2} as P-fertilizer on the field (Figure 20 B) compared to greenhouse experiments (Figure 16 E and Figure 18 D) was probably due to different chemical compositions of the ashes (Table 1), the forms of P and environmental conditions. Contrary to greenhouse pot experiments (Figure 18), increasing dosage of BA_{NG2} resulted in comparable grain yields and uptakes of P as in conventionally fertilized soybeans (Figure 20 B). The contradiction was probably due to limited pot volume and highly regulated watering of plants in greenhouse experiments, which reduced the leaching of minerals, e.g. Na, to a minimum. In contrast to greenhouse experiment, leaching of minerals could have occurred under field conditions resulting in less sever or even no nutrient imbalance for soybeans. However, more studies are needed.

Compared to the fertilization with 40 kg P_2O_5 ha^{-1} in the form of TSP, 160 kg P_2O_5 ha^{-1} from BA_{NG2} tended to increase the grain yield by 4% (Figure 20 A), which can be referred to higher effectiveness of 160 kg P_2O_5 ha^{-1} from BA_{NG2} as P-fertilizer (Figure 20 B). Although, 160 kg P_2O_5 ha^{-1} in the form of BA_{NG2} tended to a better P-fertilization than TSP, the fertilization of soybeans with BA_{NG2} is not economically feasible, as 10.2 t ha^{-1} of bagasse ash as P-fertilizer would be needed and only 1 t ash ha^{-1} remains after combustion of bagasse (calculation was based on FAO 2014, Almazan et al., 1998 and Rocha et al., 2015).

Blends of bagasse ash with TSP reduced the consumption of TSP by soybeans under greenhouse conditions. According to Vance et al. (2019), blending of poultry litter ash with TSP saved one part of P from TSP without delaying the bermudagrass development in early stage. This was in agreement with soybeans growing in Oxisol soil under greenhouse conditions for 42 d (Figure 19 A and B) and on the field in Brazil for 121 d (Figure 20 A). Under greenhouse conditions, the leaf areas of soybeans receiving around 100 mg P kg^{-1} Oxisol soil from cBP/ TSP$_{45P:55P}$ (55% of P sourced from TSP) and 115 mg P kg^{-1} Oxisol soil from TSP significantly increased compared to no-P control plants 14 d after fertilization beginning (Figure 19 A). The leaf areas of soybeans fertilized with cBP/ TSP$_{45P:55P}$ and TSP did not statistically differ, albeit tended to decrease in soybeans receiving P from cBP/ TSP$_{45P:55P}$ compared to TSP fertilized plants (Figure 19 A). The trend remained over course of the growth and was also represented by total dry biomasses of plants (Figure 19 B), which can be referred to significantly lower uptake of P from cBP/ TSP$_{45P:55P}$ (Figure 19 C). However, under field conditions with regular heavy rains as in Brazil, the lower solubility of P, could prevent against runoff of P (Vance et al., 2019) and ensure longer availability of P (Vance et al., 2019) to soybeans.

Regardless of lower content of P from TSP, i.e. 30% of total P, the fertilization with 32 kg P_2O_5 ha^{-1} in the form of BA_{NG2}/ TSP$_{70P:30P}$ allowed to produce the same amount of grain yield under field conditions in Brazil (Figure 20 A). In agreement with Fageria et al. (2013), the grain yield highly correlated with the uptake of P (Figure 20 C) and subsequently allowed to save around 70% of P from TSP. Even more, the fertilization with 65 kg P_2O_5 ha^{-1} in the form of BA_{NG2}/ TSP$_{70P:30P}$ (20 kg P_2O_5 ha^{-1} from TSP) resulted in yield increase by 11% (Figure 20 A). The relative efficiency of P from 65 kg P_2O_5 ha^{-1} in the form of BA_{NG2}/ TSP$_{70P:30P}$ was higher by 10% than from TSP (Figure 20 B), so that 50% of P from TSP can be saved by blending 45 kg P_2O_5 ha^{-1} from BA_{NG2} with 20 kg P_2O_5 ha^{-1} from TSP. Although, the pH levels did not statistically differ between varying fertilization treatments (Table 18), which was probably due to dilution effect of the soil from the fertilized furrow and beside of it (2.4.6), application of BA_{NG2} could have corrected the soil pH around TSP and made the P longer available to soybeans (Table 18). Furthermore, the higher uptake of P from BA_{NG2}/ TSP$_{70P:30P}$ could have related to less leaching of P as it was demonstrated for bermudagrass fertilized with poultry litter ash and TSP at varying P ratios (Vance et al., 2019).

