Biogas residues: Elemental composition, effects on organic matter mineralisation and P dynamics in soil

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Summary

The recently growing biogas sector generates large amounts of by-products, the digestates, which should be applied to arable land in order to recycle valuable nutrients. However, knowledge about the composition of digestates and their fate in soils, especially under root-caused alkaline conditions, is not sufficient so far.

Thus, the aims of this thesis were (1) to examine the elemental composition of different digestates (from sewage sludge, slurry or maize), especially the temporal variability and alteration during fermentation, (2) to investigate the effects of digestates on soil organic matter (SOM) under artificial rhizosphere conditions and (3) to test whether the anion exchange of a model root would increase P availability in a digestate amended soil.

These objectives were pursued by a one-year analysis of digestates and their feedstock with subsequent element balancing and by incubation experiments with digestates and soil including anion exchange resins as root models.

Digestates showed a great variability depending on their feedstock but also over time. For an element balancing from feedstock to digestate, two, five or ten sampling dates depending on the particular digestate were needed for reliable results. During fermentation, depletions of nitrogen (N), sulphur (S), magnesium (Mg), zinc (Zn) and cadmium (Cd) were determined, probably due to volatilisation or precipitation, while attrition of stirring devices may have resulted in iron (Fe) and manganese (Mn) accumulations. The mineralisation of digestates in soil was increased by resins (the root models) releasing hydrogen carbonate (HCO_3^-). Ambivalent mineralisation dynamics were observed for digestates from the same biogas

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plants, but sampled at different times from these plants. Indications for priming, i.e. an extra mineralisation of SOM due to the addition of OM to soil, occurred for digestates from sewage sludge and maize, but not for each biogas plant sampling date. Presumably, digestate stability after fermentation and thus their potential to promote priming in soils vary over time, as well. The release of HCO_3^- by the resins in exchange for anions from the digestate amended soils resulted in an increase of pH and P mineralisation. Consequently, a peak of P concentration moving away from the resin with time occurred.

In conclusion, the heterogeneous composition and stability of digestates over time impede simple predictions of their fate in soils and thus decisions about their use as fertilisers. Moreover, models for P transport to roots should account for additional supply of P by OM mineralisation.

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Zusammenfassung

Die Biogasbranche ist in den letzten Jahren stark expandiert und produziert große Mengen von Gärresten, die zur Schließung von Nährstoffkreisläufen auf landwirtschaftliche Flächen ausgebracht werden sollten. Bislang sind die Kenntnisse über die Zusammensetzung der Gärreste und ihre Wirkung im Boden, vor allem in wurzelbedingt alkalischem Milieu, jedoch nicht ausreichend.

Die Ziele dieser Arbeit waren daher (1) die Bestimmung der Elementzusammensetzung verschiedener Gärreste (aus Klärschlamm, Gülle oder Mais), insbesondere die zeitliche Variabilität and Veränderung während der Fermentation, (2) die Untersuchung der Wirkung von Gärresten auf die organische Bodensubstanz (OBS) unter künstlichen wurzelnahen Bedingungen und (3) die Prüfung, ob der Anionenaustausch durch eine Modellwurzel die P-Verfügbarkeit in einem mit Gärrest gedüngten Boden erhöht.

Zur Erreichung dieser Ziele wurden Elementanalysen von Gärresten und ihrem Ausgangsmaterial über ein Jahr zusammen mit einer anschließenden Elementbilanzierung vorgenommen, sowie Inkubationsexperimente mit Gärresten, Böden und Anionenaustauscherharzen als Modellwurzeln durchgeführt.

Die Gärreste wiesen eine große Variabilität in Abhängigkeit von ihrem Ausgangsmaterial, aber auch über die Zeit auf. Für eine Elementbilanzierung vom Ausgangsmaterial zum Gärrest sind je nach Gärrest zwei, fünf oder zehn Beprobungstermine erforderlich, um verlässliche Aussagen treffen zu können. Die Bilanzierung ergab eine Abreicherung von Stickstoff (N), Schwefel (S), Magnesium (Mg), Zink (Zn) und Cadmium (Cd) während der

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Vergärung, vermutlich durch Ausgasung bzw. Ausfällung, während der Abrieb von Rührwerksvorrichtungen zur Anreicherung von Eisen (Fe) und Mangan (Mn) geführt haben kann. Die Mineralisierung der Gärreste im Boden wurde durch die Austauscherharze (die Modellwurzeln), welche Hydrogencarbonat (HCO_3^-) freisetzten, verstärkt. Gärreste der gleichen Biogasanlage, aber von verschiedenen Beprobungsterminen, zeigten widersprüchliche Mineralisierungsdynamiken. Für die Gärreste aus Klärschlamm und aus Mais wurden Hinweise auf Priming, eine zusätzliche Mineralisierung der OBS aufgrund der Zugabe von organischem Material zum Boden, beobachtet, nicht jedoch für jeden Termin der Gärrestbeprobung von den Biogasanlagen. Vermutlich variieren die Stabilität der Gärreste und somit ihr Potential, Priming im Boden zu begünstigen, ebenfalls über die Zeit. Die Abgabe von HCO_3^- durch die Harze im Austausch für Anionen der mit Gärresten gedüngten Böden führten zu einem Anstieg des pH-Wertes und der P-Mineralisierung. Infolgedessen entstand ein P-Konzentrations-Maximum, dessen Entfernung vom Harz mit der Zeit anstieg.

Die Ergebnisse verdeutlichen, dass die zeitliche Heterogenität der Zusammensetzung und Stabilität der Gärreste einfache Vorhersagen über ihre Wirkung in Böden und somit Entscheidungen über ihre Verwendung als Dünger erschweren. Zudem sollten Modelle über den P-Transport zu Wurzeln die P-Nachlieferung durch Abbau organischer Substanz berücksichtigen.

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1 General Introduction

1.1 Background and rationale

Global warming as a consequence of anthropogenic activities is controversially discussed for decades. However, the majority of scientists support the hypothesis of human-made climate change (Oreskes 2004), being mainly caused by the emission of industrial and agricultural greenhouse gases (GHG). Indeed, the temperature of the earth has increased by about 0.6 °C over the last 100 years, and one of the main warming periods – from 1976 to now – has a warming rate greater than at any other time during the last 1,000 years (Walther 2002). The ecological impacts of global warming are manifold and include draughts, climatic extremes, rising sea levels and many more.

In order to counteract these undesirable developments, great effort has been made by scientists and politicians towards a reduction of GHG emissions. The International Panel on Climate Change (IPCC 2014) identified the energy supply sector to be the largest contributor to global GHG emissions and points to the production of renewable energy as one option to mitigate undesirable carbon dioxide (CO_2) emissions. Renewable energy is also required due to a growing energy demand in the world and the fact that most of the fossil fuel reserves are exhaustible and concentrated in politically unstable regions (Weiland 2010). Thus, the European energy and climate policy aims to cover 20 % of the energy demand by renewable energy until 2020 (FNR 2012). The German Renewable Energy Sources Act (Erneuerbare-Energien-Gesetz, EEG) from 2000 and its amendments in the following years often serve as a benchmark for other European countries which also expand the production of renewable energy (Emmann 2013). The act governs the preferential feed-in of energy from renewable

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sources and guarantees constant tariffs for producers over a period of 20 years.

The production of biogas is one of the ways to gain renewable energy. While about 1,600 biogas plants existed in Germany in 2002, the number has increased to more than 7,000 in the year 2011 (FNR 2012). The installed electrical power of biogas plants in Germany was 2,904 MW in 2011 (ibid.). About 18 % of renewable energy used for the production of electricity was delivered by biogas in 2013 (Musiol et al. 2014, Figure 1.1).

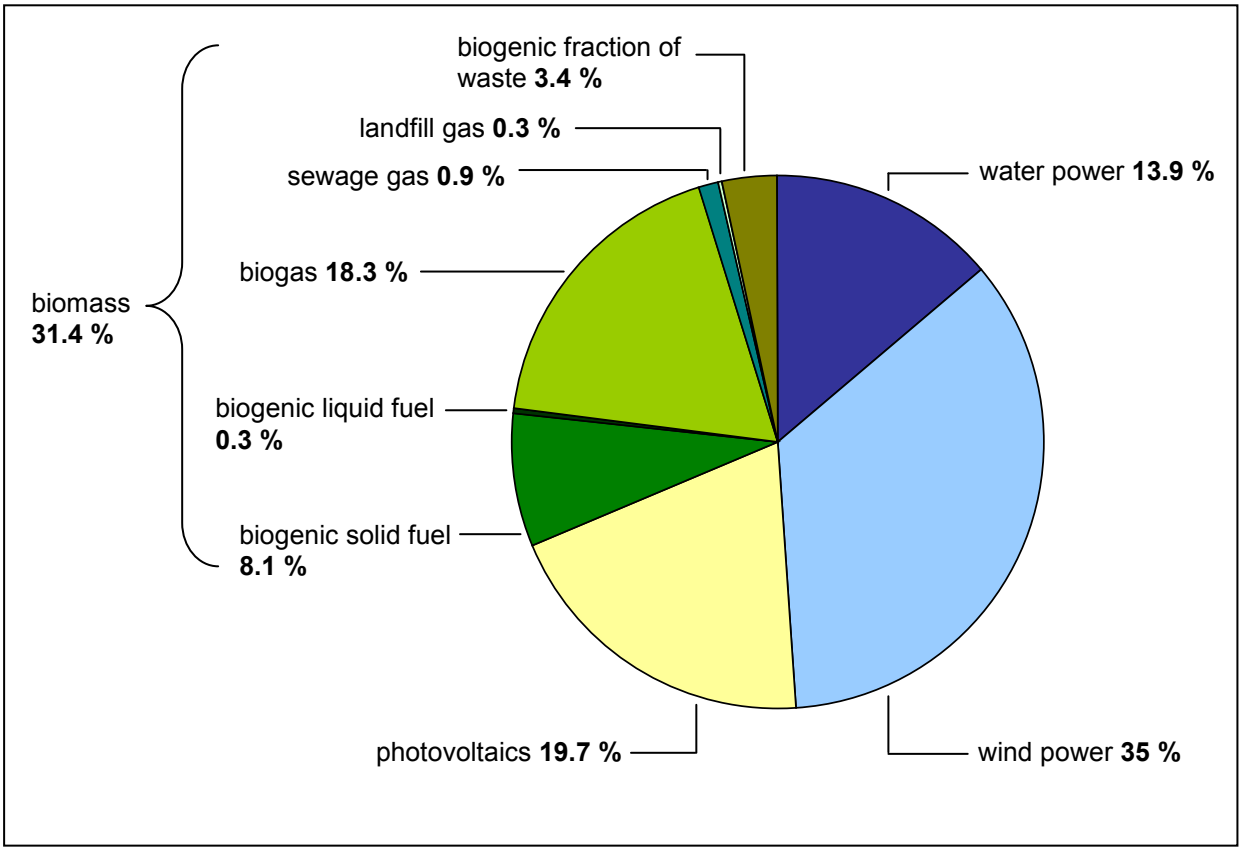

Figure 1.1: Renewable energy for the production of electricity in Germany, 2013 (according to Musiol et al. 2014).

During the production of biogas, an organic by-product – the digestate – incurs in great quantities (Grigatti 2011). These digestates contain large amounts of valuable nutrients (Galvez 2012, Garfi 2011). The nutrients can be properly recycled when digestates are

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applied to arable land as a fertiliser. In order to ensure an environmentally harmless fertilising, the composition of digestates and their fate in soils should be known. The anaerobic digestion process alters the properties of the biomass, and thus the characteristics of digestates cannot simply be concluded from the feedstock material. The degradation of organic matter (OM) leads to a mineralisation of nutrients, such as nitrogen, for example. Hence, the availability of these nutrients is increased during anaerobic digestion. Moreover, the mineralisation kinetics of digestates in soils and the availability of nutrients provided by the digestates play an important role for an effective and sustainable use of digestates as fertilisers in agriculture. Many of these issues are not or not sufficiently investigated so far and research in this area is needed.

1.2 Biogas production

1.2.1 Feedstock

A wide range of biomass can be used for the production of biogas (Boulamanti 2013). The largest resource is delivered by animal husbandry in the form of slurry from pig and cattle production units, as well as manure from poultry, horse and other livestock farming (Holm-Nielsen 2009). Many farmers conduct a biogas plant on their own farm since the anaerobic digestion of animal slurries does not only generate renewable energy, which can be sold or used for own requirements, but can also minimise odours and reduce pathogens (Salminen 2001).

Another important and increasing source of biomass for anaerobic digestion is given by energy crops, such as maize, grain and grass crops. The EEG amendment of 2004 introduced a financial incentive for the production of energy crops (Emmann 2013). This

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resulted in a strong expansion of energy crop cultivation and biogas plants on cropping farms (ibid.). In Germany, about 900,000 ha were cultivated with energy crops in 2011 and more than 70 % of this area was cropped with energy maize (ibid.). Consequently, a “food or fuel discussion” arose dealing with the competition of food and energy crops for agricultural space. Additionally, the promotion of energy crop production resulted in land-use changes and maize monocultures which may have negative ecological effects, such as soil erosions and the loss of biodiversity (ibid.).

Therefore, the fermentation of organic residues like animal slurries seems preferable. Other possible residues for anaerobic digestion are, for example, cuttings from landscape management, food residues, organic fractions of municipal solid wastes, industrial wastes or sewage sludge. All feedstocks can be digested solely or co-fermented with each other. Frequently, co-digestion of different feedstocks is used to gain higher methane yields and thus increase the efficiency of biogas plants (Álvarez 2010, Weiland 2010, Holm-Nielsen 2009). However, an application of co-digested biomass to agricultural land is not always advisable and allowed. In case digestates contain large amounts of contaminated fractions, such as sewage sludge or industrial residues, legal threshold values may inhibit a use as fertiliser. Figure 1.2 shows possible ways of biomass used for anaerobic digestion. Crops can serve directly for anaerobic digestion as energy crops but can also serve as fodder for animals whose excreta again can be used for anaerobic digestion. The resulting digestates can be re-used as fertilisers in agriculture. When crops are used as food for humans or for industrial purposes, the resulting waste and sludge can also be used for fermentation but should carefully be examined before re-use as fertiliser.

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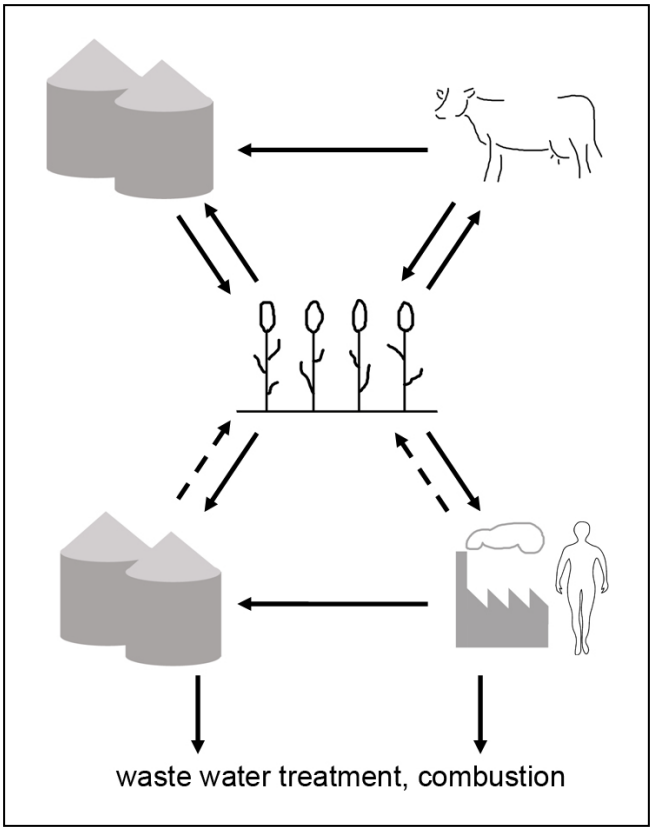

Figure 1.2: Simplified scheme of possible ways of biomass used for anaerobic digestion (dashed arrows show ways that are possibly inhibited by law).

1.2.2 Anaerobic digestion process

Biogas can be gained by dry and wet fermentation. Wet fermentation is conducted with a content of total solids of less than 10 % and is the dominating form of anaerobic digestion in the agricultural sector (Weiland 2010). Nearly 90 % of the modern biogas plants in Germany use vertical continuously stirred tanks for wet fermentation (ibid.). Mostly, anaerobic digestion is conducted at temperatures between 25 and 40 °C (mesophilic) or between 40 and 60 °C (thermophilic). The psychrophilic stage (<25 °C) is rather rarely used and requires a longer retention time of the organic matter. The anaerobic digestion proceeds in four main steps: hydrolysis, acidogenesis, acetogenesis and methanation (Figure 1.3, Gujer and Zehnder 1983).

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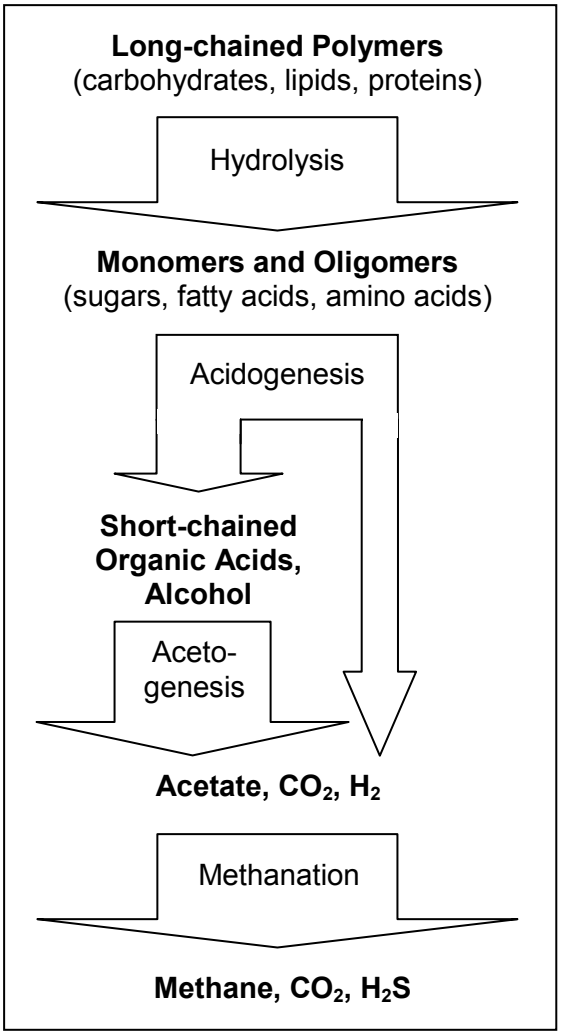

Figure 1.3: Simplified process of anaerobic digestion (according to Gujer and Zehnder 1983).

In a first step, microorganisms break down complex polymers to low-molecular compounds by excretion of various hydrolytic enzymes which attach water to the polymer compounds. Polymeric carbohydrates are mainly decomposed to monosaccharides, e.g. glucose. Proteins are degraded to amino acids and peptides while lipids are degraded to long-chained fatty acids and glycerine. Second, the resulting products are converted to low-molecular organic acids and alcohol. Additionally, acetate, hydrogen and carbon dioxide are generated in the acidogenesis phase and form initial products for the methanation. Hydrolysis and acidogenesis can be conducted separately from the following phases (two-stage fermentation) or together with acetogenesis and methanation in one fermenter (single-stage

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fermentation). During the acetogenesis, organic acids and alcohols are also transformed to acetic acid, hydrogen and carbon dioxide. The increasing hydrogen partial pressure inhibits the metabolism of acetogenic bacteria and is essentially needed for the last step, the methanation (Weiland 2010). Acetate, hydrogen and carbon dioxide are finally used by methanogenic bacteria to produce methane. Methanogenics are strictly anaerobic and require a low redox potential and nearly neutral to slightly alkaline pH conditions (ibid.).