Furthermore, blends of BA_{NG2} ash with cattle manure compost (BA_{NG2}/ CMC$_{50P:50P}$, 50% of P sourced from BA_{NG2}) were tested as organic fertilizer for soybeans under field conditions. The blends of BA_{NG2} ash with CMC produced higher grain yields than TSP

fertilized soybeans, albeit depended on the dosage of P. While soybeans fertilized with 40 kg P_2O_5 in the form of BA_{NG2}/ $CMC_{50P:50P}$ produced significantly less grain yield than its control treatment with the same dosage of P from TSP, 80 kg P_2O_5 in the form of BA_{NG2}/ $CMC_{50P:50P}$ allowed to forego the use of TSP as P-fertilizer for soybeans and even increased the grain yield by 12% (Figure 20 A). Phosphorus from BA_{NG2}/ $CMC_{50P:50P}$ was more effective as P-fertilizer for soybeans by 13% compared to P from TSP (Figure 20 B). This was probably due to pH effect and different forms of P, as the pH levels were alkaline in both BA_{NG2} and CMC (Table 1 and Table S1) and were reported to contain P with different solubilities. Whalen et al. (2000) reported solubility of P by around 75% of total P in cattle manure, and the biomass ash is known for its low solubility of P (Tan and Lagerkvist, 2011, Müller-Stöver et al., 2018, Vance et al., 2019, see also discussion 4.1 and 4.2). Furthermore, the application of BA_{NG2} and CMC as fertilizer could have altered the microbiotic activity in the soil (Sharma et al., 2015, Das et al., 2017) facilitating the dissolution of Ca and Mg bound P from the ash and improved the water household for plants, because both BA and CMC contained organic carbon (Table 1 and Table S1). According to Withers et al. (Withers et al., 2018), supply of organic fertilizers increased water holding capacity, improved soil properties and reduced fixation of P.

4.3.2 Bagasse based organic products as fertilizer for soybeans

Based on the field experiment in Brazil and the positive effect of BA_{NG2}/ CMC as P-fertilizer for soybeans, organic fertilizer products were created from combusted bagasse pellets (cBP) ash and organic chicken manure (CM; FRAUNHOFER UMSICHT and BAM, Germany). The fertilizers products met approximately the need of NPK for soybeans growing in centre west of Brazil (FAO, 2007) and were either granulated (BAM, Berlin, Germany) or pelletized (FRAUNHOFER UMSICHT, Sulzbach-Rosenberg, Germany) for its better distribution on the field. Under greenhouse conditions, the fertilization effects of granulated and pelletized bagasse ash/ chicken manure products were tested for fertilization effects on biomass accumulation of soybeans after 59 d growth.

Compared to unfertilized soybeans, fertilizations with both granulated and pelletized fertilizer products significantly increased the total dry biomass of plants (Figure 21 A) and indicated its capability to be used as fertilizer for soybeans. However, its efficiencies differed, so that soybeans fertilized with granulated product tended to increase the total dry biomass by 1.2 times compared to pelletized products (Figure 21 A). According to Irfan et al. (2018), water penetrates the fertilizer product and release the nutrient(s), which diffuse to the surface of the fertilizer product. In current experiment, the better fertilization effect of granulated fertilizer product was probably related to smaller size and lower stability of granulates compared to pelletized products, as the granules partially decomposed whereas the pellets remained the original shape after 59 d of growth (Figure 21 C). While the smaller size, and thus larger surface area, could have facilitated faster release of nutrients from granulated fertilizer surface, lower stability of granules enhanced the release of nutrients by continuous decay and increase of the surface area. According to Purnomo

et al. (2018), low pelletizing pressure and low temperature of drying may decrease the release of N, but not of K, from pelletized bagasse ash-based matrix. Purnomo et al. (2018) constituted that the release behaviour of minerals from ash-based matrix depend on each mineral individually and the different preparation parameters. Although, bagasse ash/ chicken manure pellets could serve as slow-release and granules as fast-release fertilizer for soybeans, the mechanism of fertilization effects of bagasse/ chicken manure pellets and granules is not clear and more studies are needed. However, the release of nutrients from bagasse-based fertilizers depended on the shape and production parameters of the fertilizer product.

5 Conclusion

Even though bagasse ash contains all the minerals relevant for the nutrition of soybeans and is thus reasonable for consideration as a potential fertilizer, it cannot compete with conventional fertilizers. This was primarily due to low availability of P from bagasse ash to soybeans and seemed unrelated to the availability of K.

In agreement with hypothesis (ii), the modification of processing conditions to produce bagasse ash altered the availability of P for soybeans. This could be related to the formation of different P compounds during processing and co-combustion process of bagasse with chicken manure. Nevertheless, persistence of low total and plant-available P content does not allow bagasse ash to be designated as fertilizer for soybeans.

Fertilization of soybeans with large doses of bagasse ash did incrementally increase the total dry biomass and grain yield, of plants. This allowed production comparable to that of conventionally fertilized plants. However, the application of such large amounts of bagasse ash as P fertilizer is not feasible from either an economic or an ecological point of view. Transportation and distribution costs of bagasse ash to the fields would exceed the costs of mineral fertilizer, and the frequent traffic of trucks would increase air pollution and soil compaction. In addition, the leaching of minerals into natural water bodies could contaminate ground water and aquifers.

Co-processing of bagasse with nutrient-rich residues was tested as an option to increase the total concentration of P in the ash. Results show that the availability of P from bagasse-based ashes to soybeans depended (i) on processing condition, (ii) the chemical composition of co-processed biomass, and (iii) the presence of crystalline P compounds. In conclusion, only co-combustion of bagasse with alkali-rich biomasses increased the availability of P from bagasse-based ashes to soybeans due to formation of easily soluble Ca-alkali P. The co-combustion of bagasse with chicken manure made the thermochemical post-treatment of alkali-rich ash with Na_2SO_4 and Na_2SO_4/ K_2SO_4 redundant. Under greenhouse conditions, the availabilities of P from bagasse-based ashes and thermochemical products for soybeans remained significantly lower than from TSP. The fertilization with bagasse ash and thermochemical products could have supplied suboptimal ratios and concentrations of minerals needed for the growth of soybeans. The effect of suboptimal conditions remained probably over course of the growth, because the watering of plants was highly controlled and no leaching of minerals occurred.