The anaerobic digestion process modifies properties of the feedstock. Dry matter and C content, for instance, are reduced due to the production of methane (Möller and Müller 2012). This leads to a relative concentration of mineral nutrients and heavy metals in the digestates. Additionally, process failures can contribute to different degrees of alteration. Substrate overloading, for example, promotes an accelerated hydrolysis and acidogenesis. This is accompanied by a decreasing pH which inhibits methanogenic bacteria (Weiland 2010). Thus, the production of biogas decreases and the relative concentration of elements other than C only partly occurs. Process failures can also include excessive foam formation (Moeller et al. 2012) as well as floating layers, sedimentations and stagnant zones due to insufficient or disturbed stirring (Lienen et al. 2013). Technical incidents, such as damages of the stirrer or pump apparatus, may also facilitate an accumulation of heavy metals in the digested material (Trzcinski and Stuckey 2011).

1.2.3 Open questions and objective 1 of this thesis

As described in Chapter 1.1, the composition of digestates should be known before their use as fertilisers in agriculture. Chapter 1.2.2 has shown that anaerobic digestion alters the properties of digestates and that knowledge about the characteristics of the *feedstock* material is not sufficient for the assessment of the suitability of digestates as fertilisers. The

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characteristics of the digestates *after* the anaerobic digestion process have to be known. However, data about chemical characteristics of digestates – especially about the elemental composition of digestates – are scarce. The few existing data mainly refer to contents of nitrogen (N), phosphorus (P) and potassium (K). Therefore, the first objective of this thesis was to contribute to the data base about the elemental composition of digestates. Chapter 2 deals with the elementary analysis of different digestates from four full-scale biogas plants in Germany and the temporal variability of the elemental composition of these digestates. Moreover, the differences in the elemental composition of digestates from varying feedstock are investigated and a statistical method to identify element losses or accumulations during anaerobic digestion without knowledge about the mass of produced biogas is developed.

1.3 Digestates as fertilisers

1.3.1 Digestate mineralisation in soils

One aspect deciding, whether an organic substrate is suitable as fertiliser, is its effect on the soil humus balance. An adequate humus supply is essential for satisfying plant growth, because humus delivers necessary nutrients, improves soil structure and increases water storage capacity. Since anaerobic digestion alters the C content and availability of digestates, their contribution to soil humus had to be investigated. Some authors conducted incubation experiments with different digestates and soil and measured the emission of CO_2 as an indicator for the degradability of the digestates in soils. A low degradability may strongly improve soil structure, but leads to only low amounts of released nutrients. A high degradability improves nutrient availability, but leads to a fast depletion of the organic matter. Moreover, the risk of nutrient leaching increases when nutrient supply exceeds nutrient uptake by plants. Chen et al. (2012) compared CO_2 emissions from soil amended with maize straw on the one hand and digested maize on the other hand in a 21-day incubation

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experiment at 19 °C. The authors subtracted CO_2 emissions of the control (unamended soil) and found 30 % of the added C mineralised by the maize straw amended soil while only about 6 % of the added C was mineralised by the digestate amended soil. Likewise, Galvez et al. (2012) observed, that 5-7 % of the added C by digested pig slurry was mineralised after 30 days at 20 °C. They incubated a variety of different organic fertilisers with an alkaline and a slightly acidic soil. The mineralisation of the digestate ranged in the middle of the mineralisation of fertilisers like composts, sewage sludge, biochar, rapeseed meal or bioethanol residues. Marcato et al. (2009) studied the influence of anaerobic digestion on the quality of organic matter and incubated raw and digested pig slurry with soil at 28 °C. After 49 days, 18 % of added C by raw slurry and 12 % of added C by digested slurry had been mineralised. They also found relatively less NDS (neutral detergent soluble) substances in the digestate and more hemicellulose- and lignin-like compounds than in raw slurry which indicates, that the anaerobic digestion stabilised the OM of the slurry and leads to reduced mineralisation in soil and reduced CO_2 emissions. Ambivalent results are reported by Cayuela et al. (2010), who observed, that digested cattle manure did not show higher recalcitrance than undigested cattle manure after 60 days of incubation with soil at 20 °C, but the anaerobic digestion of pig slurry could reduce the amount of mineralised C in soil from 58 % to 40 % of added C. The liquid and solid fractions of digested dedicated crops were incubated with soil at 25 °C for 66 days by Grigatti et al. (2011). Although, the amount of emitted CO_2 by the solid fraction amended soil was about four times the amount of CO_2 emitted by the liquid fraction amended soil, the proportion of emitted C related to added C was only 15 % for the solid fraction while it was 60 % for the liquid fraction. The authors assume that decaying microbial biomass from the anaerobic digestion process promotes microbial growth and thus respiration in soil. High proportions of emitted C related to added C by digestates after subtraction of emitted C by the control have also been found by Alburquerque et al. (2012a). The authors incubated six digestates (pig or cattle slurry as the main feedstock) with soil for 56 days at 26 °C and found proportions of 15-60 %, and even 105 % in one case. The latter means that more soil organic matter (SOM) must have been

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mineralised from the digestate amended soil than from the unamended soil (control). This phenomenon is described as priming and has already been observed by Bernal and Kirchmann (1992) for digestates from pig manure. They compared the mineralisation of undigested, digested and composted pig manure in an incubation study at 25 °C for 60 days. In accordance to Alburquerque et al. (2012a), the fraction of mineralised C related to added C by the digestate after subtraction of emitted C by the control was 105 %.

1.3.2 Priming

The term “priming” comprises different mechanisms in soil: On the one hand, it describes short-term changes in the turnover rate of SOM due to plant activities, such as root exudation and photosynthesis (Kuzyakov 2002). This mechanism is called rhizosphere priming. On the other hand, changes of SOM turnover can be promoted by treatments of the soil, such as fertilising (usually organic C, but also mineral N), drying and re-wetting or tilling (Kuzyakov et al. 2000). Positive priming means, that SOM of a treated soil is decomposed to a greater extent than SOM of an untreated control, while negative priming means, that SOM of a treated soil is decomposed to a lower extent than SOM of an untreated control.

Priming is mainly investigated with regard to C and N, but can also occur for P, S and other nutrient elements (ibid.). The literature on priming distinguishes between real and apparent priming. While real priming describes changes in SOM decomposition, apparent priming can refer to accelerated CO_2 evolution due to the activation of microbial metabolism and higher microbial biomass turnover without effects on SOM decomposition (Blagodatskaya and Kuzyakov 2008). Other reasons for apparent priming are isotopic displacement or pool substitution (Jenkinson et al. 1985). Apparent C priming due to isotopic displacement can be measured after the addition of labelled C, for example. Microorganisms replace ^{12}C of their

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own biomass with the added ^{13}C and release their ^{12}C as CO_2 (Sullivan and Hart 2013, Fontaine et al. 2011). However, apparent priming mostly occurs for N only (Kuzyakov et al. 2000). Pool substitution, for instance, means that native soil N, which would otherwise have been immobilised, denitrified or taken up by plants (like in the unamended control), is now replaced by added ^{15}N, so that soil N is measured and misinterpreted as primed N (Jenkinson et al. 1985). Experimental errors, such as mistakes in CO_2 trapping or labelling or enhanced CO_2 evolution due to more favourable soil moisture conditions, may also result in extra CO_2 release. These phenomena are called “artificial priming” (Blagodatskaya and Kuzyakov 2008).

This thesis focuses on positive C priming since it contributes to an undesirable increase of CO_2 in the atmosphere. The addition of fresh organic matter can lead to positive C priming due to different reasons. When the added material mainly consists of easily decomposable organic matter, microorganisms are stimulated and increase their population, especially r-strategists. The depolymerisation of easily decomposable compounds requires the release of extracellular enzymes, which may also affect SOM. Depending on the competition for fresh OM with r-strategists, slower growing K-strategists benefit from less decomposable substances and produce SOM decomposing enzymes, not differentiating between fresh organic matter and SOM (Fontaine et al. 2003). Thus, SOM is co-metabolised by microorganisms and priming occurs (Blagodatskaya and Kuzyakov 2008). When the added material is rich in easily available C, but poor in mineral N, microorganisms try to cover their need for N by exploiting SOM. This results in an additional release of soil C and N, i.e. in positive C and N priming. Another option to promote priming is the addition of material with less available C, but highly available N. In this case, microorganisms start decomposing SOM to gain C as substrate and energy source (Kuzyakov et al. 2000).

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Besides the quality of the added material, the quantity also affects the extent and direction of priming. Smaller amounts of added C (0-15 % of microbial biomass C) lead to short-term apparent priming, while higher amounts (>50 % of microbial biomass C) promote real priming (Blagodatskaya and Kuzyakov 2008). However, further increase of added C in relation to microbial biomass C mitigates priming and increases preferential substrate utilisation, i.e. negative priming (ibid.). Other influencing factors concern soil characteristics. Priming due to the addition of fresh OM containing a high amount of easily available C and less available N can get an extra push by a low availability of N in the soil. The need for microorganisms to exploit SOM to gain N is even higher in this case (Kuzyakov 2002). Especially K-strategists, which favour priming due to their production of SOM decomposing enzymes, take advantage of low N availability in soil over r-strategists (Fontaine et al. 2004). Furthermore, neutral or alkaline soils exhibit high microbial activity and may therefore particularly be prone to priming (Blagodatskaya and Kuzyakov 2008). The aggregate stability of the soil may affect the extent of priming because of higher or less availability of SOM (ibid.). Priming is also controlled by the presence of plants and the resulting competition for nutrients between plant roots and microorganisms (Kuzyakov 2002).

In the case of rhizosphere priming, plant roots release exudates, i.e. easily available OM, in order to make nutrients available for example. These exudates also influence the decomposition of SOM and can lead to priming (Kuzyakov 2002). Beyond that, plant roots emit cations and/or anions into the surrounding soil after uptake of nutrient cations and/or anions to compensate charge discrepancies (Figure 1.4). In case of preferential anion nutrition, plants accordingly release a high amount of anions in return, such as OH^- or HCO_3^-. As a result, pH in the rhizosphere can increase by up to two units compared to root-free soil zones (Marschner 1995, Dakora and Phillips 2002). Since priming is promoted by a high pH, it may particularly occur in the near of plant roots, even without the release of organic root exudates, but with regard to the addition of fresh OM, as for example digestates from biogas production. Anaerobic digestion reduces the C/N ratio of the fermented organic matter

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(Kirchmann and Witter 1992). As described above, priming can be a consequence of the addition of organic matter containing high amounts of N, but less available C. When digestates are applied to soil, microorganisms could exploit SOM to gain C as an energy source.

1.3.3 Open questions and objective 2 of this thesis

Since priming leads to an increase of CO_2 emission from soil, it should thoroughly be investigated with regard to climate change. As shown in Chapter 1.3.2, digestates may cause priming in soils due to their low C/N ratio. Especially at high pH, as for instance in the near of anion exchanging plant roots, priming could strongly be promoted by digestates. Thus, the second aim of this thesis was to test whether priming occurs in soils fertilised with digestates from biogas production under artificial rhizosphere conditions. This subject is dealt with in Chapter 3. The mineralisation of digestates in soil and possible priming were investigated in incubation experiments with integrated anion exchange resins which were intended to simulate the anion exchange of plant roots.

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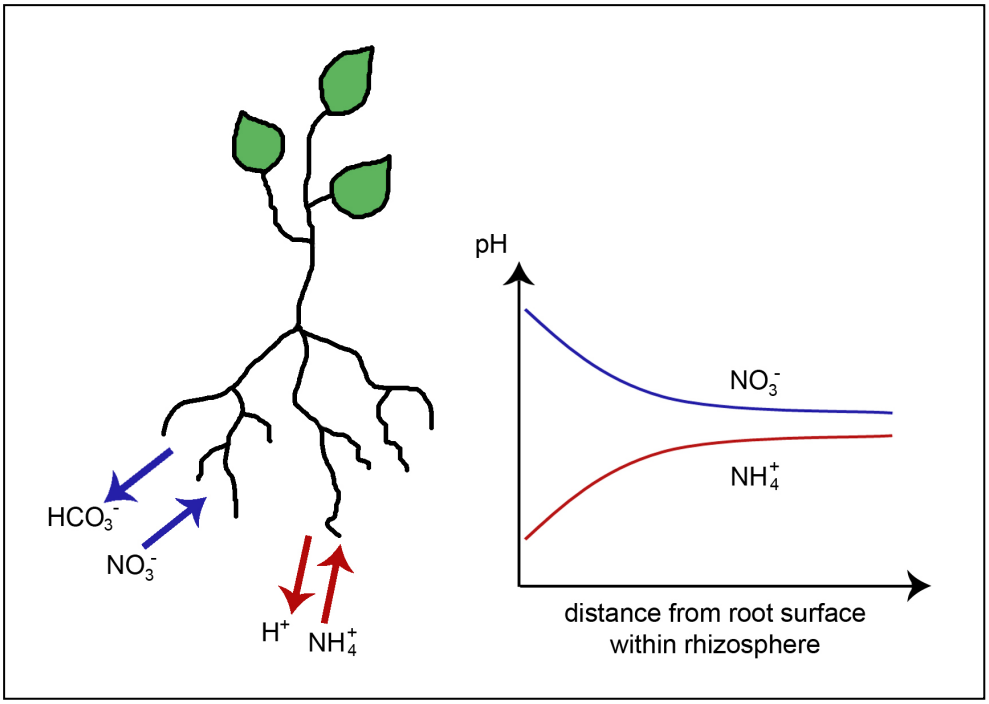

Figure 1.4: Schematic of cation and anion exchange in the near of plant roots and the influence on pH within the first millimetres from the root surface (according to Gisi et al. 1997).

1.3.4 Nutrient availability of digestates in soils

Another important factor deciding, whether digestates are suitable fertilisers, is their nutrient supply for crops. Many studies have been conducted to investigate the effect of digestates on crop yield and nutrient availability compared to unfertilised controls, to conventional organic or to mineral fertilisers. A good deal of these studies appeared in the last three years. Digestates mostly turned out to be adequate fertilisers in terms of nutrient availability and crop yield. In a three-month field trial, Baba et al. (2013) tested the fertilising effect of anaerobically digested crude glycerol and observed an increased grass yield by 1.2 times compared to unfertilised plots. A field trial of Garfí et al. (2011) showed that anaerobically digested guinea pig manure caused an increase in potato yield compared to the unfertilised control. This increase even exceeded the increase achieved by undigested manure compost. Clements et al. (2012) also found increased ryegrass yield in a glasshouse trial after

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application of digested dairy cattle slurry compared to undigested slurry and could attribute this to an improved N availability of the digestate. These findings are supported by Johansen et al. (2013), who discovered an increased N availability by 30-40 % after fertilisation with digested cattle slurry and maize silage compared to undigested cattle slurry. Herrmann et al. (2013) even obtained a similar maize yielding performance and a higher N efficiency by digested energy crops, i.e. a purely vegetable digestate, in comparison to conventional pig and cattle slurry in a two-year field experiment. Furthermore, Garg et al. (2005) detected positive effects of digestates on soil in addition to satisfying crop yield, as for instance a reduced bulk density and improved water retention capacity compared to the unfertilised control. Walsh et al. (2012) found less potential for loss of nutrients via leaching in pots fertilised with digested cattle slurry than in pots with inorganic fertiliser. Positive effects of digestates on nutrient availability and crop yield were also reported by Vaneeckhaute et al. (2013), Odlare et al. (2011) and Bougnom et al. (2012). Although, digestates have a lower viscosity than undigested slurries or manures and should thus infiltrate quickly into the soil and reduce the risk of gaseous N losses (Weiland 2010, Chantigny et al. 2009), it has been shown, that increased N availability and crop yield could only be achieved when digestates had been incorporated into soil immediately after spreading (Möller et al. 2008).

Some authors described rather ambivalent experiences with digestates in soils. Grigatti et al. (2011) found a great amount of easily available N only in the liquid fraction of the digested dedicated crops and Alburquerque et al. (2012b) observed that the readily available nutrients only lasted for the summer crops but had been depleted in the following winter. Sieling et al. (2013) compared the fertilising effect of digested pig slurry and maize silage to mineral fertiliser and undigested cattle slurry in a two-year field trial. In contrast to Baba (2013) and Clements (2012), they observed lower grass yield and N availability for digestate amended plots, and in contrast to Alburquerque et al. (2012b) a higher N balance than for undigested cattle slurry and mineral fertilisers. Finally, Svensson et al. (2004) could not confirm, that digestates from source-separated domestic waste reached similar or even higher crop yield

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than mineral fertilisers, and recommends combining digestates with P fertiliser.

Only few authors report on negative effects of digestates used as fertiliser. The high amount of available N is not always compensated via uptake by plants, so that N losses due to leaching may occur (Haraldsen et al. 2011, Goberna et al. 2011). In contrast, Bernal and Kirchmann (1992) found up to 40 % of the ammonium-N in digestates immobilised. Likewise, N availability was remarkably lower in a soil fertilised with digested cattle slurry and maize silage compared to a soil fertilised with undigested cattle slurry in a microcosm study of Ernst et al. (2008). The authors attribute this to the lower amount of degradable organic matter in the digestate. Lastly, in some cases, digestates can be unstable and contain large amounts of organic acids and ammonia being phytotoxic for plants (Salminen et al. 2001). This can be reduced by composting of digestates prior to application to soils (ibid., Abdullahi et al. 2008). A positive side effect of composting of digestates is the suppression of pathogens (Bustamante et al. 2012). However, the reduction of ammonia during composting can be a result of undesirable volatilisation. Efficient pH control could be a way to reduce these N losses (Salminen et al. 2001).

1.3.5 Open questions and objective 3 of this thesis

Chapter 1.3.4 reveals that there are numerous studies on the nutrient availability of digestates in soils. However, these studies mainly focus on nitrogen. Phosphorous availability of digestates is not investigated as thoroughly, although P is regarded as a short-running resource (Cordell et al. 2011) and plays a major role in plant nutrition. Therefore, Chapter 4 of this thesis focuses on the P availability of digestates. In particular, the kinetics of P transport to plant roots was examined. Hitherto existing models about P transport to plant roots include mass flow and diffusion as dominating processes, but do not account for

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additional supply of P due to the decomposition of OM and its effect on P transport kinetics. However, since large amounts of OM get into the soil with the application of digestates, this additional supply of P due to the mineralisation of the OM could play an important role for P transport. Hence, the third objective of this thesis was to find out, whether additional P supply by mineralised OM influences P transport to an artificial plant root. Chapter 4 deals with the hypothesis that the presence of an anion sink (HCO_3^- source) and the consequentially increasing pH induces enhanced mineralisation of organic P and that this increased mineralisation influences the diffusive P transport. A digestate amended soil was incubated with an anion exchange resin intended to simulate P removal by plant roots. Moreover, a new model conception for P transport to plant roots is introduced that combines a diffusion model and a term accounting for additional P supply due to OM mineralisation.