In conclusion, while sugarcane bagasse ash used alone as a P fertilizer for soybeans was only able to replace rock-P based fertilizer if unreasonably large amounts of ash were applied, its blends with commercial mineral and organic P-sources was able to achieve a more than 50% reduction of required TSP while retaining high grain yield under field

conditions. Thus, future research investigating the application of bagasse ash as a fertilizer should aim to identify the processes responsible for these observations.

6 Outlook

In the current study, the positive effects of bagasse ash and bagasse ash-based fertilizers were described for soybeans under greenhouse and field conditions.

Under greenhouse conditions, the use of ash as a P-fertilizer for soybeans incrementally increased the pH level of Oxisol soil, which was suggested to be at least partly responsible for the decreased fertilization effects of bagasse ash-based thermophosphates. The fertilization effects of bagasse ash-based products need to be tested under field conditions, as Yoorin (Yoorin Fertilizants, Poços de Caldas-MG, Brazil), a commercially available thermophosphate, is used for fertilizing soybeans and sugarcanes (Yoorin Fertilizantes, 2020) in Brazil.

Contrary to soybeans, perennial plants, such as sugarcane, could benefit from a slow release of P from bagasse and bagasse ash-based fertilizers. This slow release leads to reduced leaching and thus more continuous availability of P for plants. Subsequently, the bagasse and bagasse ash-based fertilizers should be tested as P fertilizers for sugarcane with the aim of closing nutrient cycles under field conditions in Brazil.

However, the dynamics of P release from ash and the associated rhizosphere processes facilitating the dissolution of P by soybeans are still unknown. To this end, better understanding of the role of rhizobia on P dynamics, as well as rhizosphere processes related to soybean morphology and physiology during ash-fertilization, would allow the formulation of a fertilizer for soybeans meeting the demand of P by plants at specific times during growth and development.

Finally, life cycle analyses and economic assessment should be conducted to investigate the impact of bagasse and bagasse ash-based fertilizers on the environment and the economic advantage of these fertilizers over conventional fertilizer.

A Supplementary

Table S1: Elementary compositions of bagasse pellets (BP), chicken manure (CM), sewage sludge (SSL) and cattle manure compost (CMC).

Elements	Abbrev.	Unit	BP water free	CM water free	SSL water free	CMC water free
Carbon	C	wt%	46.04	38.48	33.16	
Hydrogen	H	wt%	4.98	4.51	4.41	
Oxygen	O	wt%	37.55	30.68	17.65	
Nitrogen	N	wt%	0.22	5.85	5.55	33.7
Sulphur	S	wt%	0.03	0.43	1.10	2.50
Phosphorus	P	wt%	0.03	1.48	3.60	0.69
Potassium	K	wt%	0.12	2.04	0.26	3.20
Magnesium	Mg	wt%	0.05	0.68	0.72	0.15
Calcium	Ca	wt%	0.11	5.60	4.20	0.23
Manganese	Mn	wt%	0.01	0.06	0.08	
Iron	Fe	wt%	0.37	0.09	3.30	
Sodium	Na	wt%	0.01	0.15	0.07	
Aluminium	Al	wt%	0.27	0.10	2.50	
Chlorine	Cl	wt%	0.04	0.24	0.07	
Strontium	Sr	wt%	0.00	0.01	0.02	
Titanium	Ti	wt%	0.01	0.00	0.01	
Ash		wt%	10.90	19.70	38.10	
$pH_{1:5}$ in 0.01 M $CaCl_2$						7.44

Table S2: Modified 1/3 strength Hoagland's solution.

Chemicals	Concentrations
KNO_3 (1 M)	1.67 mM
$Ca(NO_3)_2$ (1 M)	1.67 mM
$MgSO_4$ (1 M)	0.67 mM
K_2SO_4 (0.5 M)	0.8 mM
$FeSO_4$ x 7 H_2O	10.0 μM
$MnCl_2$ x 4 H_2O	3.33 μM
$CuSO_4$ x 5 H_2O	0.33 μM
$ZnSO_4$ x 7 H_2O	0.33 μM
H_3BO_3	16.66 μM
$NaMoO_4$ x 2 H_2O	0.17 μM

Table S3: Effect of ash from combustion of bagasse pellets (cBP) on root growth and dry biomass accumulation of soybeans. The values were not statistically different and only trends are presented (n = 3). Abbreviations: root length fraction (RLF) in fertilizer depot area 13 and 21 d after fertilization application (AFA), total dry matter (TDM) of soybeans from shoot and root organs, biomass partitioning to the roots represented by root mass fraction (RMF), phosphorus (P) and triple-superphosphate (TSP).