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2 Elemental composition of biogas residues: Variability and alteration during anaerobic digestion[1]

2.1 Abstract

Biogas production and the amount of thereby incurred digestates increased remarkably in the last decade. Digestates should be used as soil fertilisers to close nutrient cycles. However, knowledge about the elemental composition of digestates from biogas production and element losses or accumulations during fermentation process is insufficient so far. Intending to enlarge the database for the elemental composition of digestates and to investigate element in- and outputs of biogas fermenters, we measured the concentrations of C, N, P, K, S, Ca, Mg, Fe, Mn, Zn, Cu, Pb, Cd, Ni, Mo and Se of digestates and feedstock (ingestates) of four full-scale biogas plants in Germany monthly over a one year period. Ingestates were sewage sludge, fat and mash (SEW1), sewage sludge and fat (SEW2), pig slurry, treacle and food residues (SL) and maize silage (M). We developed a statistical method to calculate the number of required sampling dates which have to be integrated for the calculation of reliable element budgets between ingestates and digestates for the case when information about the amount and composition of the produced biogas is not available. Our results suggest that two (SEW2), five (SEW1, M) and ten (SL) sampling dates had to be integrated for reliable balances. All fermenters revealed losses of N, most likely due to volatilisation of NH_3. Losses of S (probably H_2S), Mg (precipitation of struvite), Cd and Zn (precipitation of sulfides) could be detected in some cases. Iron and Mn accumulations can be attributed to attrition of the stirrer.

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2.2 Introduction

Whilst the worldwide growing demand for energy has to be satisfied, the concentration of greenhouse gases in the atmosphere steadily increases due to emissions from fossil fuel combustions (Weiland 2010). Thus, in recent decades, the urgent need for the production of renewable energy has been well-known. Biogas production, for example, expanded rapidly in the last 10 years. In Germany, the number of biogas plants increased from nearly 1,600 in 2002 to more than 7,000 in 2011 (FNR 2012). This development is especially driven by the German policy of promoting renewable energies by the “EEG” (Erneuerbare-Energien-Gesetz) in year 2000 and its amendments in the following years. Biogas can replace fossil fuels in power and heat production and can also be used as gaseous vehicle fuel (Weiland 2010). However, production of energy crops for anaerobic digestion competes against food production for agricultural land. Additionally, the increasing cultivation of energy crops provokes land-use changes and monocultures which often involve environmental disadvantages such as loss of biodiversity or soil erosion (Emmann et al. 2013). Therefore, wastes and edible organic residues are preferable feedstocks for anaerobic digestion. An additional benefit of waste fermentation is the reduction of odour and mass reduction of landfilled agricultural and municipal waste due to the conversion of organic material to methane (CH_4) and carbon dioxide (CO_2, Teglia et al. 2011a) .

For a life cycle assessment of biogas production the whole process chain has to be considered (Alburquerque et al. 2012a). This also includes the recirculation of digestates and nutrients contained therein to the soil where new biomass is cultivated. Alburquerque et al. (2012a) even assume advantages of digestate fertilisation compared to non-fermented organic fertilisers, such as a greater microbial stability and hygiene as well as greater available nitrogen (N). But before utilization as fertiliser or amendment, the elemental composition of digestates has to be known to evaluate fertilising effects and to prevent contamination of soils. Since anaerobic digestion alters the chemical properties of the initial

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biomass (ingestate), knowledge about the composition of the initial material is not sufficient. However, investigations about the elemental composition of the resulting digestates are scarce (Teglia et al. 2011a, Tambone et al. 2010, Whelan et al. 2010). Table 2.1 shows some chemical characteristics of various digestates which can be found in the literature so far. Furthermore, a number of non-peer-reviewed publications about characteristics of digestates exist but are not cited here.

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Table 2.1: Digestates and analysed chemical parameters in the literature (FS: Fermenter scale, F: Full-scale biogas plant, L: Laboratory or pilot scale fermenter, [a]: dm (dry matter) in %, [b]: dm in g l^{-1}, [c]: total C, [d]: average over 2 different samples, [e]: average over three different samples, ±: standard deviation over different samples).

author	ingestate	FS	pH	dm	TOC	N	P	K	S	Ca	Mg	Fe	Mn	Zn	Cu	Pb	Cd	Ni	Mo	Se
								g kg^{-1} related to dm							mg kg^{-1} related to dm					
Albur-querque et al. 2012c	cattle slurry + 4 % glycerine	L	5.6	38[b]	465	49	13	47	4.6	40	7	3.0	358	473	282	-	-	-	-	-
	cattle slurry + 6 % glycerine	L	7.4	73[b]	587	32	5	22	3.6	24	5	2.3	235	388	178	-	-	-	-	-
	cattle slurry + 5 % orange peel residues	L	7.9	24[b]	385	59	8	45	4.6	41	11	1.2	246	316	115	-	-	-	-	-
	pig slurry + 0.6 % pasteurised slaughterhouse waste	L	8.0	21[b]	276	138	24	105	10	38	15	2.4	543	4019	681	-	-	-	-	-
	pig slurry + 1 % sludge from slaughterhouse wastewater treatment plant + 6.5 % biodiesel wastewater	F	8.2	20[b]	303	205	10	103	35	11	3	1.1	149	1779	206	-	-	-	-	-
	cattle slurry + 4.3 % cattle manure + 11.6 % maize-oat silage	F	7.5	90[b]	374	44	9	34	5	45	8	3.3	305	307	120	-	-	-	-	-
Albur-querque et al. 2012b	pig slurry + 1 % sludge from slaughterhouse wastewater treatment plant + 6.5 % biodiesel wastewater	F	8.3	1.9[a]	247	200	6	52	-	26	10	1	158	1158	211	2	0.5	-	-	-
Haraldsen et al. 2011	source-separated biodegradable household waste	F	8.0	1.5[a]	-	152	16	78	7	50	10	-	<0.7	79	13	-	-	-	-	-
Kirchmann and Witter 1992	cattle slurry	L	8.5	-	500	42	9	13	3.6	20	6	2.0	164	137	44	-	-	-	-	-
	pig slurry	L	7.3	-	551	43	20	18	4.6	34	12	1.0	245	1304	121	-	-	-	-	-
	poultry slurry	L	7.8	-	452	67	24	24	5.3	92	6	1.8	663	578	105	-	-	-	-	-
Möller et al. 2008	cattle slurry	L	7.7	9.2[a]	355[c]	43	7	47	-	-	18	-	-	-	-	-	-	-	-	-
Selling et al. 2008	maize	L	-	-	-	-	-	-	-	-	-	-	-	34	29	1.3	0.05	5	-	-
	horse manure	L	-	-	-	-	-	-	-	-	-	-	-	43	14	3.2	0.3	3.8	-	-

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author	ingestate	FS	pH	dm	TOC	N	P	K	S	Ca	Mg	Fe	Mn	Zn	Cu	Pb	Cd	Ni	Mo	Se
				g kg^{-1} related to dm									mg kg^{-1} related to dm							
Tambone et al. 2010	80 % OFMSW + 20 % pig slurry [e]	F	-	4±1[a]	481 ±30	144±8	5.4±2	23±2	-	-	-	-	-	-	-	-	-	-	-	-
	48 % pig slurry + 24 % milk serum + 14 % cow slurry + 10 % maize silage + 4 % rice residues [d]	F	-	4±1[a]	483 ±13	93±14	11	13.5±2	-	-	-	-	-	-	-	-	-	-	-	-
	65 % pig slurry + 20 % blood industry residues + 15 % maize silage [e]	F	-	6 ±0.5 [a]	488±9	88±4	12±1	28±6	-	-	-	-	-	-	-	-	-	-	-	-
Teglia et al. 2011b	sewage sludge [d]	F	-	20± 1[a]	291 ±18	42± 11	32±5	-	-	-	-	-	-	-	-	-	-	-	-	-
	17 % fat + 75 % rabbit manure and urban sludge + 8 % duck slaughterhouse sludge	F	-	20[a]	365	25	14	-	-	-	-	-	-	-	-	-	-	-	-	-
	70 % cattle manure + 7 % rabbit manure + 3 % garden wastes + 17 % fruits and vegetables	F	-	24[a]	330	20	8	-	-	-	-	-	-	-	-	-	-	-	-	-
	source-selected fraction of municipal solid wastes [d]	F	-	44± 2[a]	274 ±104	14±1	6±5	-	-	-	-	-	-	-	-	-	-	-	-	-
Trzcinski and Stuckey 2011	organic fraction of municipal solid waste	L	-	-	-	-	-	-	-	-	-	-	-	77	25	5	0	50	-	-
Vintiloiu et al. 2012	25 different mixtures of pig and/or cattle slurry, manure and maize and/or grass silage	F	-	-	-	-	-	-	4.4 ±0.7	-	-	2.6 ±1.6	-	-	-	-	-	6.2 ±3.3	3±1	0.5 ±0.3
Walsh et al. 2012	cow slurry	F	8.6	5.2[a]	274[c]	22	1	17	-	20	-	-	-	-	-	-	-	-	-	-
	Mean				**399**	**76**	**11**	**42**	**8**	**37**	**9**	**2**	**279**	**764**	**153**	**2.9**	**0.21**	**16**	**-**	**-**
	Standard deviation				**104**	**60**	**6**	**30**	**9**	**21**	**4**	**0.8**	**187**	**1079**	**173**	**1.6**	**0.23**	**23**	**-**	**-**

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Scanning the literature reveals that mostly only a few chemical characteristics have been investigated so far, with most authors focussing on N, P and K. In particular, heavy metals like lead (Pb), cadmium (Cd) or nickel (Ni) are underrepresented. Thus, more extensive data for digestate characteristics are needed. Table 2.1 also shows that element concentrations of digestates are highly variable and depend on the kind of ingestate. Additionally, the elemental composition of digestates is influenced by digestion process characteristics. During fermentation, varying amounts of N and S can volatilise from the ingestate (Vintiloiu et al. 2012, Kaltschmitt et al. 2009) and heavy metals can accumulate in the ingestate due to attrition from the stirrer, for example (Trzcinski and Stuckey 2011). A few authors (Banks et al. 2011, Pognani et al. 2012) studied the fate of nutrients during fermentation and calculated element mass balances. However, again these studies only focussed on N, P and K. Furthermore, data about the mass of produced biogas are not always available as in the mentioned studies (Banks et al. 2011, Pognani et al. 2012), i.e. another way to account for the mass losses due to biogas emission from the substrate has to be found to conduct an element balance in this case.

Thus, the aims of our study were:

1. to give an overview of the elemental composition, particularly heavy metal concentrations, of digestates from four full-scale biogas plants in Germany
2. to investigate the temporal variability of digestates' elemental composition within one biogas plant
3. to investigate the difference in digestates' elemental composition between different biogas plants
4. to identify element losses or accumulations during the digestion process.

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2.3 Materials and methods

2.3.1 Digestates and element analysis

We collected digestates and ingestates from four biogas plants (wet fermentation) at intervals of one month over one year. The main process parameters of the biogas plants and digestate abbreviations used in this study are given in Table 2.2.

Table 2.2: Main characteristics of digestates and main process parameters of corresponding biogas plants (dm (dry matter): means of all sampling dates, pH: means of 3 (SEW1), 7 (SEW2), 4 (SL) and 2 (M) sampling dates).

ingestate	fermentation structure	fermenter volume (m^3)	operating temperature (°C)	stirring device	nutrient addition	dm (%)	pH	digestate abbr.
about 55 % sewage sludge, 13 % fat, 32 % mash	single stage process	2,300	47	fluid jet	not known	2.77 (0.4-3.85)	7.35 (7.25-7.53)	SEW1
sewage sludge, 0.2 % fat	single stage process	8,000	38	fluid jet	seldom lime	3.32 (1.26-4.2)	7.33 (7.21-7.49)	SEW2
pig slurry, treacle, food residues	two-stage process	2,100	37	central stirrer	none	3.50 (1.34-6.19)	7.76 (7.46-7.89)	SL
maize, seldom grass	single stage process	2,500	39	submersible stirrer	trace elements	6.97 (2.87-8.29)	7.53 (-)	M

Digestates and ingestates were freeze-dried and ground in the laboratory. Carbon (C), N and sulfur (S) were measured by a CNS Analyser (Elementar Vario EL III). Additionally, digestates and ingestates were digested with 10 ml 69 % HNO_3 (suprapur, Roth, Karlsruhe, Germany) for 15 min at 180 °C in a microwave pressure apparatus (Mars Xpress, CEM, Kamp-Lintfort, Germany). All samples were digested in duplicate. Concentrations of calcium (Ca), magnesium (Mg), potassium (K), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) were analysed with flame AAS (Perkin Elmer Atomic Absorption Spectrophotometer 1100B). Lead, Cd and Ni were determined by a Varian graphite furnace AAS (SpectrAA 880Z), molybdenum

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(Mo) and selenium (Se) by an ICP-OES (iCAP 6000 Series, Thermo Scientific) and phosphorus (P) by a Continuous Flow Analyser (880 nm wavelength, Skalar San plus system 5120).

2.3.2 Correction of nitrogen analysis

Mineral N is mainly present as ammonium (NH_4^+) in digestates due to anaerobic conditions in the biogas fermenter. High pH of the digestates can facilitate gaseous losses of N as ammonia (NH_3, Pain et al. 1990). Ammonia emissions during digestate preparation in the laboratory can therefore lead to an underestimation of N concentrations in the digestates. Previous acidification of the digestates with hydrochloric acid (HCl) could be a suitable method to prevent NH_3 emissions during sample preparations. Exemplarily, we acidified the digestates prior to any further treatment at six different sampling dates and compared N concentrations to those of non-acidified digestates in order to find the average potential for N losses of the digestates and to get a correction factor which we applied to our N concentration data obtained from non-acidified digestates. We added 1 M HCl to the digestates until a pH of 6 was reached. Under these conditions the amount of NH_3 should be decreased to 0.1 % and the majority of mineral N should be present as non-volatile NH_4^+ (Scheffer and Schachtschabel 2002): $NH_3 + HCl \rightarrow NH_4^+ + Cl^-$. The samples were subsequently dried, ground and measured as described above.

Acidified digestates prior to drying exhibited significantly higher N concentrations than non-acidified (SEW1: $p=0.002$, SEW2: $p<0.001$, M: $p<0.001$). When acidified, SEW1 showed 1.53 times, SEW2 1.35 times and M 1.29 times the N concentration of non-acidified references. It is assumed that about 35 % of the nitrogen of SEW1, about 26 % of the nitrogen of SEW2 and about 22 % of the nitrogen of M is lost by NH_3 volatilisation, when digestates are not acidified prior to drying. Nitrogen concentrations determined without pre-

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acidification of the digestates were corrected by the corresponding factors. Nitrogen losses of SL could not be determined since digestate material was not available due to biogas plant close-down when we conducted the acidification tests. Thus, we calculated the mean (1.39) of the factors for N losses of the other digestates (SEW1, SEW2 and M) and applied it to the N concentrations of SL.

We did not determine potential N losses of ingestates during sample drying. It is assumed that maize silage did not contain much NH_4^+ and thus, no N emissions occurred during the preparation of the M ingestate samples. In contrast, ingestates of SEW1, SEW2 and SL may have contained NH_4^+ which was likely to volatilise as NH_3 during sample preparation. Various authors report on ingestates containing about 20 % less NH_4^+ than the corresponding digestate (e.g. Möller et al. 2008, Johansen et al. 2013, Seppala et al. 2013). Based on this we adjusted the factors gained by acidification of the digestates and got the factors 1.39 (SEW1), 1.27 (SEW2) and 1.29 (SL) which we applied to the N concentrations of the corresponding ingestates.

While *preventing* gaseous losses of N, acidification of the samples *enhanced* losses of C and by trend of S. Carbon concentrations of acidified samples were lower than those of non-acidified samples (SEW: p=0.004, SEW2: p=0.041, SL: p not determinable, M: p=0.032). Acidification reduced C concentrations by 5-17 % (SEW1), 0-16 % (SEW2), 10 % (SL, only one replicate) and 0-10 % (M). This can be attributed to CO_2 emissions resulting from reactions of the added HCl with carbonates. Gaseous losses of S due to previous sample acidification were only significant for SEW2 (p=0.003). Addition of HCl resulted in 13-27 % lower S concentrations for SEW2. We assume that hydrochloric acid converted sulfides present in the digestate into hydrogen sulfide which volatilised. Therefore, the previous acidification of digestates is not suitable for total C (if carbonates are present in the materials) and S analyses.

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2.3.3 Statistical analysis

For the detection of element losses or accumulations during the fermentation process, the element concentrations of the ingestate and the digestate have to be compared to each other. However, due to the temporal heterogeneity of the composition of the feedstock a sampling of the ingestate and the directly corresponding digestate is not realisable. That means an ingestate sampled only once cannot be compared to a digestate sampled only once. Therefore, we first needed to investigate, how many samplings of ingestates and digestates we have to integrate to get representative element concentrations for ingestates and digestates. Hence, we divided the element concentration of the digestate by the element concentration of the ingestate and stepwise integrated element concentrations of further sampling dates:

$$C_{\mathrm{dig\,1}}^{\mathrm{el}} \Big/ C_{\mathrm{ing\,1}}^{\mathrm{el}} = q_1^{\mathrm{el}} \qquad \text{(Eq. 2.1)}$$

$$\left(C_{\mathrm{dig\,1}}^{\mathrm{el}} + C_{\mathrm{dig\,2}}^{\mathrm{el}}\right) \Big/ \left(C_{\mathrm{ing\,1}}^{\mathrm{el}} + C_{\mathrm{ing\,2}}^{\mathrm{el}}\right) = q_2^{\mathrm{el}} \qquad \text{(Eq. 2.2)}$$

…

$$\sum_{i=1}^{i=n} C_{\mathrm{dig\,i}}^{\mathrm{el}} \Big/ \sum_{i=1}^{i=n} C_{\mathrm{ing\,i}}^{\mathrm{el}} = q_{\mathrm{n}}^{\mathrm{el}} \qquad \text{(Eq. 2.3)}$$

where, C^{el} [mg kg^{-1}] is the concentration of a specific element; dig means digestate; ing means ingestate, q [-] is the resulting quotient and n ist the number of sampling dates. We expected the quotients to tend to a specific value which will stay constant after the necessary number of integrated sampling dates. It is possible that the necessary number of sampling dates for a comparison of ingestate and digestate differs for different elements. For an examination of the *whole* elemental composition of the digestates and ingestates, an average

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of the necessary number of sampling dates for *all* investigated elements had to be found. This was determined as follows: We calculated the standard deviation (*Sd*) of the digestate/ingestate quotient (*q*) of all elements and expected the standard deviation to decrease with increasing number of integrated sampling dates:

$$Sd_1\,(q_1^{\mathrm{Ca}}, q_1^{\mathrm{Mg}}, q_1^{\mathrm{K}}, q_1^{\mathrm{P}}, \ldots) > Sd_2\,(q_2^{\mathrm{Ca}}, q_2^{\mathrm{Mg}}, q_2^{\mathrm{K}}, q_2^{\mathrm{P}}, \ldots) > \ldots > Sd_{\mathrm{n}}\,(q_{\mathrm{n}}^{\mathrm{Ca}}, q_{\mathrm{n}}^{\mathrm{Mg}}, q_{\mathrm{n}}^{\mathrm{K}}, q_{\mathrm{n}}^{\mathrm{P}}, \ldots)$$

We also expect the standard deviation to tend to a minimum which should stay constant after the necessary number of integrated sampling dates was approached. Subsequently, we intended to compare the element concentrations of the ingestate to the element concentrations of the digestate after integration of the previously determined number of sampling dates. However, concentrations cannot be simply compared to each other since they refer to differing dry masses due to gaseous losses of matter during the fermentation process. Thus, a reference remaining constant during fermentation processes had to be found and we considered K to meet this requirement. This assumption is in accordance with Banks et al. (2011) who showed that 96.4 % of the K in the ingestate was recovered after anaerobic digestion. We compared the ratio of K concentration and element concentration of the ingestate to the ratio of K concentration and element concentration of the digestate. Concentrations for this were averages from the previously determined number of sampling dates. These averages were generated in five replicates, always using another set of sampling dates, in order to test the precision of the determination of sampling dates. Then we calculated the quotient q_K of the ingestate ratio and the digestate ratio. If the result was nearly 1, no accumulation or losses had occurred. If the result was greater than 1, an accumulation had occurred during the fermentation process, and if it was less than 1, an element loss had occurred.

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$$\sum_{i=1}^{i=n} \frac{C_{\text{ing i}}^{\text{K}}}{C_{\text{ing i}}^{\text{el}}} \Bigg/ \sum_{i=1}^{i=n} \frac{C_{\text{dig i}}^{\text{K}}}{C_{\text{dig i}}^{\text{el}}} = q_{\text{K}} \qquad \text{(Eq. 2.4)}$$

General statistical analysis (mean value, variation, t-test, Mann-Whitney rank sum test) was conducted with Microsoft Excel and SigmaPlot.

2.4 Results and discussion

2.4.1 Variability of the elemental composition of digestates

The elements with the highest concentrations were C, N, K and Ca in all analysed digestates (Figure 2.1). This corresponds to most of the literature data (Table 2.1). The lowest concentrations were found for Cd, Mo, Pb and Ni. Selenium concentrations are not shown here since most of them were below detection limit (0.75 mg kg^{-1} related to dm). Molybdenum concentrations did not reach optimal conditions for methanogenic microorganisms (≥7.5 mg kg^{-1} related to dm, Bauer et al. 2009) for digestate M and for SL at some sampling dates. Furthermore, digestates M, SL and SEW1 at some sampling dates revealed a deficit of Ni for microorganisms (limit: ≥30 mg kg^{-1} related to dm, Bauer et al. 2009). A lower methane yield can be the consequence of these low trace element concentrations. However, requirements of methanogens for micronutrients heavily depend on the type of ingestate (Demirel and Scherer 2011).

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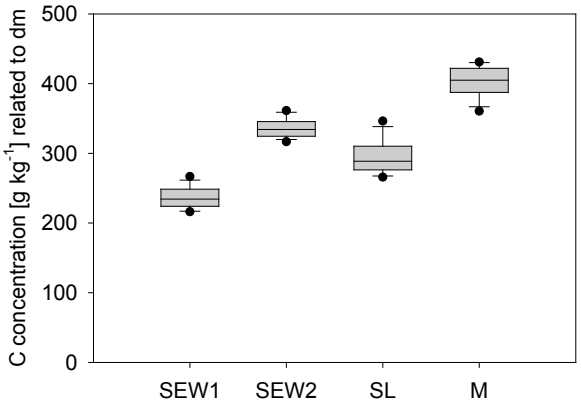

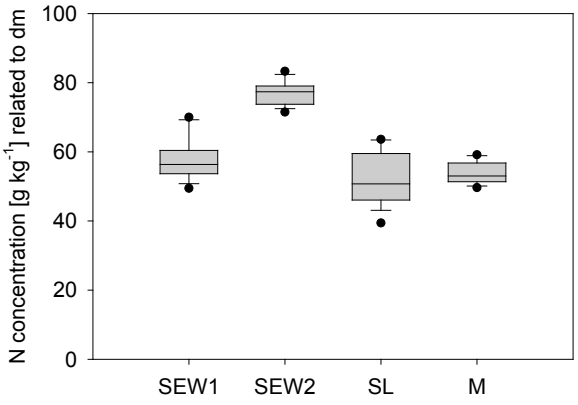

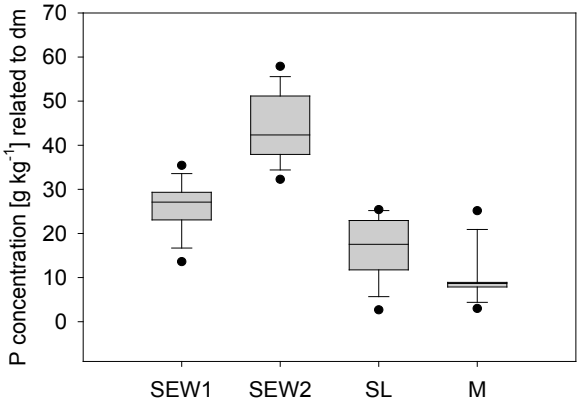

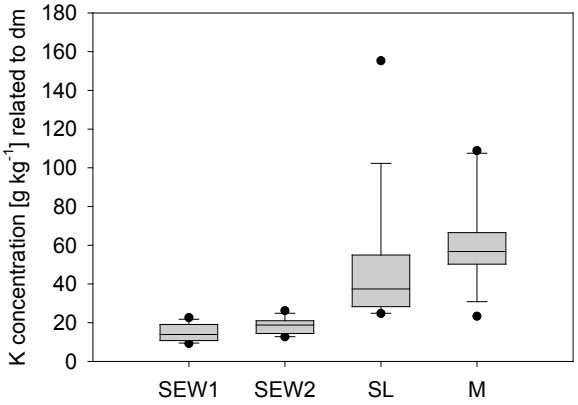

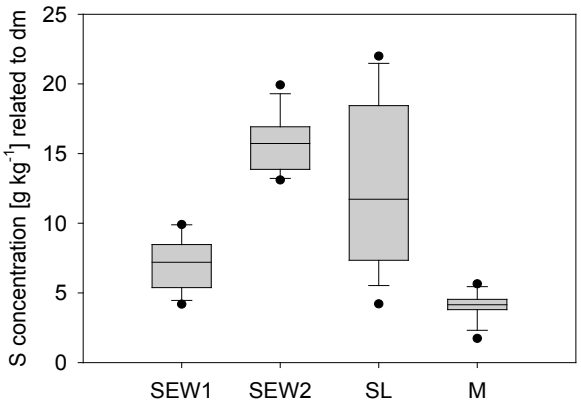

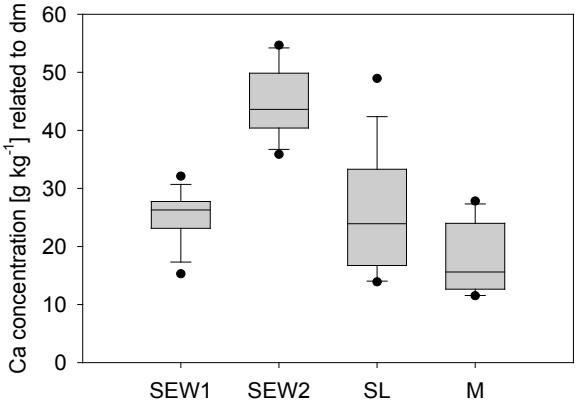

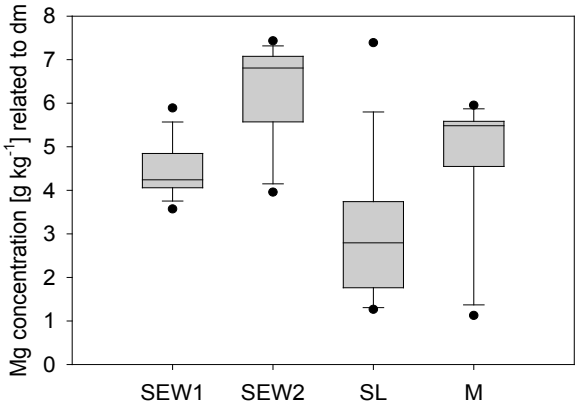

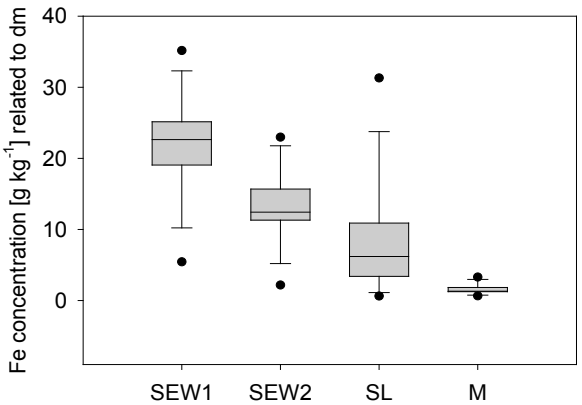

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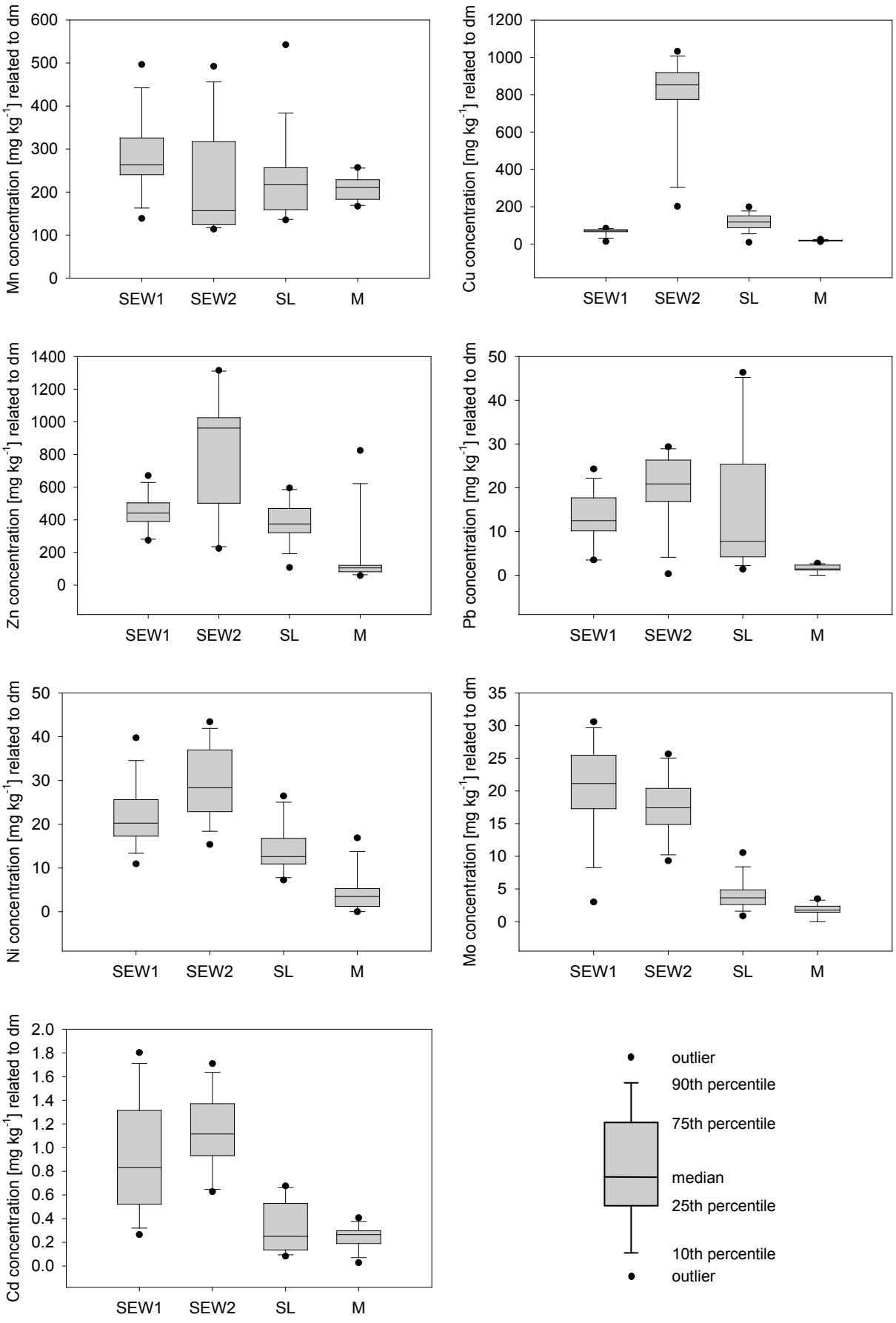

Figure 2.1: Statistics of elemental composition of digestates (sampling dates in months for SEW1+SEW2: 13, SL: 16, M: 11).

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As expected for sewage sludge, digestates SEW1 and SEW2 showed high P concentrations. Phosphorus concentrations of SEW2 (up to 60 g kg^{-1} related to dm) even exceeded those found in the literature (Table 2.1, 1-32 g kg^{-1} related to dm). This can be attributed to the fact that nearly 100 % of the ingestate of SEW2 was sewage sludge. In comparison to SL, M and literature data, SEW1 and SEW2 additionally exhibited high Fe concentrations which are also typical for sewage sludge material since Fe is often applied in order to precipitate P during wastewater treatment. Iron concentration for the only sewage sludge derived digestate found in the literature was not determined by the authors cited in Table 2.1. Digestates SEW1 and SEW2 also showed highest concentrations of other heavy metals like Zn, Pb, Cd and Ni. Copper concentrations of SEW2 (up to 1.2 g kg^{-1} related to dm) partly exceeded the range of literature data (0.01-0.7 g kg^{-1} related to dm) and threshold value of the German limits for sewage sludge (0.8 g kg^{-1} related to dm, AbfKlärV 1992). Copper (up to 0.2 g kg^{-1} related to dm) and Zn (up to 0.6 g kg^{-1} related to dm) concentrations of SL were also above threshold values of the German decree for bio-wastes which has to be consulted in this case (Cu: 0.1 g kg^{-1} and Zn: 0.4 g kg^{-1} related to dm, BioAbfV 2012). These high Cu and Zn concentrations in SL are most likely a result of Cu and Zn addition to pig feed. Another possible reason is the co-fermentation of slurry with other wastes and residues. The latter may have increased Cu and Zn concentrations in SL to a minor degree as well. Compared to the other digestates, digestate M showed low heavy metal concentrations but particularly high C and K concentrations which is typical of plants, such as maize, the ingestate of digestate M. It is less decomposed than sewage sludge or slurry which have already passed a human or animal digestive tract. Other element concentrations of M were unremarkable compared to literature data. With the exception of concentrations of P, Fe, Cu, Pb and Cd for SEW2 and in some cases SEW1, element concentrations of the investigated digestates were in the wide range of literature data.

Our results indicate that the elemental composition of digestates varied remarkably depending on the kind of ingestate and fermentation procedure. This is in accordance with

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findings of Alburquerque et al. (2012a, c) and Schattauer et al. (2011). The latter found variations of 1-2 orders of magnitude concerning the trace element concentrations of the digestates of 10 different biogas plants. However, variation does not only occur between digestates of different biogas plants but also between digestates of one single biogas plant over time. Temporal variations were particularly pronounced for SL, followed by SEW2 and SEW1. These digestates derived from co-fermentation of different ingestates, which were mixed in varying proportions. Digestate M originated from predominantly homogenous ingestate and accordingly showed the lowest temporal variations in elemental composition.

2.4.2 Ingestate-digestate relationship

Figure 2.2 exemplarily shows the ratio of element concentrations of the digestate and ingestate of SEW1 (Eq. 2.3). As expected, the data tended to approach a specific value with increasing number of integrated sampling dates. In the case of Mg, K, P and Fe, the ratios reached a quite constant value after integration of at least five sampling dates (dotted lines). The disturbance in the data of Zn occurred due to a particularly high Zn concentration in the ingestate at the seventh sampling date. Nevertheless, the integration of more sampling dates finally resulted in standard deviations approaching a constant value.

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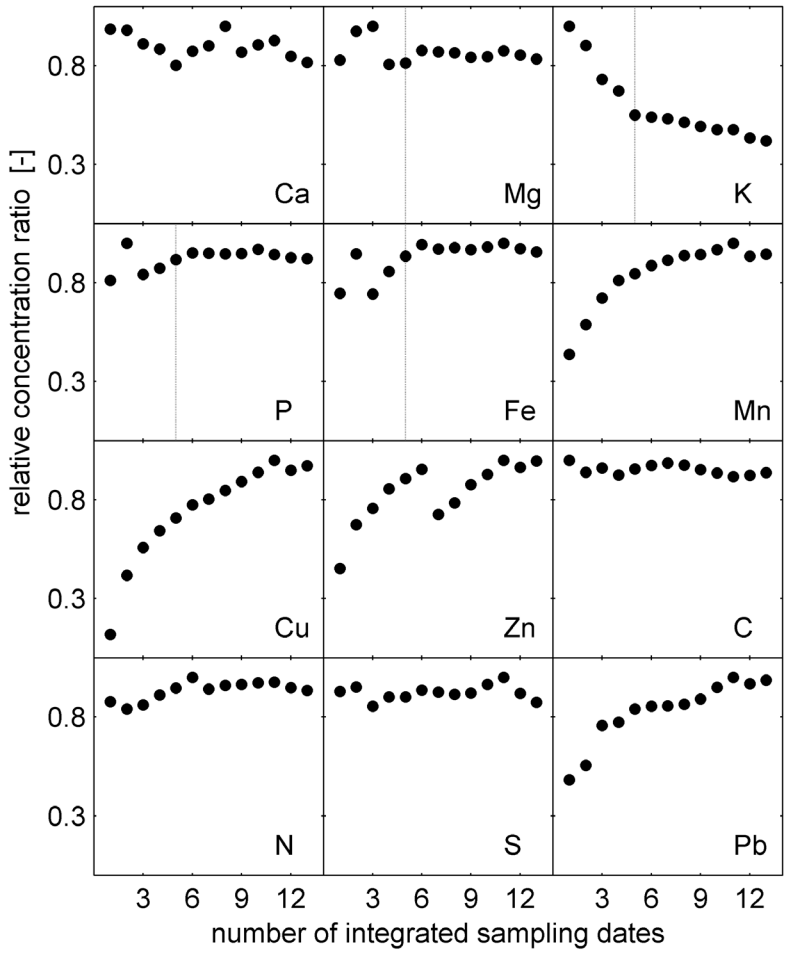

Figure 2.2: Relative ratio of element concentration of the digestate SEW1 and element concentration of the ingestate with increasing number of integrated sampling dates (Relative means, ratio values were divided by the maximum value).

An estimation of the necessary number of sampling dates for *all* elements can be made by observing the standard deviation (Sd) of the digestate/ingestate ratios of all elements with increasing number of integrated sampling dates (Figure 2.3). Standard deviation for SEW1 and M was almost constant after integration of five sampling dates. That means we needed at least five sampling dates at intervals of one month to reach relatively constant q values for all elements and thus to allow conclusions of whether element losses or accumulations have occurred during fermentation. The need for more than one sampling for investigations of

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digestates is supported by Teglia et al. (2011a). These authors characterised digestates and assumed that representativeness of one single sample may not be reliable. Figure 2.3 also shows that at least two sampling dates were needed for SEW2 and ten sampling dates for SL.

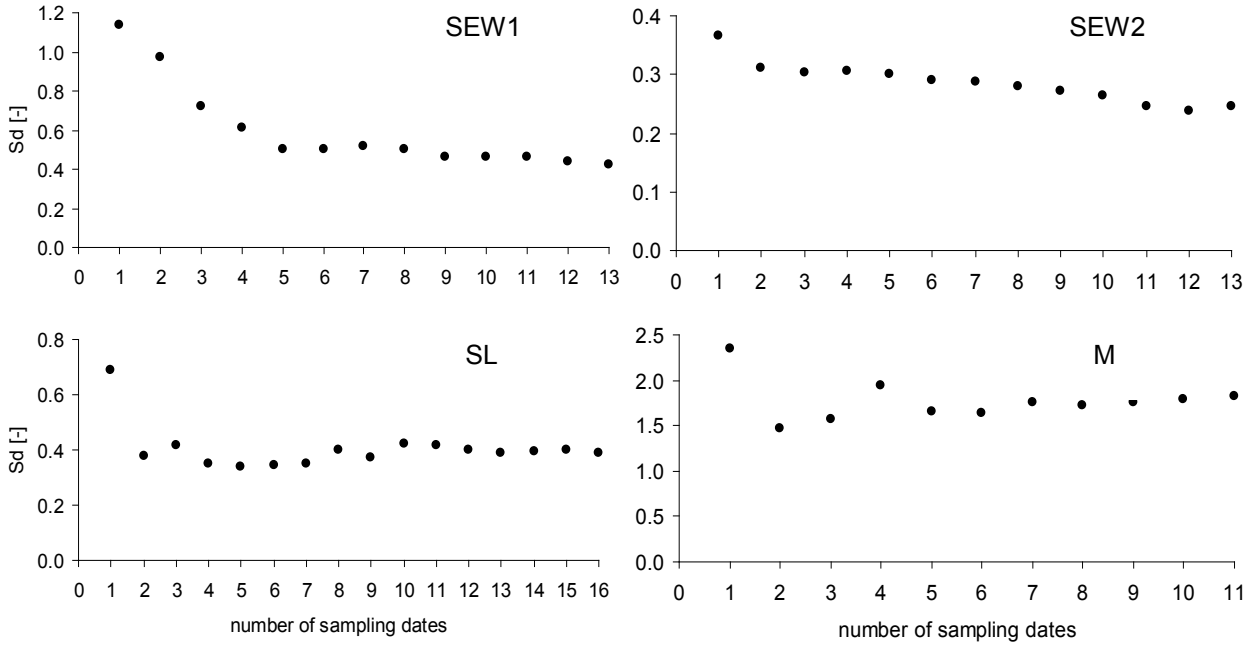

Figure 2.3: Standard deviation of ratios of element concentration of the digestate and element concentration of the ingestate with increasing number of integrated sampling dates for all four investigated biogas plants.