Trait	ANOVA	No P	cBP ash	TSP
$RLF_{13\ d\ AFA}$ in depot area (cm cm^{-1} total root length)	$p \leq 0.20$	4.90	10.00	3.50
$RLF_{21\ d\ AFA}$ in depot area (cm cm^{-1} total root length)	$p < 0.20$	5.10	12.00	10.60
TDM	$p \leq 0.95$	1.41	1.36	1.41
RMF (g g^{-1} TDM)	$p \leq 0.97$	0.41	0.40	0.40

Table S4: Effect of fertigation (fert.) with all nutrient but no P on total dry matters (TDM) from shoot and root organs, biomass partitioning to the roots represented by root mass fractions (RMF), and uptakes of phosphorus (P) and potassium (K) from unfertilized nutrient-poor substrate, combusted bagasse pellets (cBP) ash and triple-superphosphate (TSP) by soybeans 35 d after fertilizer application (Tukey's test, $p \leq 0.05$, n = 3).

| Fertilizations mg P/ mg K kg^{-1} substrate	TDM (g) No fert.	Fert.	RMF (g g^{-1} TDM) AVG (no fert. & fert.)	P uptake (mmol) AVG (no fert. & fert.)	K uptake (mmol) No fert.	Fert.
No P (488 K)	2.24 cd	1.96 d	0.24 a	0.01 b		
cBP ash (73 P/ 488 K)	2.88 bc	3.41 b	0.24 a	0.05 b		
TSP/ K$_2$SO$_4$ (73 P/ 995 K)	4.96 a	5.51 a	0.18 b	0.97 a		
No P (488 K)	2.24 b	1.96 b	0.24 a	0.01 c	0.69 d	0.57 d
cBP ash (170 P/ 995 K)	5.16 a	5.09 a	0.21 a	0.14 b	1.07 c	1.19 c
TSP/ K$_2$SO$_4$ (73 P/ 995 K)	4.96 a	5.51 a	0.18 b	0.97 a	3.45 b	4.14 a
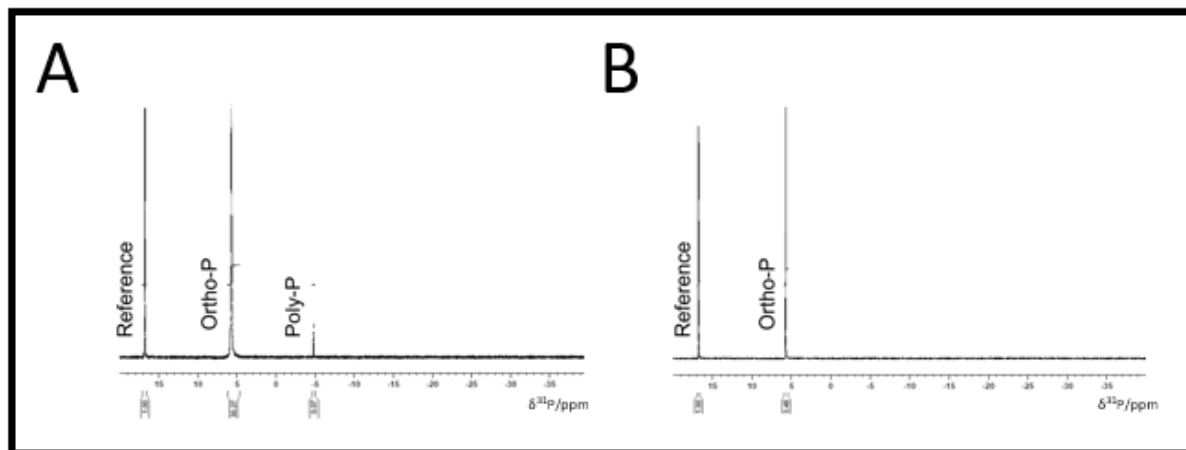
Figure S1: Phosphorus (P)-species determined by ^{31}P NMR spectroscopy in ashes from gasification of (A) bagasse pellets (gBP) and (B) bagasse pellets and chicken manure at a ratio of 85:15 by dry weight (gBP/ CM$_{85:15}$).

Table S5: Effect of fertigation with all nutrients but no phosphorus (P) on nutrient uptakes from 0 and 73 g cBP ash kg^{-1} nutrient-poor substrate and from conventional fertilization after 35 d growth. Conventional fertilization received P and potassium (P) from triple-superphosphate and potassium sulphate. The interactions between minerals and fertigation with all nutrients but no P were not statistically supported. (n = 3).