Table 2.3 presents quotients q_K of ratios of K concentrations (reference, which stays constant during fermentation) and element concentration of the ingestate and corresponding ratios of the digestate (Eq. 2.4). Figure 2.4 shows quotients q_K integrating all sampling dates available from our one year lasting measurements (y-axis) correlated to quotients q_K integrating the previously (Figure 2.3) obtained number of necessary sampling dates (x-axis). A slope of nearly 1 would mean that the previously obtained number of necessary sampling dates (Figure 2.3) gave information similar to the information given by *all* sampling dates available

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from one year. Thus, the previously obtained number of necessary sampling dates (Figure 2.3) could be regarded as sufficient for an estimation of possible element losses or accumulations. Correlations for all investigated biogas plants revealed slopes of nearly 1 (SEW1: 0.9382, SEW2: 0.9711, SL: 0.9038 and M: 0.9064). We can thus confirm that element concentrations of ingestates and digestates have to be analysed at least 5 times in intervals of one month for SEW1 and M, at least 2 times for SEW2 and 10 times for SL to allow an evaluation of the element balance during fermentation.

Table 2.3: Quotient q_K of ratios of K concentration and element concentration of the ingestate and corresponding ratios of the digestate for a) the number of necessary sampling dates obtained from Figure 2.3 and for b) all available sampling dates (C is excluded due to great losses during methanogenesis, Pb is excluded for SL due to high outlier values, Pb and Ni are excluded for M since concentrations were too close to detection limit in some cases, so that division by 0 would have occurred).

	Number of sampling dates	N	P	S	Ca	Mg	Fe	Mn	Zn	Cu	Pb	Cd	Ni
SEW1	a) 5	0.74	0.92	0.78	0.93	1.05	1.13	1.01	0.91	0.93	1.09	0.82	1.00
	b) 13	0.76	1.00	0.80	0.89	1.04	1.18	0.97	0.88	0.89	1.01	0.76	0.90
SEW2	a) 2	0.73	1.01	1.01	0.92	0.82	0.98	1.22	0.90	0.90	0.85	0.94	0.89
	b) 13	0.71	0.99	0.99	0.94	0.81	0.98	1.18	0.82	0.92	0.84	0.96	0.89
SL	a) 10	0.50	0.67	0.57	0.67	0.43	0.82	0.61	0.72	0.75	-	1.09	0.87
	b) 16	0.50	0.67	0.63	0.68	0.43	0.85	0.61	0.71	0.75	-	0.97	0.95
M	a) 5	0.74	1.12	0.47	1.08	0.76	1.70	1.03	0.48	1.18	-	1.27	-
	b) 11	0.72	1.16	0.46	1.00	0.71	1.50	0.79	0.46	1.15	-	1.30	-

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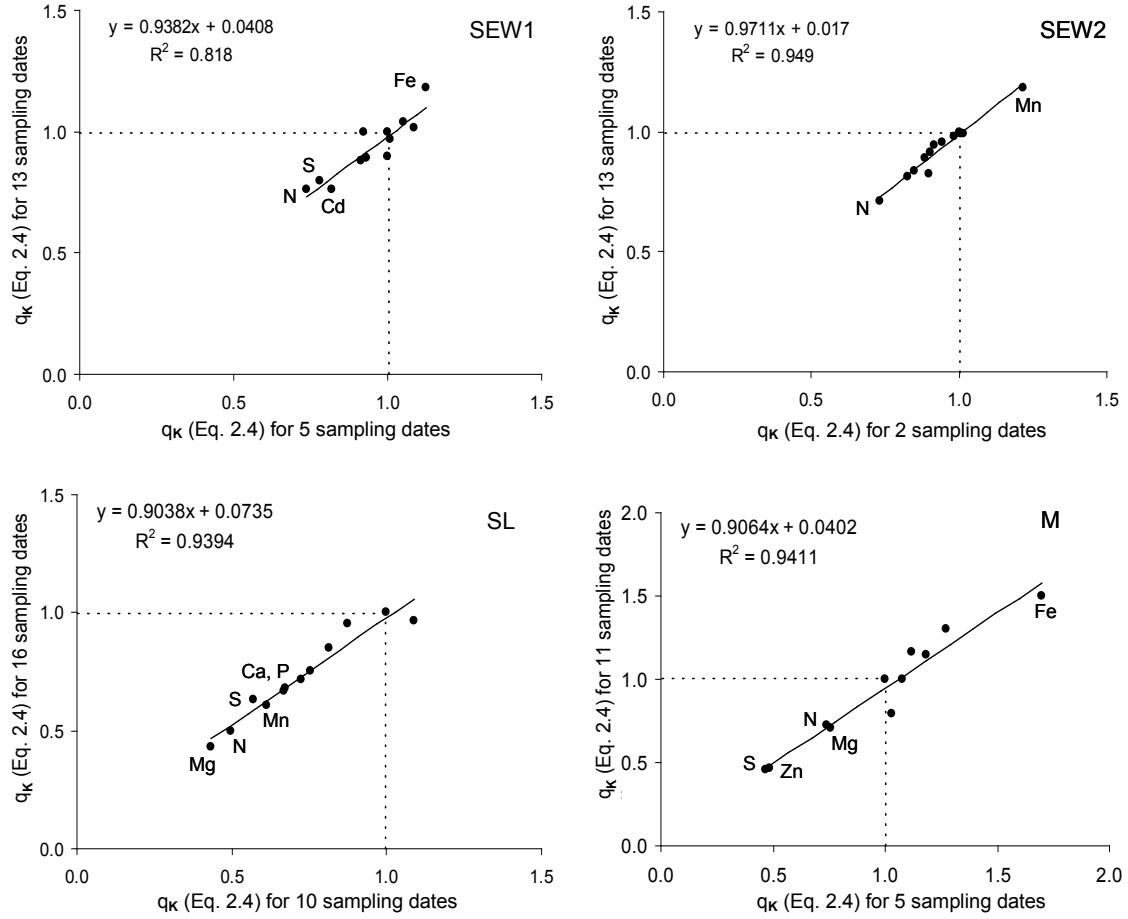

Figure 2.4: Quotient q_K of ratios of K concentration and element concentration of the ingestate and corresponding ratios of the digestate: Correlation of all available sampling dates and number of necessary sampling dates obtained from Figure 2.3 (C is excluded due to great losses during methanogenesis, Pb is excluded for SL due to high outlier values, Pb and Ni are excluded for M since concentrations were too close to detection limit in some cases, so that division by 0 would have occurred).

Element losses or accumulations during fermentation can be estimated from Figure 2.4, as well. Dotted lines in the figure mark a q_K of 1 which means that the ratio of K concentration and element concentration of the ingestate would be equal to the ratio of K concentration and element concentration of the digestate and thus, no element loss or accumulation would have occurred. Quotients far away from 1 are most likely to indicate such loss or accumulation. For SEW1, quotients farthest from 1 are those for N, S and Cd lying below 1 and the one for Fe lying above 1. We conclude that *losses* of N, S and Cd and an

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accumulation of Fe happened during digestion processes of SEW1. Nitrogen losses also occurred in the fermenters of all the other three biogas plants and S was also depleted in fermenters of SL and M. This can be attributed to NH_3 and H_2S emissions mostly taking place during the anaerobic digestion process (Vintiloiu et al. 2012, Kaltschmitt et al. 2009). Losses of Cd in the fermenter of SEW1 as well as of Zn in the fermenter of M can be explained by precipitation of Cd and Zn sulfides, respectively. Since Cd and Zn sulfides have a low solubility and a high density (CdS: 4.82 g cm^{-3}, ZnS: 4.01 g cm^{-3}), these precipitates are most likely to form and to sediment to the fermenter ground.

Trzcinski and Stuckey (2011) previously reported on accumulations of Ni and Cr in digestates because of attrition of the stainless steel stirrer. We assume that accumulations of Fe in fermenters of SEW1 and M, as well as accumulation of Mn in the fermenter of SEW2 may also have taken place due to attrition of the submersible stirrer in the fermenter of M and attrition of the pump apparatus of the fluid jet in fermenters of SEW1 and SEW2. Figure 2.4 shows that fermenters of SL and M also indicated a loss of Mg which can be explained by precipitations of struvite ($NH_4MgPO_4 \cdot 6H_2O$). Banks et al. (2011) investigated the fate of N, P and K during anaerobic digestion of food waste and reported on losses of N and P participating in the formation of struvite in the biogas fermenter. Although losses of Mg also participating in struvite precipitation occurred in the case of SL and M, we could not confirm corresponding losses of P in the fermenter of M. We attribute this to the much higher P concentrations in digestate M compared to P concentrations in the digestates of Banks et al. (2011). Phosphorus losses due to struvite formation are negligible when P concentrations are relatively high. Phosphorus concentrations of SL were also much higher than P concentrations of the digestates of Banks et al. (2011) as well. Nevertheless, losses of P were observable in the case of SL. Possibly, Ca (which was also depleted in this fermenter) and P precipitated as apatite and could not be recovered and measured in the digestate samples. In line with this suggestion, Kleyböcker et al. (2012) found aggregates consisting of P, Ca and carbon rich organic matter in fermenters following a process failure. Finally, many

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of the accumulated elements in the fermenter of M can also be explained by additions of mineral nutrients by the biogas plant operators to achieve favorable conditions for methanogenic microorganisms.

2.5 Summary and conclusions

The elemental composition of digestates varies greatly and depends very much on the feedstock material and the kind of fermentation procedure. Even within one biogas plant, digestates show a distinct heterogeneity in their characteristics over time. For representative statements about the elemental composition of digestates and for an element balancing during anaerobic digestion processes, a single sampling point is not sufficient. Our statistical analysis has proven suitable to determine the necessary number of sampling dates for an element balancing and to discover element losses or accumulations during fermentation processes of the investigated biogas plants. Depending on the diversity of ingestates and the variation of ingestates over time, a sampling of at least two to five, and in one case even ten, is needed. Within the limits of the four selected biogas plants, a loss of N and in three cases of S during the anaerobic digestion process could be detected. Losses of Cd, Zn and Mg occurred as well, probably due to precipitations of metall sulfides and struvite. Accumulations of metals like Fe and Mn cannot be excluded. Further investigations should cover an expanded scope of digestates with regard to elemental characterisations and therefore necessary number of sample takings.

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2.6 Acknowledgements

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3 Carbon mineralisation of digestates in soils under artificial rhizosphere conditions: Incubation experiments with anion exchange resins[2]

3.1 Abstract

The worldwide expanding biogas sector generates increasing amounts of digestates that are generally applied to soils in order to recycle nutrients. Digestates deliver high amounts of nitrogen and could thus induce priming. In case of anion-dominated nutrition, the pH is increased in the rhizosphere, which may additionally favour priming. The aim of our study was to investigate the mineralisation dynamics of digestate amended soils and their potential to promote priming in the presence of an anion-sink intended to simulate plant roots. We conducted three laboratory incubation experiments and measured CO_2 emissions over 60 days from a mixture of sandy soil and digestates from: 1. sewage sludge (SEW), 2. pig slurry (SL) and 3. maize (M). The first experiment included integrated anion exchange resins (AER) to simulate root ion exchange. The second experiment conducted one year later included washed AER to reduce the increase in pH due to excess HCO_3^-, and digestates SEW and M previously stored at 4 °C were used. Effects of digestate aging were constrained by a third experiment using freshly sampled digestates SEW and M from the same biogas plants as in experiment 1, but sampled one year later. The amount of respired C decreased in the order: incubated with AER > washed AER > without AER. We mainly attribute these patterns to an

[2] To be submitted, Zirkler, D., Peters, A., Kayler, Z. and Kaupenjohann, M.

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increase in pH due to HCO_3^- exchange from the AER, which most likely stimulated microbial activity resulting in organic matter (OM) mineralisation. In the first experiment, the M amended soil showed a greater mineralisation than the SEW amended soil, and indications for priming triggered by M were observed. In the third experiment, the SEW amended soil showed both greater mineralisation than M and indications for priming. We attribute this discrepancy between the experiments to a high variability in the stability of digestates. In the second experiment, the amount of respired C was near basal respiration of the unamended soil. We infer that the easily available compounds of the digestates have been converted to more stabile ones during storage. Our results suggest that priming in this case may occur when a sufficient amount of easily degradable OM is added in combination with mineral N. When evaluating the mineralisation dynamics of digestates, a single sample taking of a digestate and its incubation with soil does not necessarily give reliable results since digestate properties and stability in one biogas plant can vary highly over time.

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3.2 Introduction

The production of biogas is expanding worldwide, especially in Germany (Sieling et al. 2013, Alburquerque et al. 2012c), and the amount of organic by-products of the anaerobic digestion (digestates) is increasing (Grigatti 2011). These digestates are normally returned to arable land as amendments and fertilisers in order to recycle valuable nutrients (Vaneeckhaute et al. 2013, Pezzolla et al. 2012, Alburquerque et al. 2012b, c) and to avoid additional loads for landfills or carbon dioxide (CO_2) emissions due to digestate combustion. Many studies show, that digestates are suitable as soil amendments. They exhibited similar or even higher nutrient availability compared to conventional fertilisers (Johansen et al. 2013, Alburquerque 2012b, Clements et al. 2012, Goberna et al. 2011, Haraldsen et al. 2011, Tambone et al. 2010), they improved soil physical properties (Garg et al. 2005), and stimulated soil microbial activity (Odlare et al. 2008). Furthermore, organic fertilisers do not only serve as nutrient

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suppliers for crops but should also increase the humus content in soils. The effect of an organic fertiliser on soil humus balance depends on its mineralisation in soils, for example.

Many authors conducted incubation experiments to investigate the decomposition of digestates in soils (e.g. Alburquerque et al. 2012a, Grigatti 2011, Galvez 2012). In those studies, digestates were incubated at temperatures between 19 and 28 °C for 21-80 days. The mineralised fraction of added carbon (the fraction respired by microbes) ranged from 5 to 60 % for most digestates. The authors calculated that by subtracting the amount of carbon (C) mineralised by the untreated control from the amount of C mineralised by the soil-digestate-mixture. Alburquerque et al. (2012a) observed in one case of the six digestates they investigated that even more C was mineralised from the soil-digestate-mixture within 56 days than has been added with the digestate (105 %). This is consistent with earlier findings of Bernal and Kirchmann (1992) who studied the C mineralisation of anaerobically treated pig slurry mixed into soil. The authors interpret this as priming: Addition of the digestate had accelerated the mineralisation of soil C.

Priming can be induced by short-term changes in the turnover of soil organic matter (SOM) due to treatments of the soil, as for instance organic or mineral fertilising (Kuzyakov et al. 2000). One important factor deciding whether priming occurs or not, is the composition and stability of the fertiliser material. When easily decomposable organic matter is added to the soil, microbial biomass grows and decomposing activity increases. After the depletion of the easily available substances, microorganisms intensify the turnover of less degradable organic compounds and co-metabolise SOM (Blagodatskaya and Kuzyakov 2008). Microorganisms also try to meet their requirements for nutrients by decomposing SOM when fertilisers contain only low amounts of nutrients like nitrogen (N). Another factor promoting priming is the addition of mineral N. In that case, microorganisms may use soil organic matter as a substrate and energy source (Kuzyakov et al. 2000). This could also apply with

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regard to the addition of digestates to soils. Anaerobic digestion alters the C/N ratio of biomasses (Kirchmann and Witter 1992). The methanogenesis turns easily degradable organic C to methane (CH_4) while inorganic N is relatively accumulated. When fertilised with digestates, the soil thus receives organic matter (OM) with less easily degradable C but more easily available N compared to undigested organic fertilisers. Soil microorganisms could exploit the inorganic N but were in need of available C which they would gain from the soil C pool. Thus, the addition of digestates to soils could promote priming and hence undesirable greenhouse gas (CO_2) emissions.

The most important intermediate steps to priming are enhanced microbial activity or biomass (Kuzyakov et al. 2000), which is promoted by favourable conditions for microorganisms, for instance high pH. Blagodatskaya and Kuzyakov (2008) already reported on priming arising more often under rather neutral than acidic conditions. It is well known that pH in the vicinity of plant roots can be up to two units higher than in soil zones farther from roots (Marschner 1995, Dakora and Phillips 2002). Depending on the kind and amount of nutrients and especially on the available N-species plant roots release equivalent amounts of hydrogen ions (H^+) and/or hydroxide/hydrogen carbonate ions (OH^-/HCO_3^-) when taking up nutrients. An excessive uptake of nutrient anions over cations leads to higher OH^- or HCO_3^- release and thus to a higher pH, which could promote priming in the rhizosphere.

Existing incubation studies do not account for these conditions influenced by the rhizosphere. Therefore, we conducted three incubation experiments including anion exchange resins as a model for roots taking up a surplus of anions. In agricultural and environmental research, ion exchange resins are normally used to determine the ion bioavailability in soils (Qian and Schoenau 2002). In this study, the resin was intended to adsorb the anions from the soil solution and release bicarbonate ions into the soil solution in turn. We expected that the release of bicarbonate would increase pH. In a phenomenological approach, we investigated

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1. the mineralisation dynamics of soil-digestate-mixtures and 2. whether digestates can cause priming in soils under conditions that are similar to those near plant roots.

3.3 Materials and methods

3.3.1 Materials

We conducted three incubation experiments with digestates from different full-scale biogas plants. The digestates were mixed into two silty sand soils and homogenised by hand. These soils were collected from the Ap horizons of haplic luvisols at an experimental site of the Leibniz Centre for Agricultural Landscape Research (ZALF) in Dedelow, Germany. After drying to a water content of 7 %, the soil was sieved to a grain size of 5 mm and stored at -18 °C.

The digestates were collected in three mesophilic biogas plants conducting wet fermentation of sewage sludge, fat and mash (SEW), pig slurry, treacle and food residues (SL) and maize silage (M), and were stored at 4 °C. We also used fresh (MF) and rotted manure (MR) as reference being well examined in the literature. The main characteristics of the materials are summarised in Table 3.1.

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Table 3.1: Main characteristics of the materials used in this study (All concentrations are related to dry matter. Digestates for the second experiment were the same as for the first, but one year stored at 4 °C).

	Soil 1	Soil 2	First experiment					Third experiment	
			Digestate 1	Digestate 2	Digestate 3	Manure 1	Manure 2	Digestate 4	Digestate 5
Origin	Ap horizon	Ap horizon	55 % sewage sludge, 13 % fat, 32 % mash	pig slurry, treacle, food residues	maize, seldom grass	cattle, a few days old	cattle, rotted	55 % sewage sludge, 13 % fat, 32 % mash	maize, seldom grass
Dry matter [%]	93	93	2.6	3.2	7.7	21	23	2.9	7.4
pH	5.8	5.8	7.4	7.8	7.5	-	-	7.3	7.5
C_{org} [g kg^{-1}]	6.4	8.9	240	328	413	390	410	225	382
Total C [g kg^{-1}]	6.9	9.7	241	330	417	392	411	227	388
Total N [g kg^{-1}]	1.1	1.3	57	48	52	22	32	47	43
C/N [-]	6.3	7.7	4.2	6.9	8.1	17.8	12.8	4.9	9
Total Fe [g kg^{-1}]	3.8	5	17	7.3	1.1	2	1.9	13	1.8
Total Mn [mg kg^{-1}]	194	174	300	379	168	153	275	157	184
Total Cu [mg kg^{-1}]	3.4	3.7	62	171	12	17	25	45	15
Total Zn [mg kg^{-1}]	12	13	421	629	91	84	136	324	96
Total Pb [mg kg^{-1}]	4	3.4	4.7	2.6	1.7	2.7	3.7	25	6
Total Cd [mg kg^{-1}]	0.2	0.2	0	0	0.3	0.1	0.3	0.6	0.3
Abbreviation	S1	S2	SEW_1	SL	M_1	MF	MR	SEW_3	M_3

3.3.2 Experimental setup

The first incubation experiment was conducted to get a first impression about magnitude and course of the mineralisation processes. Digestates SEW_1, SL and the manures were mixed with soil (S1) collected at a field that had been cultivated with maize (C4 plant) for at least 10 years, and digestate M_1 was mixed with soil (S2) from a field that has always been cropped with C3 plants. These combinations should lead to soil $^{13}C/^{12}C$ ratios differing from the $^{13}C/^{12}C$ ratios of the corresponding digestates or manures and should thus allow a detection of possible priming (see below). For a better comparability, the chosen soils were similar in most of the other characteristics (Table 3.1). Digestates and manures were applied to soil corresponding to a nitrogen addition of 170 kg ha^{-1}, based on a plough layer of 30 cm and a

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soil bulk density of 1.5 g cm^{-3}. This application rate was chosen as fertilisers are usually applied to soils with respect to N supply in agriculture. We added deionised water to the mixtures to adjust 63.5 % of the water holding capacity (WHC). The homogenised mixtures were filled into glass jars lined with aluminium foil (50 g fresh matter (FM) per jar) and compacted to a bulk density of 1.35 g cm^{-3} (soil column diameter: 4.6 cm, height: 2 cm). To avoid anaerobic conditions during our incubation experiments, we chose the density of 1.35 g cm^{-3} instead of the mostly greater bulk density in the field.

In order to test the mineralisation of digestates and their potential to promote priming under conditions similar to plant roots, we also integrated 4 g of an anion exchange resin (AER) in the HCO_3^- form (Merck Ion exchanger III, strongly basic) at the bottom of the jar beneath the soils, soil-digestate- and soil-manure-mixtures. The resin was separated from the mixture by a glass fibre filter (Pall, Type A/D, 3 µm pore diameter, 254 µm thick) and should simulate the function of plant roots taking up nutrient anions and releasing HCO_3^- in return. The jars were placed in polypropylene beakers and closed by screw caps. Each soil-digestate- and soil-manure-mixture was run in triplicate and both soils without amendment were used as controls. Additionally, we prepared the soil-SEW_1-digestate-mixture and the corresponding control *without* integrated AER for the incubation experiment.

All samples were incubated in a respirometer with a water basin of constant temperature (20 °C) for 60 days. According to Franko and Oelschlägel (1995), a biological active time of 60 days (under optimal laboratory conditions) corresponds to approximately 1.5 years under field conditions in the region where the soils were collected. A small vessel with 15 mL of 0.6 M potassium hydroxide (KOH) solution in each sample beaker absorbed the emitted CO_2 (Figure 3.1). The electrical conductivity in the KOH solution was recorded every half hour. The amount of emitted CO_2 was quantified by the change in electrical conductivity of the KOH solution resulting from CO_2 absorption. Potassium hydroxide solutions were exchanged

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when the ratio of the initial electrical conductivity to the actual electrical conductivity was smaller than 0.6. All samples were aerated in adequate intervals ensuring sufficient oxygen supply.

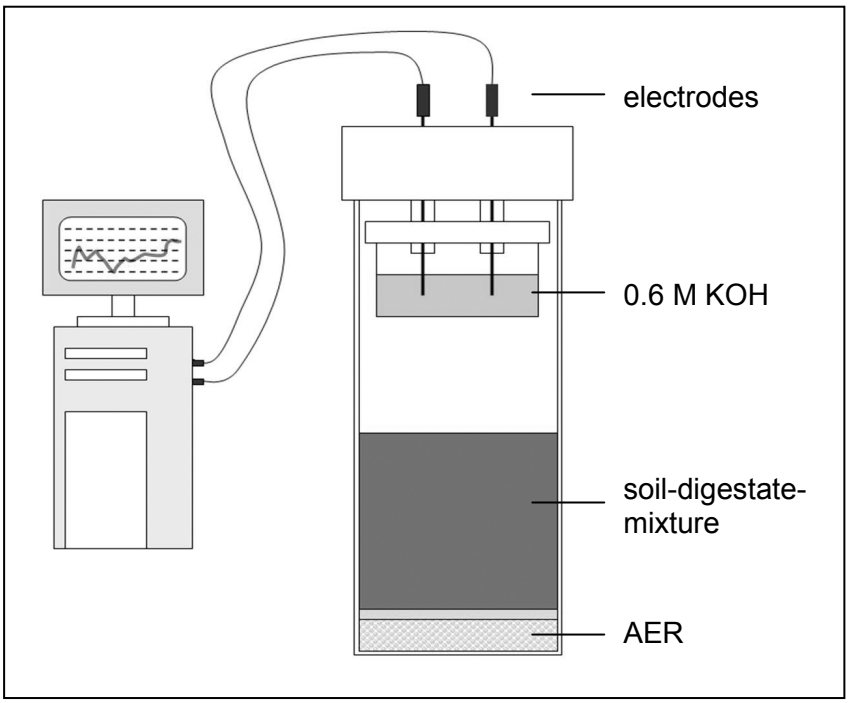

Figure 3.1: Scheme of an incubation vessel

We conducted a second experiment to estimate the general effect of the resin on mineralisation and the effect of resin washing and non-washing on mineralisation. The whole procedure of the first experiment was repeated with the same soils and digestates SEW_2 and M_2, extended by a set of the same samples with integrated *washed* AER, i.e. the resin has been washed with deionised water prior to incubation to remove excess HCO_3^- from the resin. In this second experiment, we prepared soil-digestate-mixtures and the corresponding controls *without* any resin for *both* digestates, not only for SEW. In addition, we incubated washed and non-washed AER (4 g each, three replicates) without any soil or digestate to gain information about the C emission from the pure resins.

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Since the digestates used for the second experiment have already been stored for twelve months after the first experiment, and aging effects could not be excluded, we conducted a third experiment with the same soils and resin versions like for the second experiment, but we used *fresh* digestates (SEW_3 and M_3) from the same biogas plants.

3.3.3 Modelling

Emission of CO_2 was described by a simple two-pool model with first order kinetics:

$$C_{\mathrm{em}}(t) = C_{\mathrm{org}}^{0,\mathrm{A}} \cdot (1 - e^{-k_{\mathrm{A}} t}) + C_{\mathrm{org}}^{0,\mathrm{B}} \cdot (1 - e^{-k_{\mathrm{B}} t}) \qquad \text{(Eq. 3.1)}$$

where C_{em} [mg kg^{-1}] is the amount of emitted C related to incubation sample dry matter, $C_{\mathrm{org}}^{0,\mathrm{A}}$ [mg kg^{-1}] is the initial concentration of easily degradable organic carbon in the incubation sample, $C_{\mathrm{org}}^{0,\mathrm{B}}$ [mg kg^{-1}] the initial concentration of less degradable organic carbon in the incubation sample, k_{A} [d^{-1}] and k_{B} [d^{-1}] the corresponding decomposition rate constants and t [d] is the incubation time. In the following, C_{em} is regarded to be equivalent to mineralised organic carbon. Half lives for the single pools are calculated by:

$$h_{\mathrm{x}} = \ln(2) / k_{\mathrm{x}} \qquad \text{(Eq. 3.2)}$$

where the subscript x stands for either pool A or pool B.

Equation 3.1 was fitted to the data by minimising the sum of squared residuals (SSR) between model and data. In cases where the performance of the two-pool model was not different from the performance of the one-pool model, $C_{\mathrm{org}}^{0,\mathrm{A}}$ was simply set to 0. The parameter estimation procedure was subject to the constraint that the sum of $C_{\mathrm{org}}^{0,\mathrm{A}}$ and $C_{\mathrm{org}}^{0,\mathrm{B}}$ was less or equal to total measured organic carbon in the sample. Selection of either one or

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two-pool model was done by using the Akaike Information Criterion (AIC, Akaike 1974):

$$AIC = n\ln(SSR/n) + 2r \quad \text{(Eq. 3.3)}$$

where r is the number of adjustable parameters. The first term of Eqation 3.3 penalises a poor fit and the second term the number of parameters.

3.3.4 Determination of priming

3.3.4.1 Isotope analyses

We determined the $\delta^{13}C$ signal of the soils, the digestates, the manures, the HCO_3^- sorbed to the resin and the KOH solutions after 60 days of incubation. $\delta^{13}C$ [‰] is defined as follows (Craig 1953):

$$\delta^{13}C = \left(\frac{\frac{^{13}C}{^{12}C}\text{sample} - \frac{^{13}C}{^{12}C}\text{standard}}{\frac{^{13}C}{^{12}C}\text{standard}} \right) * 1000 \quad \text{(Eq. 3.4)}$$

where the standard is Vienna Pee Dee Belemnite (VPDB). The solid samples (soils and digestates) were combusted by a Thermo-Finnegan Flash HT elemental analyser converting carbon to CO_2. The sample gas was flushed via a con-flow IV to a Thermo-Scientific, Delta V advantage isotope ratio mass spectrometer. Analysis of internal laboratory standards ensured that the estimates of the isotopic values were accurate to within 0.1 ‰. The liquid samples (HCO_3^- sorbed to the AER, and KOH in which emitted CO_2 is dissolved) were transferred to oxygen free, N_2 -flushed glass vials (Labco, 12 mL, UK) containing 100 µL phosphoric acid (85 %, Merck, Darmstadt, Germany) for acidification. Exetainer vials of sampled gas were loaded onto Combi-Pal auto-sampler attached to the GasBench-II, a continuous flow interface. Helium pushed the sample air out of the exetainer into the GasBench where it was separated on a PoraPLOT Q gas chromatograph column, dried with

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a nafion drier and then passed to the mass spectrometer via an open split. Internal precision of analyses based on internal standards was 0.22 ‰.

The $\delta^{13}C$ signals of digestates SEW, SL and the manures did not differ sufficiently from the $\delta^{13}C$ signals of the soil. Therefore, determination of possible priming via isotope analysis could only be done for digestate M. We calculated the fraction of soil-derived and the fraction of digestate-derived C by a three pool mixing model which was reduced to a two pool mixing model, since the third pool – the resin – was independently determined by C measurement (see below):

$$S + D + R = 100\ \% \qquad \text{(Eq. 3.5)}$$

$$\delta^{13}C_{tot} = S * \delta^{13}C_S + D * \delta^{13}C_D + R * \delta^{13}C_R \qquad \text{(Eq. 3.6)}$$

where S [%] is the fraction of soil-derived emitted carbon, D [%] is the fraction of digestate-derived emitted carbon, R [%] the fraction of resin-derived emitted carbon (by releasing HCO_3^- due to nutrient anion sorption), $\delta^{13}C_{tot}$ [-] is the $\delta^{13}C$ signal of the total emitted C dissolved in the KOH solution, $\delta^{13}C_S$ [-] the $\delta^{13}C$ signal of the soil, $\delta^{13}C_D$ [-] the $\delta^{13}C$ signal of the digestate and $\delta^{13}C_R$ [-] the $\delta^{13}C$ signal of the HCO_3^- sorbed to the resin. The fraction of resin-derived C was determined by measuring the amount of C sorbed to the resin at the beginning and at the end of the incubation experiment and calculating the difference between the two. This could only be done for washed resins. Carbon was measured with a Total Organic Carbon Analyser (TOC-5050A, Shimadzu). If the fraction of soil-derived C in the KOH of digestate-amended soils was greater than the fraction of soil-derived C in the KOH of the control (without digestate), priming by digestate application would have occurred.

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3.3.4.2 Calculations of mineralised C related to added C

Indications of priming can also be gained by calculating the portion of mineralised C related to added C by the digestate:

$$F = 100(C_{tot} - C_c)/C_a \quad \text{(Eq. 3.7)}$$

where F [%] is the fraction of C emitted only by digestates related to added C, C_{tot} [mg kg^{-1}] is C emitted in total by soil, digestate and – if included – resin, C_c [mg kg^{-1}] is C emitted by the control (only soil and – if included – resin) and C_a [mg kg^{-1}] is C added by the digestate. When F exceeds 100 %, priming is likely to have occurred. An important assumption for this method is that the amount of C emitted by the resin in the digestate amended soil is nearly the amount of C emitted by the resin in the unamended control.

3.3.5 Characterisation of materials

Dry matter of the used materials was determined after freeze-drying for digestates and oven-drying (105 °C) for soils. We also measured the concentrations of C and N in freeze-dried (digestate) or oven-dried (soil) samples after grinding, using a CNS Analyser (Elementar Vario EL III). We analysed both samples before and after combustion with a muffle furnace (600 °C). The difference between C in the samples before combustion (total C) and C in the samples after combustion (inorganic C) was the content of organic C (C_{org}).

Gaseous emissions of N during drying of digestates cause an underestimation of N concentration in digestates. The acidification of digestates prior to drying can prevent gaseous N losses. In a pretest, digestates from the biogas plants we took digestates for this study from were acidified prior to drying and correction factors for non-acidified digestates

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were determined (Zirkler et al. 2014). We corrected measured N concentrations of the digestates in this study by these factors (1.53 for SEW, 1.39 for SL and 1.29 for M).

Freeze- and oven-dried samples were also digested in duplicate with 10 ml 69 % HNO_3 (Suprapur, Roth, Karlsruhe, Germany) for 15 min at 180 °C in a microwave pressure apparatus (Mars Xpress, CEM, Kamp-Lintfort, Germany). The concentrations of iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn) in the digested solutions were determined with flame AAS (Perkin Elmer Atomic Absorption Spectrophotometer 1100B) as well as lead (Pb) and cadmium (Cd) with a Varian graphite furnace AAS (SpectrAA 880Z). The pH measurements were conducted with a Knick pH-meter (761 Calimatic) directly in the untreated liquid digestate samples and in suspensions of soil and 0.01 M $CaCl_2$ at a ratio of 1:2.5. Furthermore, the soil-digestate-mixtures of the first experiment were extracted with 0.0125 M $CaCl_2$ at a mass ratio of 1:4 at days 16, 32 and 60 (30 minutes horizontal shaking at 200 rounds per minute) and the corresponding resins were shaken two times with 200 ml of 1 M NaCl (each for 1 hour at 200 rounds per minute). Nitrate was measured in the extracts with a Continuous Flow Analyser (550 nm wavelength, Skalar San plus system 5120).

3.4 Results

3.4.1 First experiment

In the first incubation experiment the amount of mineralised C (C_{em}) from the different treatments influenced by an AER after 60 days of incubation increased in the following order: S2 (910-913 mg kg^{-1}) < S1 (915-1073 mg kg^{-1}) ≤ SEW_1 (1008-1085 mg kg^{-1}) < MR (1250-1314 mg kg^{-1}) ≤ SL (1300-1335 mg kg^{-1}) < M_1 (1357-1422 mg kg^{-1}) ≤ MF (1341-1584 mg kg^{-1}, Figures 3.2+3.3). A similar distribution – but on a C_{em} level of approximately

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1000 mg C related to kg dry matter less – could be observed for SEW_1 and SL *without* AER (Figure 3.3). The amount of C_{em} from the SEW_1 amended soil without AER (103-170 mg kg^{-1}) was greater than that of the control without AER (76-98 mg kg^{-1}) and was even exceeded by the amount of C_{em} from SL amended soil without AER (228-368 mg kg^{-1}). The fraction (F) of mineralised C related to added C (C_a) after subtraction of C_{em} of the control (C_c) was 17 % for digestate SEW_1, 50-60 % for the two manures, about 85 % for digestate SL and 116 % for digestate M_1 after 60 days (Figure 3.4), given that C_{em} of the resins in the amended soils resembled C_{em} of the resins in the unamended control.

The data for C mineralisation of samples with AER could be well described by a two-pool model and data for samples without AER were sufficiently described by a one-pool-model (Figures 3.2+3.3, Table 3.2). As Table 3.2 shows, the amount of C being easily degradable is highest for the SL amended soil with AER. Among samples with AER, the SEW_1 amended soil exhibited the smallest easily degradable C pool and the lowest half-life for both, the easily and the less degradable C pool. The greatest half-lifes for both C pools were predicted for the sole soil S1 and for the MR amended soil.

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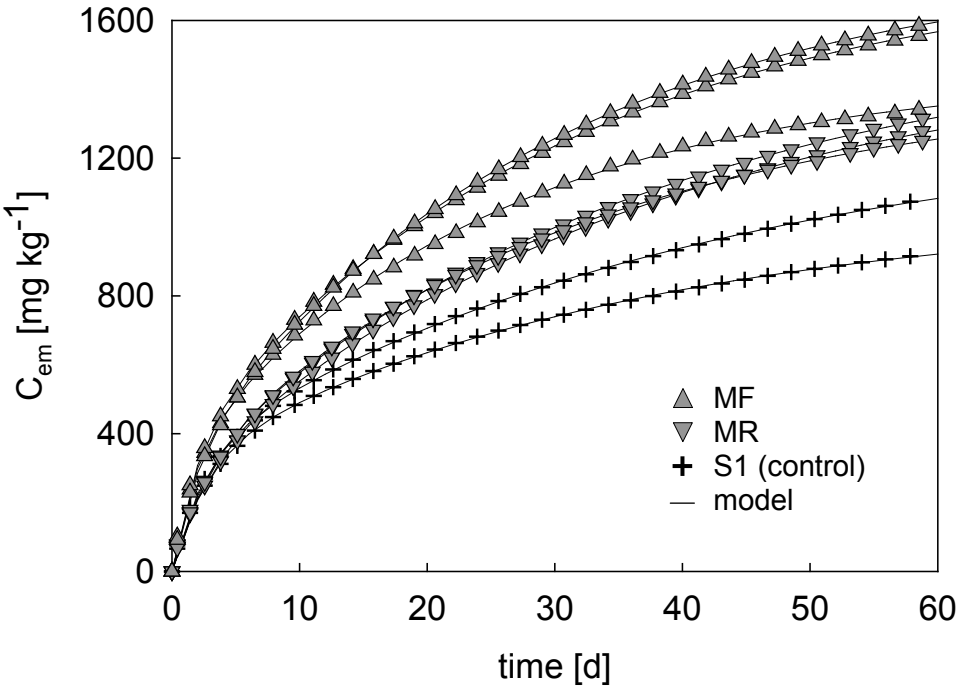

Figure 3.2: Mineralised C related to kg dry matter: Measured data (measured every half hour but thinned for better visibility) and fitted model for manures MF and MR in the first experiment.