| Elements	Abbrev.	Fertigation	Not fertilized	73 g cBP ash kg^{-1} substrate	Conv. fertilization
Nitrogen	N	No	0.57	0.79	1.37
	N	Yes	0.71	0.77	1.45
Phosphorus	P	No	0.02	0.03	0.19
	P	Yes	0.02	0.03	0.19
Potassium	K	No	0.35	0.22	0.71
	K	Yes	0.33	0.25	0.77
Sulphur	S	No	0.06	0.04	0.09
	S	Yes	0.06	0.04	0.10
Magnesium	Mg	No	0.08	0.13	0.09
	Mg	Yes	0.09	0.14	0.10
Calcium	Ca	No	0.16	0.26	0.22
	Ca	Yes	0.17	0.26	0.24
Manganese	Mn	No	0.69	0.94	1.09
	Mn	Yes	0.56	0.72	0.84
Boron	B	No	0.67	1.10	0.43
	B	Yes	1.07	1.24	0.86
Silicon	Si	No	17.38	24.98	20.31
	Si	Yes	20.58	20.90	19.82
Iron	Fe	No	2.00	2.21	2.40
	Fe	Yes	3.24	2.34	3.06
Aluminium	Al	No	2.46	2.12	1.91
	Al	Yes	6.21	2.04	1.35
Chlorine	Cl	No	0.16	0.03	0.03
	Cl	Yes	0.04	0.03	0.04
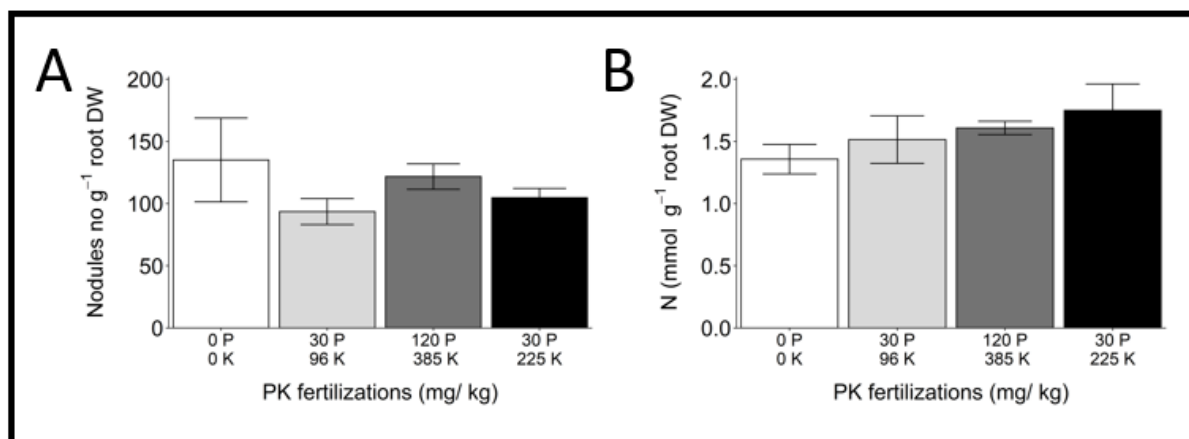
Figure S2: Nodules numbers (A) and concentrations of nitrogen (N) in roots of differently fertilized soybeans (B). The plants were fertilized with 0 mg phosphorus (P)/ 0 mg potassium (K) kg^{-1} nutrient-poor substrate, 30 mg P/ 96 mg K and 120 mg P/ 385 mg K kg^{-1} nutrient-poor substrate from combusted bagasse pellets (cBP) ash, and 30 mg P/ 225 mg K from triple-superphosphate and potassium sulphate. The error bars indicate standard errors of the means. The differences were not statistically significant (p ≤0.65, n = 5).

Table S6: ANOVAs to measurements conducted in experiment 1 (2.3.3.1 Effect of bagasse ash on rooting media). Measured traits were water holding capacity (WHC), water infiltration time (WIT), relative water retention capacity (WRC) after incubation at room temperature or 60 °C, pH levels measured in 0.01 M $CaCl_2$ (1:2.5, w/v) and electric conductivity (EC). Included factors for ANOVAs were the dosage effects of BA_{SP} ash in sand, Oxisol soil and nutrient-poor substrate, respectively.

		ANOVAs Pr (>F)		
Traits	Factors	Sand	Oxisol soil	Nutrient-poor substrate
WHC	Dosages	3.67e-06 ***	9.69e-05 ***	0.83 n.s.
WIT	Dosage	1.18e-08 ***	0.313 n.s.	0.142 n.s.
WRC	Dosage	3.7e-06 ***	0.71 n.s.	0.911 n.s.
pH	Dosage	<2e-16 ***	1.86e-10 ***	0.631 n.s.
EC	Dosage	<2e-16 ***	9.66e-06 ***	0.802 n.s.

Table S7: ANOVAs to measurements conducted in experiment 2 (2.3.4.1 Germination-Elongation test). Measured traits were germination rate (GR), radicle elongation (RE) and germination index (GI) of garden cress seedlings. Included factors for ANOVAs were the treatment effects, i.e. applications of no ash, BA_{SP} and BA_G ashes, dosage effects, i.e. application of 0 - 22.5 mg ash ml^{-1} ultra-pure water, and their interactions.

Traits	Factors	ANOVAs Pr (>F)
GR	Treatments (T)	0.0905 n.s.
	Dosages (D)	0.2992 n.s.
	T x D	0.9649 n.s.
RE	Treatments (T)	9.47e-08 ***
	Dosages (D)	0.000112 ***
	T x D	0.263978 n.s.
GI	Treatments (T)	0.147 n.s.
	Dosages (D)	0.996 n.s.
	T x D	0.917 n.s.

Table S8: ANOVAs to measurements conducted in experiment 2 (2.3.4.2 Root response to bagasse ash as fertilizer). Measured traits were root length fractions (RLF) in fertilizer depot areas determined 13 and 21 days (d) after fertilizer application (AFA), total dry matter (TDM) and root mass fractions (RMF). Included factor for ANOVA was the fertilizer treatment effect, i.e. no P supply, and application of cBP ash and triple-superphosphate as P fertilizer in depots, respectively .