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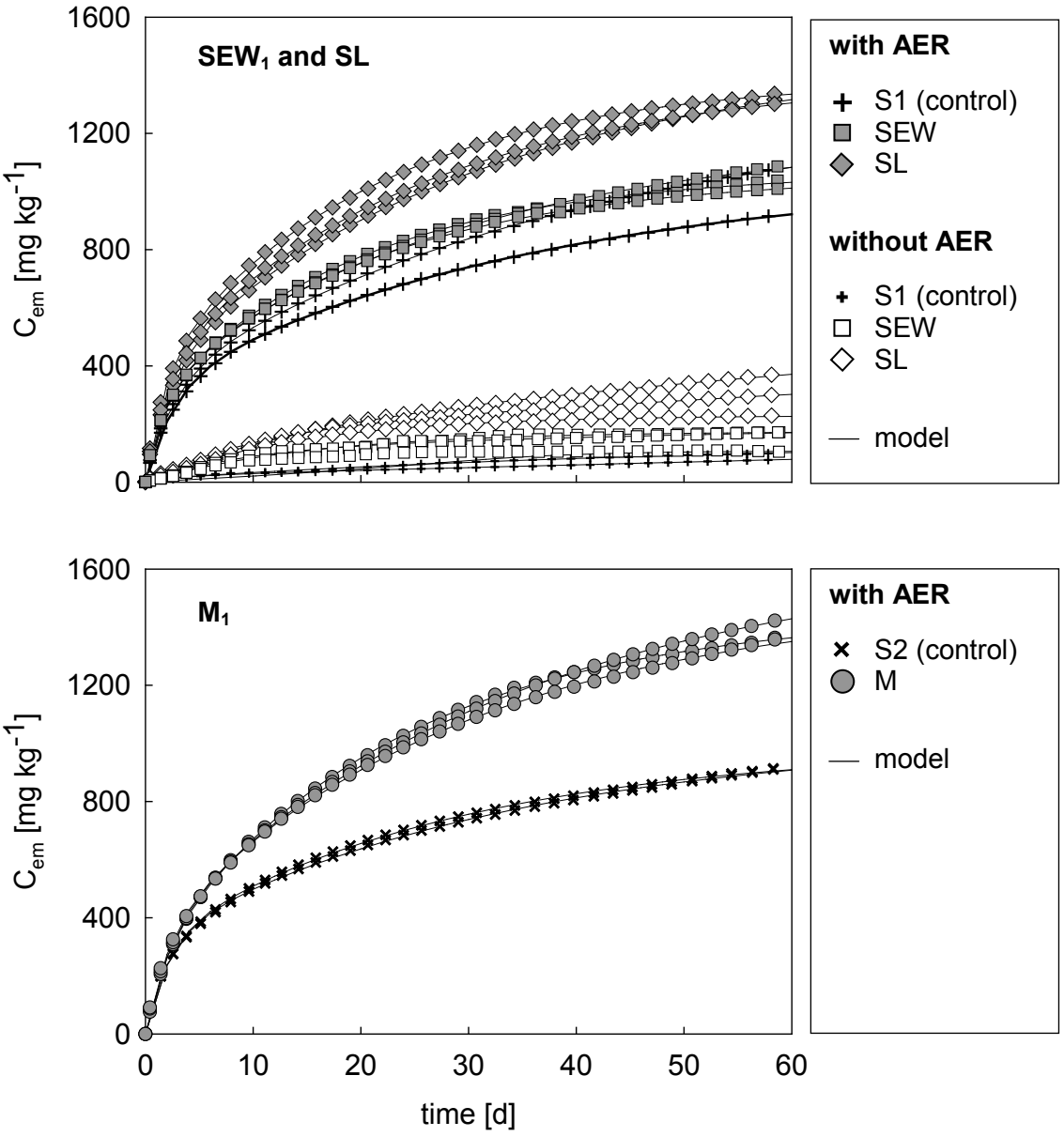

Figure 3.3: Mineralised C related to kg dry matter: Measured data (measured every half hour but thinned for better visibility) and fitted model for digestates SEW_1, SL and M_1 in the first experiment.

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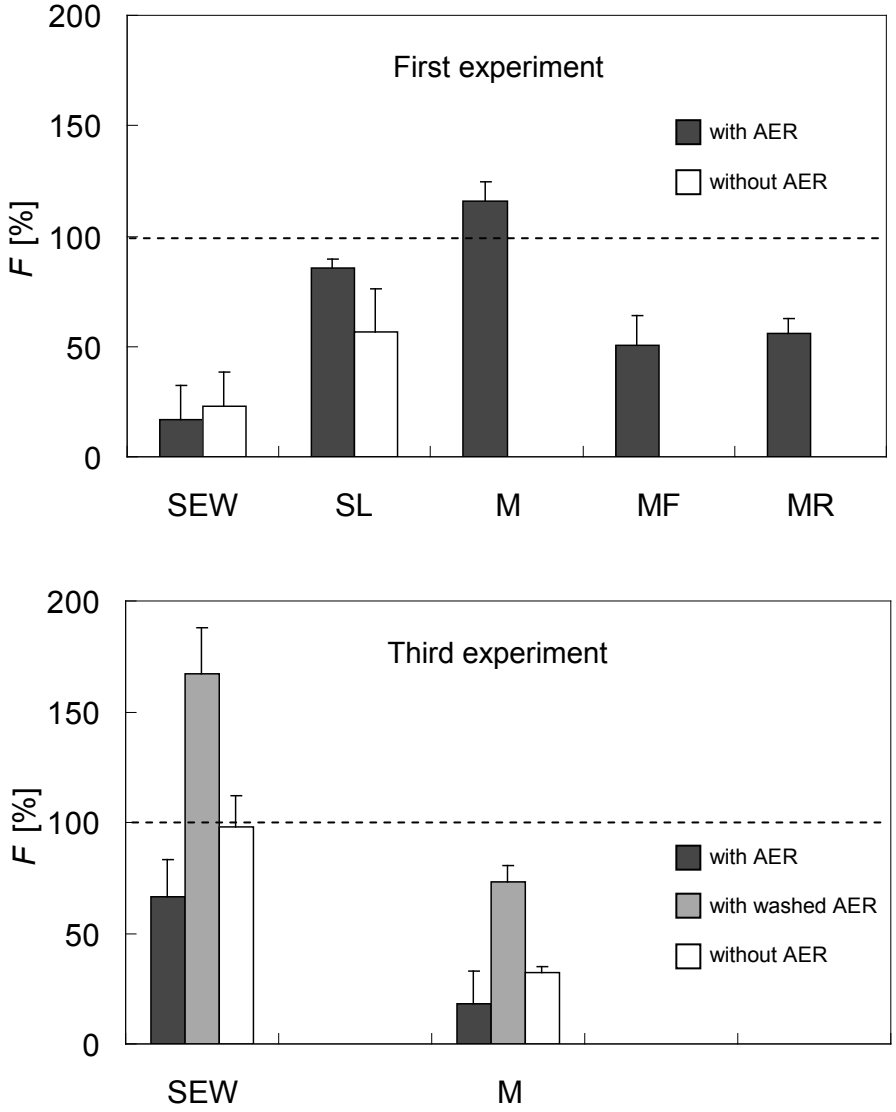

Figure 3.4: Fraction (F) of mineralised C related to C added by digestates after subtraction of C emitted by the control (Eq. 3.7).

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Table 3.2: Parameters estimated by a two-pool and one-pool model with a first order kinetics for C mineralisation and diagnostic variable R^2 rounded to four decimal places.

		$C_{org}^{0,A}$ [mg kg^{-1}]	k_A [t^{-1}]	h_A [d]	$C_{org}^{0,B}$ [mg kg^{-1}]	k_B [t^{-1}]	h_B [d]	R^2
				First experiment				
$S1_1$	with AER	**317** (312-323)	**0.393** (0.387-0.399)	**1.76** (1.74-1.79)	**864** (743-985)	**0.027** (0.025-0.029)	**26** (24-28)	0.9998-0.9999
$S2_1$		**309** (303-315)	**0.487** (0.478-0.495)	**1.42** (1.40-1.45)	**696** (685-707)	**0.033** (0.030-0.036)	**21** (19-23)	0.9992-0.9993
SEW_1		**279** (252-317)	**0.585** (0.491-0.632)	**1.20** (1.10-1.41)	**828** (788-878)	**0.045** (0.034-0.051)	**16** (14-20)	0.9996-0.9998
SL		**356** (353-361)	**0.510** (0.435-0.591)	**1.38** (1.17-1.59)	**1060** (1037-1100)	**0.041** (0.034-0.049)	**17** (14-21)	0.9998-0.9999
M_1		**299** (241-332)	**0.478** (0.418-0.551)	**1.47** (1.26-1.66)	**1235** (1177-1319)	**0.036** (0.030-0.044)	**20** (16-23)	0.9996-0.9999
MF		**312** (286-342)	**0.528** (0.501-0.567)	**1.32** (1.22-1.38)	**1351** (1141-1491)	**0.036** (0.033-0.041)	**19** (17-21)	0.9997-0.9999
MR		**268** (255-277)	**0.384** (0.355-0.401)	**1.81** (1.73-1.95)	**1244** (1150-1300)	**0.029** (0.027-0.032)	**24** (22-26)	0.9998-0.9999
$S1_1$	without AER	-	-	-	**102** (83-121)	**0.031** (0.028-0.033)	**23** (21-24)	0.9702-0.9927
SEW_1		-	-	-	**148** (107-174)	**0.082** (0.064-0.109)	**9** (6-11)	0.9861-0.9956
SL		-	-	-	**310** (229-406)	**0.053** (0.035-0.069)	**14** (10-20)	0.9907-0.9983
				Third experiment				
$S1_3$	with AER	**421** (398-445)	**0.324** (0.287-0.359)	**2.16** (1.93-2.41)	**983** (973-993)	**0.033** (0.029-0.036)	**21** (19-24)	0.9990-0.9993
$S2_3$		**256** (239-275)	**0.618** (0.552-0.693)	**1.13** (1.00-1.26)	**919** (902-946)	**0.032** (0.031-0.034)	**22** (21-23)	0.9997-0.9999
SEW_3		**635** (576-699)	**0.191** (0.183-0.196)	**3.64** (3.55-3.79)	**1111** (983-1281)	**0.024** (0.017-0.028)	**31** (25-41)	0.9993-0.9996
M_3		**424** (388-458)	**0.246** (0.206-0.276)	**2.86** (2.51-3.36)	**911** (835-1028)	**0.024** (0.021-0.028)	**30** (25-33)	0.9989-0.9992
$S1_3$	with washed AER	**135** (127-140)	**0.273** (0.212-0.324)	**2.62** (2.14-3.26)	**781** (588-909)	**0.014** (0.010-0.018)	**52** (38-68)	0.9997-1.0000
$S2_3$		**196** (182-212)	**0.215** (0.174-0.236)	**3.29** (2.93-3.98)	**1133** (972-1408)	**0.010** (0.007-0.013)	**70** (54-93)	0.9992-0.9999
SEW_3		**365** (243-497)	**0.133** (0.097-0.176)	**5.55** (3.95-7.17)	**904** (701-1017)	**0.023** (0.020-0.026)	**31** (27-35)	0.9995-0.9997
$M_{3'}$		**323** (236-383)	**0.157** (0.137-0.193)	**4.54** (3.59-5.11)	**7769** * (1096-11121)	**0.008** (0.001-0.023)	**14** (5-31)	0.9999
$S1_3$	without AER	**64** ** (63-66)	**0.140** ** (0.123-0.158)	**5.02** ** (4.39-5.65)	**448** ** (419-476)	**0.012** ** 0.010-0.013)	**60** ** (53-67)	0.9993-0.9999
$S2_3$		**87** (72-95)	**0.130** (0.111-0.141)	**5.38** (4.91-6.26)	**1106** (428-1748)	**0.006** (0.002-0.012)	**182** (58-291)	0.9991-0.9999
SEW_3		**385** (353-431)	**0.068** (0.060-0.078)	**10.37** (8.84-11.52)	**7466** (7421-7498)	**0.00038** (0.00033-0.00041)	**1860** (1684-2084)	0.9995-0.9996
M_3		**267** (222-303)	**0.065** (0.056-0.074)	**10.75** (9.38-12.35)	**8014** * (1676-11197)	**0.0009** (0.0002-0.0023)	**1926** (306-2814)	0.9998-0.9999

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Figure 3.5 shows pH-values of the soils and soil-digestate-mixtures at the different times of incubation. The pH of all samples influenced by AER increased within 10 days from about 6 to 6.8-8.8. The MR and SL amended soils exhibited the greatest pH, followed by M_1 and MF amended soils. A clearly lower pH was found for samples without AER (5.2-5.6).

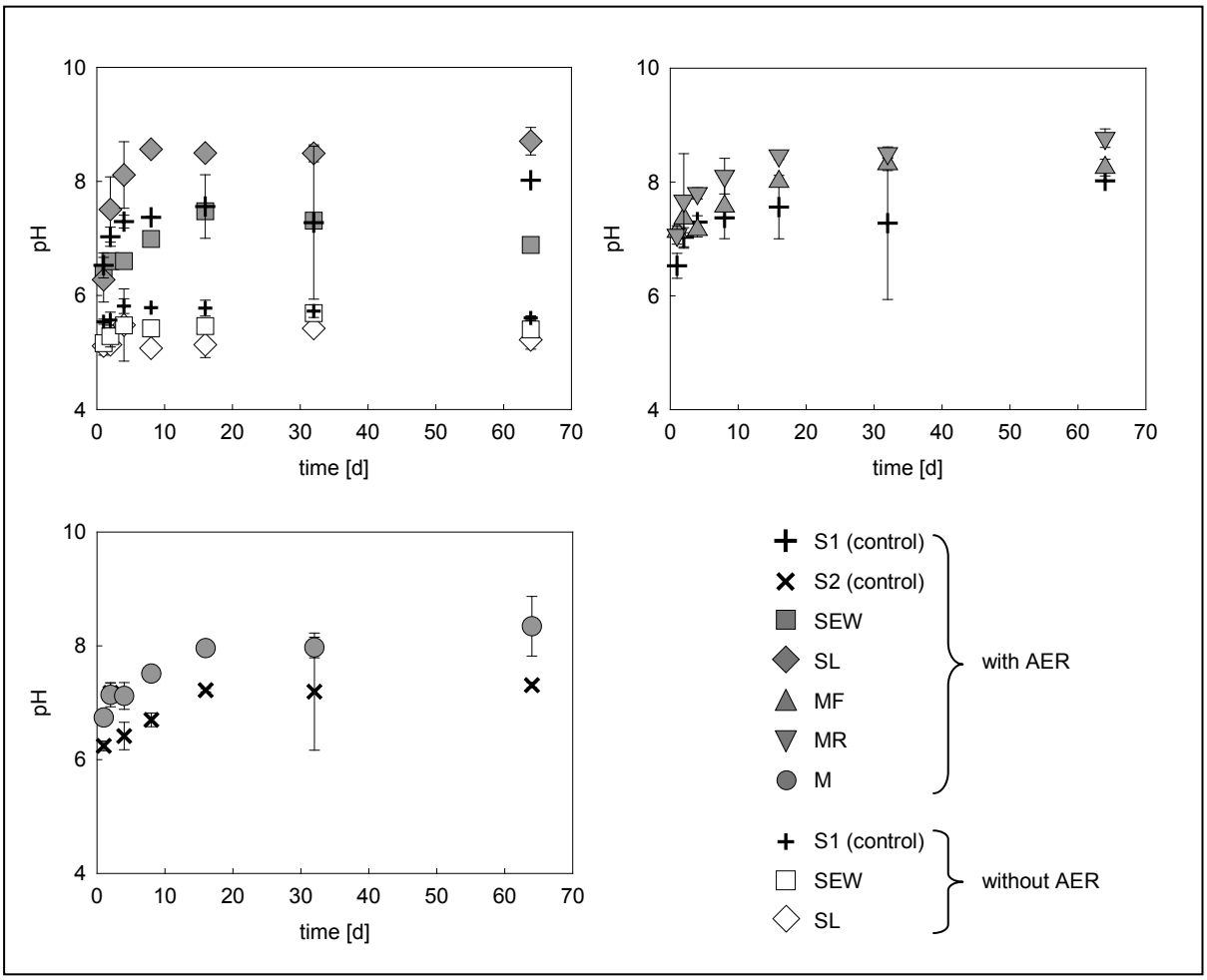

Figure 3.5: pH-values of the soils and soil-digestate-mixtures during 60 days of incubation (first experiment).

3.4.2 Second experiment

In the second experiment, the amount of C_{em} after 40 days of incubation was similar for both, SEW_2 and M_2 amended soils (Figure 3.6). About 700 mg C per kg dry matter was mineralised from samples with AER, 300-390 mg kg^{-1} from samples with washed AER and 109-

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217 mg kg^{-1} from samples without AER. The amount of C_{em} from samples with AER did not reach the amount of C_{em} from unamended controls of the first experiment. The experiment was stopped after 40 days.

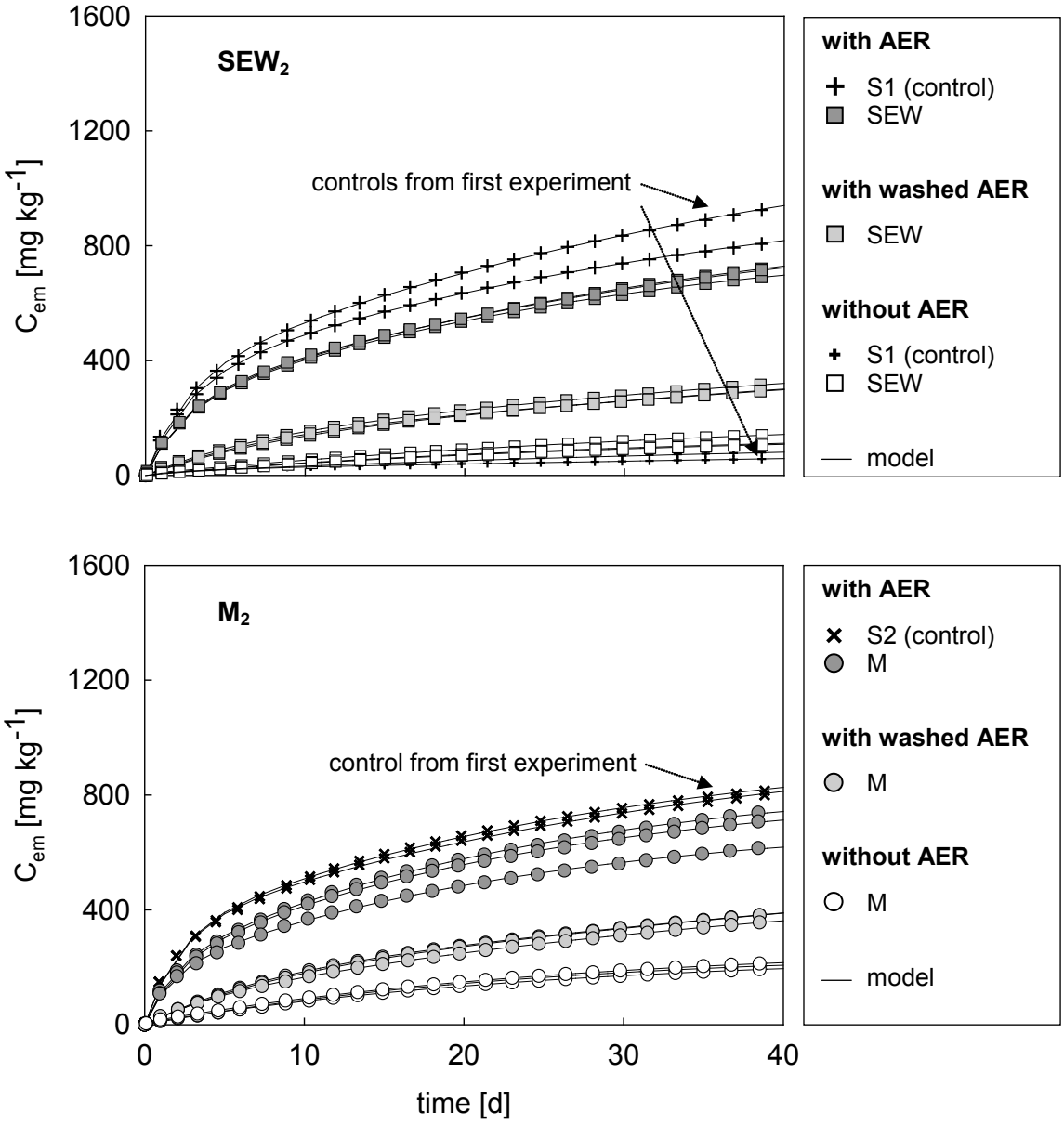

Figure 3.6: Mineralised C related to kg dry matter: Measured data (measured every half hour but thinned for better visibility) and fitted model for digestates SEW_2 and M_2 in the second experiment.