Traits	Factors	ANOVAs Pr (>F)
$RLF_{13\ d\ AFA}$ in depot area	Treatments	0.207 n.s.
$RLF_{21\ d\ AFA}$ in depot area	Treatments	0.194 n.s.
TDM	Treatments	0.933 n.s.
RMF	Treatments	0.973 n.s.

Table S9: ANOVAs to measurements conducted in experiment 3 (2.3.5.1 Bagasse ash as fertilizer for soybeans, dose-response). Measured traits were leaf area estimations, which were based on the measurements with "ScreenHouse" platform every week, total dry matters (TDM) from shoot and root organs, root mass fractions (RMF), phosphorus (P) and potassium (K) uptakes, specific nodule numbers and nitrogen concentrations in nodulated roots. ANOVAs for TDM and RMF included treatment effects, i.e. applications of six dosages of BA_{NG1} ash (0 to 31.6 g kg^{-1} nutrient-poor substrate) as P and K fertilizer for soybeans, and fertilizations with triple-superphosphate (TSP) and potassium sulphate (K_2SO_4) and no fertilization as positive and negative control treatments, respectively. ANOVAs for P and K uptakes, specific nodule numbers and nitrogen concentrations were performed only with fertilization treatments receiving 0 mg P/ 0 mg K, 30 mg P/ 96 mg K and 120 mg P/ 385 mg K kg^{-1} nutrient-poor substrate from BA_{NG1} ash and 30 mg P/ 225 mg K kg^{-1} nutrient-poor substrate from TSP and K_2SO_4. ANOVA for estimated leaf areas included treatment and measuring day effects and their interaction.

Traits	Factors	ANOVAs Pr (>F)
Estimated leaf areas	Treatments (T)	7.02e-15 ***
	Measuring days (MD)	< 2e-16 ***
	T x MD	3.27e-12 ***
TDM	Treatments	9,46 e-11 ***
RMF	Treatments	0,000937 ***
P uptakes	Treatments	7,28e-14 ***
K uptakes	Treatments	3.36e-15 ***
Specific nodule numbers	Treatments	0.437 n.s.
Nitrogen conc. in nodulated roots	Treatments	0.375 n.s.

Table S10: ANOVAs to measurements conducted in experiment 4 (2.3.5.2 Bagasse ash from controlled combustion as fertilizer for soybeans). Measured traits were leaf area estimations, which were based on the measurements with "ScreenHouse" platform every week, total dry matters (TDM) from shoot and root organs, root mass fractions (RMF), phosphorus (P) and potassium (K) uptakes. ANOVAs for TDM, RMF, P and K uptakes included fertilizer treatment effects, i.e. applications of 0 mg P/ 0 mg K, 30 mg P/ 161 mg K and 72 mg P/ 387 mg K kg^{-1} nutrient-poor substrate from cBP ash, and fertilizations with 30 mg P/ 387 mg K kg^{-1} nutrient-poor substrate from triple-superphosphate (TSP) and potassium sulphate (K_2SO_4). ANOVA for estimated leaf areas included fertilizer treatment and measuring day effects and their interaction.

Traits	Factors	ANOVAs Pr (>F)
Estimated leaf area	Treatments (T)	<2e-16 ***
	Measuring days (MD)	<2e-16 ***
	T x MD	<2e-16 ***
TDM	Treatments	7.38e-07 ***
RMF	Treatments	0.0612 n.s.
P uptake	Treatments	5.29e-11 ***
K uptake	Treatments	3.54e-16 ***

Table S11: ANOVAs to measurements conducted in experiment 5 (2.3.5.3 effect of fertigation on nutrient uptake from bagasse ash). Measured traits were the concentration of minerals in the biomass of soybeans receiving nutrients from 0, 31.3 and 73.3 g cBP ash kg^{-1} nutrient-poor substrate and conventional fertilizer forms, i.e. triple-superphosphate (TSP) and potassium sulphate (K_2SO_4). One half of replicates was fertigated with all nutrients but no P every week. ANOVAs included fertilizer treatment and fertigation effects and their interaction.

| Traits | Abbrev. | Factors | ANOVAs Pr ($>$F) | | |
			Treatment (T)	Fertigation (F)	T x F
Nitrogen	N	Treatments	4.77e-08 ***	0.216 n.s.	0.442 n.s.
Phosphorus	P	Treatments	2.42e-11 ***	0.869 n.s.	0.995 n.s.
Potassium	K	Treatments	1.45e-11 ***	0.143 n.s.	0.243 n.s.
Sulphur	S	Treatments	2.69e-10 ***	0.0826 n.s.	0.2609 n.s.
Calcium	Ca	Treatments	3.14e-06 ***	0.199 n.s.	0.621 n.s.
Magnesium	Mg	Treatments	4.46e-08 ***	0.019 *	0.453 n.s.
Boron	B	Treatments	5.96e-05 ***	0.000203 ***	0.152218 n.s.
Zinc	Zn	Treatments	1.38e-05 ***	0.000146 ***	0.021126 *
Manganese	Mn	Treatments	0.000601 ***	0.002274 **	0.596994 n.s.
Aluminium	Al	Treatments	0.280 n.s.	0.476 n.s.	0.418 n.s.
Iron	Fe	Treatments	0.769 n.s.	0.223 n.s.	0.702 n.s.
Sodium	Na	Treatments	0.000271 ***	0.006167 **	0.007968 **
Chlorine	Cl	Treatments	0.337 n.s.	0.366 n.s.	0.370 n.s.
Silicon	Si	Treatments	0.170 n.s.	0.788 n.s.	0.236 n.s.

Table S12: ANOVAs to measurements conducted in experiment 6 (2.3.5.4 Effect of co-processing bagasse pellets with chicken manure on availability of P from the ashes to soybeans). Measured traits were leaf areas estimations, which were based on the measurements with "Screen-House" platform every week, total dry matters (TDM) from shoot and root organs, root mass fractions (RMF) after 42 d growth, phosphorus (P) uptakes from beginning of fertilizer application to final harvest, i.e. 0 to 42 days (d) after fertilizer application (AFA), P uptake dynamics, i.e. P uptake from beginning of fertilizer application to 27 d AFA and from 27 d AFA to 42 d AFA. ANOVAs for TDM, RMF and P uptakes included fertilizer treatment effects, i.e. application of gBP, gBP/ $CM_{85:15}$, cBP/ $CM_{80:20}$ ashes as P fertilizer and no P supply as negative control treatment. ANOVA for P uptake dynamics included treatment and harvesting date effects and their interaction. ANOVA for estimated leaf areas included fertilizer treatment and measuring day effects and their interaction.

Traits	Factors	ANOVAs Pr ($>$F)
Estimated leaf area	Treatments (T)	1.57e-10 ***
	Measuring days (MD)	$<$ 2e-16 ***
	T x MD	2.34e-12 ***
TDM	Treatments	7.18e-08 ***
RMF	Treatments	8.91e-06 ***
P uptake	Treatments	2.22e-11 ***
P uptake dynamics	Treatments (T)	5.57e-16 ***
	Harvests (H)	7.81e-05 ***
	T x H	0.00558 **

Table S13: ANOVAs to measurements conducted in experiment 7 (2.3.6.1 Effect of co-combustion bagasse with nutrient rich residues on the availability of P from bagasse-based ashes to soybeans). Measured traits were leaf areas estimations, which were based on the measurements with "ScreenHouse" platform every week, total dry matters (TDM) from shoot and root organs, root mass fractions (RMF) and P uptakes. ANOVAs for TDM, RMF and P uptakes included fertilizer treatment effects, i.e. P from no P control treatment and cBP, cBP/ $SSL_{83:17}$ and cBP/ $CM_{80:20}$ ashes. ANOVA for estimated leaf areas included fertilizer treatment and measuring day effects and their interaction.

Traits	Factors	ANOVAs Pr (>F)
Estimated leaf area	Treatments (T)	<2e-16 ***
	Measuring days (MD)	<2e-16 ***
	T x MD	<2e-16 ***
TDM	Treatments	1.33e-10 ***
RMF	Treatments	0.00524 **
P uptake	Treatments	5.19e-11 ***

Table S14: ANOVAs to measurements conducted in experiment 8 (2.3.6.2 Availability of P from bagasse/ chicken manure ashes and its thermochemical products for soybeans compared to P from TSP, dose-response). Measured traits were total dry matters (TDM) from shoot and root organs, root mass fractions (RMF) and P uptakes. ANOVAs for TDM and RMF included fertilizer treatment effects, i.e. no P application, and P supply by cBP/ $CM_{80:20}$, cBP/ $CM_{60:40}$ ashes, cBP/ $CM_{60:40}$+Na and cBP/ $CM_{60:40}$+Na/K thermochemical products, and triple-superphosphate (TSP), P-dosage effects, i.e. application of five dosages of P from 0 to 340 mg kg^{-1} Oxisol soil, and their interaction. ANOVA for P uptake included only the fertilizer treatment effects, which included fertilizations with 0 and 340 mg P kg^{-1} Oxisol soil from cBP/ $CM_{80:20}$, cBP/ $CM_{60:40}$ ashes, cBP/ $CM_{60:40}$+Na and cBP/ $CM_{60:40}$+Na/K thermochemical products and TSP.

Traits	Factors	ANOVAs Pr (>F)
TDM	Treatments (T)	< 2e-16 ***
	Dosages (D)	1.72e-09 ***
	T x D	0.0351 *
RMF	Treatments (T)	< 2e-16 ***
	Dosages (D)	1.5e-05 ***
	T x D	0.384 n.s.
P uptake	Treatments (T)	8.23e-09 ***

Table S15: ANOVAs to measurements conducted in experiment 9 (2.3.6.3 Bagasse ash as an additive of conventional P-fertilizer). Measured traits were leaf areas estimations, which were based on the measurements with "ScreenHouse" platform every week, total dry matters (TDM) from shoot and root organs, root mass fractions (RMF), uptakes of P and total P in Oxisol soil after 41 d growth. ANOVAs for TDM, RMF, P uptake and soil P included treatment effects, i.e. no P fertilizer application, and supply of P by triple-superphosphate (TSP) and its blend with cBP ash. ANOVA for estimated leaf areas included fertilizer treatment and measuring day effects and their interaction.

Traits	Factors	ANOVAs Pr ($>$F)
Estimated leaf areas	Treatments (T)	$<$ 2e-16 ***
	Measuring days (MD)	$<$ 2e-16 ***
	T x MD	9.73e-14 ***
TDM	Treatments	3.59e-06 ***
RMF	Treatments	0.935 n.s.
P uptake	Treatments	7.95e-06 ***
Soil P	Treatments	0.148 n.s.

Table S16: ANOVAs to measurements conducted in experiment 10 (2.4 Soybean field experiment in Goiânia, Brazil). Measured traits were grain yield, P uptake, dry weights of 1000 grains, pH levels 14, 65 and 119 days (d) after fertilizer application (AFA), and P extractable in Mehlich-I solution 14, 65 and 119 d AFA. ANOVAs for grain yields and dry weights of 1000 grains included fertilizer treatment effects, i.e. fertilizations with 40, 80 and 160 kg P_2O_5 ha^{-1} from BA_{NG2} ash and its blends with cattle manure compost (CMC), 80 kg P_2O_5 ha^{-1} from CMC, 40 kg P_2O_5 ha^{-1} from triple-superphosphate (TSP), and 32, 65 and 130 kg P_2O_5 ha^{-1} from the blends of TSP with BA_{NG2} ash. ANOVA for P uptakes included fertilizer treatments with 40, 80, 160 kg P_2O_5 ha^{-1} from BA_{NG2} ash, 40 kg P_2O_5 ha^{-1} from TSP, 65 kg P_2O_5 ha^{-1} from the blend of TSP with BA_{NG2} ash, 80 kg P_2O_5 ha^{-1} from CMC and its blend with BA_{NG2} ash. ANOVAs for pH levels and P extractable in Mehlich-I solution 14, 65 and 119 d AFA included all the same fertilizer treatments as described for P uptake analyses but no 40 kg P_2O_5 ha^{-1} from BA_{NG2} ash.

Traits	Factors	ANOVAs Pr ($>$F)
Grain yields	Treatments	1.86e-11 ***
P uptakes	Treatments	6.29e-12 ***
1000 grain dry weight	Treatments	0.749 n.s.
pH 14 d AFA	Treatments	0.261 n.s.
pH 65 d AFA	Treatments	0.466 n.s.
pH 119 d AFA	Treatments	0.926 n.s.
pH 14 d AFA	Treatments	0.638 n.s.
pH 65 d AFA	Treatments	0.433 n.s.
pH 119 d AFA	Treatments	0.549 n.s.

Table S17: ANOVAs to measurements conducted in experiment 11 (2.3.6.4 Bagasse ash-based fertilizer product for soybeans). Measured traits were total dry matters (TDM) from shoot and root organs and root mass fractions (RMF). ANOVAs included fertilizer treatment effects, i.e. no fertilizer, granulated and pelletized fertilizer products containing bagasse ash and chicken manure in ratios of 90:10 by dry weight.

Traits	Factors	ANOVAs Pr ($>$F)
TDM	Treatments	2.79e-06 ***
RMF	Treatments	0.229 n.s.

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C Publications

Research paper
Herzel H*,†, Dombinov V*,†, Vogel C, Willbold S, Levandowski GV, Meiller M, Müller F, Zang JW, da Fonseca-Zang WA, Jablonowski ND, Schrey SD, Adam C. Soybean Fertilized by P-Phases from Bagasse-Based Materials: P-Extraction Procedures, Diffusive Gradients in Thin Films (DGT), and X-ray Diffraction Analysis (XRD). Agronomy 2020;10(6):895. [10.3390/agronomy10060895] (* corresponding authors, † both authors contributed equally)

Aimed publications based on presented data
Dombinov V, Meiller M, Müller F, Willbold S, Herzel H, Zang JW, da Fonseca-Zang WA, Poorter H, Watt M, Jablonowski ND, Schrey SD. Sugarcane bagasse ash as fertilizer for soybeans and the effects of co-processing bagasse with nutrient rich residues on the plant availability of phosphorus. Science of the Total Environment (in preparation)

Dombinov V, Zang JW, da Fonseca-Zang WA, Herzel H, Meiller M, Müller F, Poorter H, Watt M, Jablonowski ND, Schrey SD. Bagasse-based thermophosphates as fertilizer for sugarcane and soybeans in Oxisol soil. Biomass and Bioenergy (in preparation)

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Proceeding articles
Stahl E, Meiller M, Danz P, Dombinov V, Herzel H. Recycling of nutrients from residues of thermo-chemical processing of sugarcane bagasse and straw - handling of produced fertilizers based on biomass ash. VGB Power Tech 2018; 10: 56–59. ISSN: 1435-3199.

D Conference contributions

Oral presentations
Dombinov V. Breaking the wall of wasting valuable ash. Falling walls lab 2016 Jülich. Jülich, Germany.

Dombinov V, Zang JW, Leandro WM, Poorter H, Jablonowski ND, Schrey SD. Phosphorus availability from bagasse ash to soybeans and the effect of additional fertigation. Biomass and Bioenergy Congress 2018. Sorocaba, Brazil.

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Dombinov V, Schrey SD, Poorter H, Watt M, Jablonowski ND. Sugarcane bagasse ash as alternative nutrient source for plant nutrition. 8th International Phosphorus Workshop (IPW8) 2016. Rostock, Germany.

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