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Figure 3.7 shows that pure anion exchange resins in the non-washed form emitted 168 mg C (mean) related to a potential kg of soil or soil-digestate-mixture dry matter, while washed resins emitted a C amount of 88 mg kg^{-1} after 40 days. Since the slope of $C_{em}(t)$ is close to 0 at the end of the 40 days, we may assume similar C_{em} concentrations for an incubation time of 60 days.

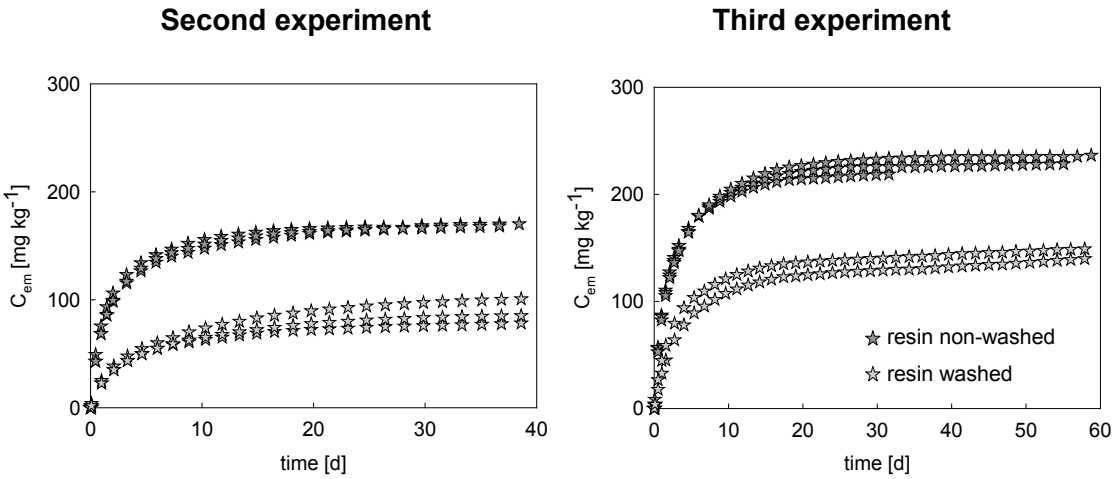

Figure 3.7: Carbon emitted by pure washed and non-washed resins related to kg potential dry matter of soil or soil-digestate-mixture (measured every half hour but thinned for better visibility, one replicate of washed resin failed completely (not shown) and two replicates of non-washed resin failed after 30 and 55 days in the third experiment due to lost cable connection).

3.4.3 Third experiment

Carbon emissions from the SEW_3 amended soil (1283-1359 mg kg^{-1}) influenced by non-washed AER exceeded those from the M_3 amended soil (949-1065 mg kg^{-1}, Figure 3.8) in the third experiment. Lower C emissions which are similar for both SEW_3 and M_3 amended soils could be observed for samples with washed AER: 860-952 mg kg^{-1} for SEW_3 amended soil and 895-946 mg kg^{-1} for M_3 amended soil. Lowest C emissions were found for samples without resin influence. The SEW_3 amended soil emitted 463-531 mg kg^{-1} and exceeded C

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emissions from the M_3 amended soil without AER (381-404 mg kg^{-1}). On condition that C_{em} of resins in amended soils is similar to C_{em} of resins in the unamended control the fraction (F) of mineralised C related to added C was 67 % for digestate SEW_3 after 60 days when influenced by a non-washed AER while F was only 18 % for digestate M_3 (Figure 3.4). The fractions were 167 % for digestate SEW_3 with washed AER and 73 % for digestate M_3. In samples without influence of AER, F was 98 % for SEW_3 and 32 % for M_3. All data of this experiment can be best described by a two-pool model (Figure 3.8). In general, the easily degradable C pool is greatest for samples with non-washed AER, smaller for samples with washed AER and smallest for samples without AER (Table 3.2). Half-lives are lower with increasing influence by the HCO_3^- from the AER.

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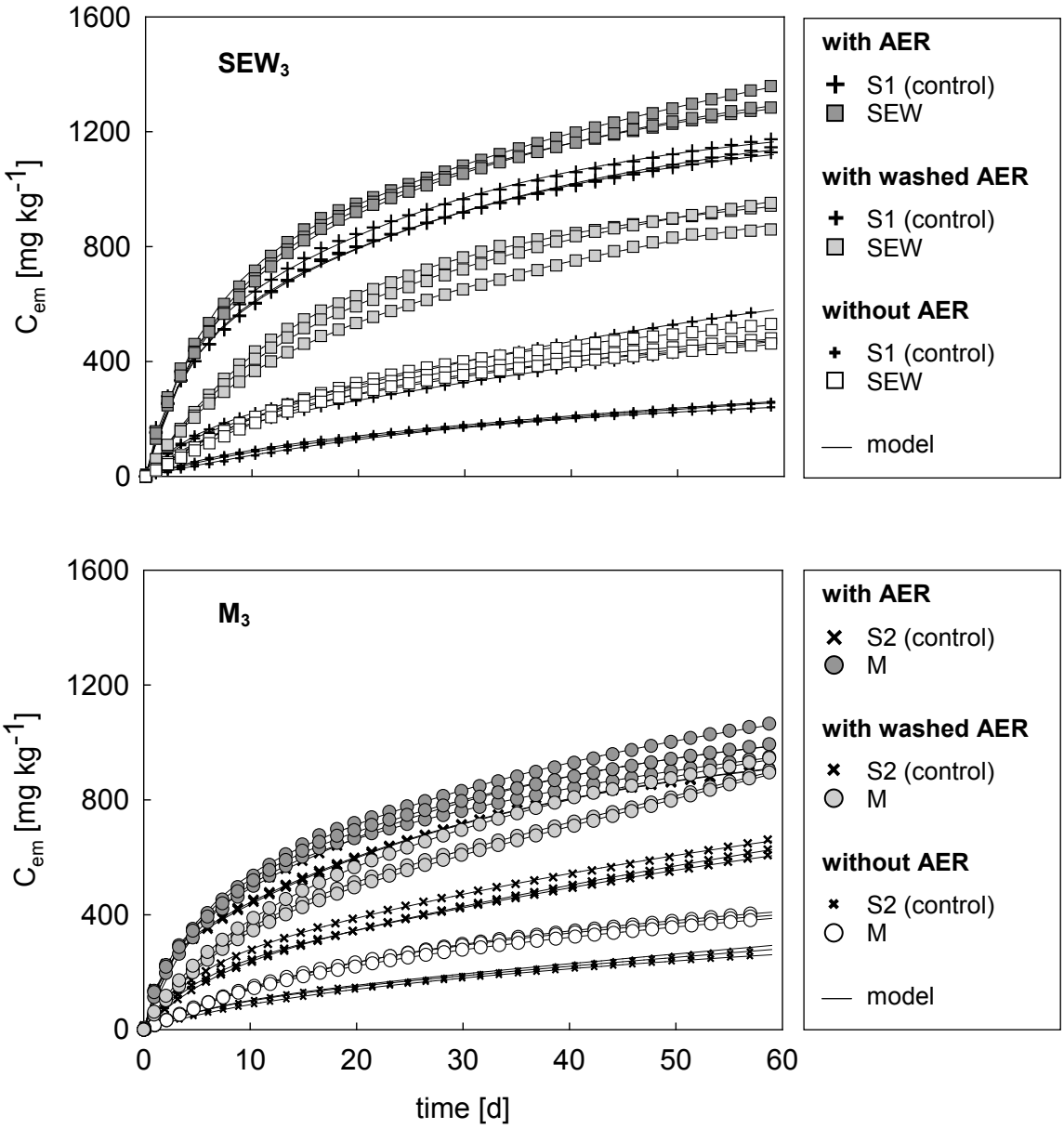

Figure 3.8: Mineralised C related to kg dry matter: Measured data (measured every half hour but thinned for better visibility) and fitted model for digestates SEW_3 and M_3 in the third experiment.

Non-washed resins without any soil or amendment emitted about 230 mg C related to kg dry matter of a potential soil or soil-digestate-mixture, and 140-200 mg kg^{-1} when pre-treated by washing (Figure 3.7). The amount of C emitted by the washed resins which have been incubated with the M_3 amended soil was 185 mg kg^{-1}. The washed resins incubated with the SEW_3 amended soil emitted a C amount of 278 mg kg^{-1}.

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The concentration of C mineralised from the soil fraction in the soil-digestate-M_3-mixture with washed AER and without AER compared to C mineralised by the soil from the corresponding control can be seen in Figure 3.9. No significant difference between M_3 amended soil and control could be detected, i.e. no priming had occurred.

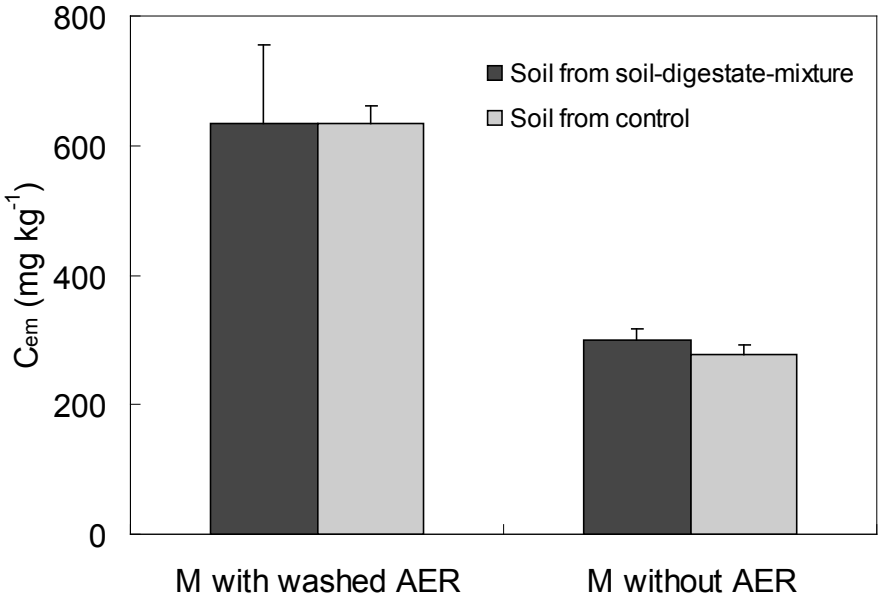

Figure 3.9: Carbon mineralised by the soil in the soil-digestate-mixture and by the soil in the control (only soil, no amendment) related to kg dry matter.

After 60 days of incubation, pH was lowest for soil-digestate-mixtures without AER and highest for soil-digestate-mixtures with non-washed AER (Table 3.3).

Table 3.3: pH-values for soil-digestate-mixtures in the third experiment after 60 days of incubation

	pH
SEW with AER	8.11 (8.02-8.23)
SEW with washed AER	6.59 (6.49-6.71)
SEW without AER	5.38 (5.35-5.41)
M with AER	7.24 (7.19-7.30)
M with washed AER	6.42 (6.39-6.45)
M without AER	5.80 (5.76-5.85)

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3.5 Discussion

3.5.1 Effect of AER on mineralisation of OM

All incubation experiments showed that the anion exchange resins had a stimulating effect on OM mineralisation. Table 3.2 shows that the modelled sizes of the easily available C pool and the mineralisation rates increased when resins were integrated. We assume that HCO_3^- from the resins was exchanged by anions from the soil-digestate-mixtures. Accordingly, pH rose due to proton association by bicarbonate. Increasing pH may have stimulated OM mineralising bacteria in the soil-digestate-mixtures. This effect was higher for non-washed resins compared to the washed ones due to their greater potential to emit HCO_3^-. Therefore, incubations influenced by non-washed resins rather represented “extreme scenarios” with pH 7 to above 8. In contrast, *washed* resins may better have simulated the anion exchange of plant roots and could thus have generated pH conditions closer to those in the real rhizosphere (in case of *anion* dominated nutrition) compared to samples without resins. However, pure resins without soil and/or digestate also emitted considerable amounts of CO_2 (Figure 3.7). These have to be accounted for when interpreting CO_2 emissions from soil-digestate-mixtures influenced by AER. Although C emissions by pure resins in the third experiment exceeded those in the second experiment by about 50 mg related to kg potential dry matter, the mineralisation intensities of the soil-digestate-mixtures are comparable within each experiment. Moreover, relations of the mineralisation between differently treated soils like SEW/M are also comparable between the experiments.

It is important to bear in mind that the estimated pool sizes in the modelling approach are not solely determined by the OM material (molecular structure, complexation with mineral compounds and occludation in aggregates) but also by the conditions, like water content, temperature and acidity. As water contents and temperatures have been kept constant for all experiments, the influence of HCO_3^- released by AER must have changed the pool sizes.

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3.5.2 Mineralisation of manures and digestates in soils

The mineralisation courses of the references MF and MR are in the range of those of digestates SL and M_1 (Figures 3.2+3.3), with MF having induced a higher mineralisation than MR. This is plausible, since the rather fresh manure MF probably still contained more easily degradable OM than the rotted MR. Accordingly, the labile C pool of MR is predicted smaller than the one of MF in our two-pool model (Table 3.2). Related to the amount of C added by the manures, 50-56 % of MF and MR were mineralised, which ranges between the portions of C mineralised in the SEW_1 and SL treatments related to added C (Figure 3.4). The mineralisation of the manures in our experiment was slightly higher compared to findings of Klimanek (1982, 44 % for manure and a soil similar to ours) which can be attributable to the influence of the AER. However, basically, mineralisation of MF and MR were in a similar range as mineralisation of manure found in the literature (Bernal and Kirchmann 1992, Ajwa and Tabatabai 1994). Therefore, we assume that our incubation experiment, though unconventionally integrating AER, gives sensible results.

The C emissions of soils incubated with digestates differed distinctly between the experiments. In the first experiment, the mineralisation of M_1 with AER was greater than mineralisation of SEW_1 with AER, whereas this was reversed in experiment 3. In the second experiment, a mineralisation of both digestates could not be observed since the amount of C emitted by the soil-digestate-mixtures did not even reach the amount emitted by the controls of experiment 1. These variations between the experiments are surprising in consideration of the fact that the same materials have been used: Digestates for experiment 2 were the same as for experiment 1, but one year stored at 4 °C, and digestates for experiment 3 were similar to the ones for experiment 1, and just sampled from the same biogas plants one year later. Generally, the differences in mineralisation between experiment 1 and 2 can be explained by proceeded mineralisation of easily available organic matter of the digestates during the one year of storage and/or the conversion of easily available OM to less

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degradable OM. Therefore, the subsequent incubation with the soil might not have led to a mineralisation further than basal respiration. The potential of digestates to continue mineralising during storage is well known (Lansche and Müller 2012, Liebetrau 2010). However, according to researchers who investigated stored digestates, the methane and CO_2 production potential as well as changes in elemental composition of digestates decreases drastically with decreasing storage temperature and when digestates are stored in closed containers (Lansche and Müller 2012, Liebetrau 2010, Paavola and Rintala 2008). Since the digestates used in our first experiment have been stored at only 4 °C in closed bottles, we assume that the conversion of easily degradable OM to a less degradable fraction was more likely the reason for low CO_2 emissions than proceeded mineralisation.

Discrepancies between results of the first and the third experiment may be caused by several reasons. However, a lot of these reasons can be excluded in our case. They are discussed in the following.

Concentrations of heavy metals, which may inhibit microbial activity (Gülser and Erdogan 2008) and hence mineralisation, were similar for digestates in experiment 1 and 3 despite Pb concentrations which were five times higher for SEW_3 in the third experiment compared to SEW_1 in the first experiment (Table 3.1). However, SEW_3 in the third experiment was exactly the digestate having been mineralised to a greater extend than SEW_1 in the first. Thus, lower CO_2 emissions due to higher contents of heavy metals can be excluded.

The pH cannot explain mineralisation differences as well since pH values of all digestates were similar to each other (Table 3.1). After application of digestates SEW_1 and SEW_3 soil pH did not change substantially in samples without AER (Figure 3.5 and Table 3.3). Nevertheless, the amount of C_{em} by the SEW_3-digestate-mixture was more than double the

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amount by the SEW_1-digestate-mixture (Figures 3.3 and 3.8). Only in samples with AER, pH goes with the amount of C_{em}.

The amount of C_{em} usually also depends on the amount of C added to the soil and on nitrate availability in the soil (Bertora et al. 2008). However, the concentration of C is similar for the digestates between the first and the third experiment (Table 3.1). Nitrate availability of SEW_1 and M_1 amended soils differed after 60 days of incubation (380 and 530 mg kg^{-1} dm for SEW_1 and M_1, respectively). This may have promoted mineralisation discrepancies between SEW_1 and M_1 in the first experiment. However, mineralisation discrepancies between samples of the different experiments cannot be explained since data about nitrate concentrations in the third experiment are not available.

The heterogeneity of the digestates themselves and their distribution in the soil may sometimes influence the extent of OM mineralisation. It is widely known that microbial activity can vary on a small scale and often occurs in the form of “hotspots” (Parkin 1987). Depending on the distribution of these hotspots within the soil-digestate-mixtures, great variations in microbial activity in spite of otherwise identical experimental conditions could actually occur. However, such variations in microbial activity would lead to high variation of replications which is not the case in our experiments.

The most likely reason for discrepancies between the first and third experiment is a great variation in digestate stability. During anaerobic digestion, easier degradable compounds like proteins, easily hydrolysable lipids and carbohydrates are primarily converted to methane while less degradable compounds like lignin remain in the digestate (Angelidaki and Ahring 2000). Hence, digestate organic matter is rather stable after anaerobic digestion compared to their feedstock material (Marcato et al. 2009, Tambone et al. 2009). This is supported by Chen et al. (2012) who observed a 34 % greater increase of soil C and a lower C turnover

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after application of anaerobically digested maize compared to non-fermented. However, several studies did not confirm these findings. Digestates (from cattle slurry) did not show higher stability after digestion (Cayuela et al. 2010) and anaerobically digested pig manure caused the highest mineralisation compared to aerobically and non-digested pig manure (Bernal and Kirchmann 1992) which was attributed to the availability of easily degradable OM. Digestates contain larger amounts of easily degradable OM under suboptimal digestion conditions. Process failures such as over-acidification or ineffective stirring are responsible for incompletely digested OM (Kleyböcker et al. 2012). Alburquerque et al. (2012a) also indicated that anaerobic digestion can lead to the production of unstable digested materials and found a correlation between biodegradability and extent of mineralisation of digestates. Taking these considerations into account, we assume that variations in the biodegradability of the digestates SEW and M between first and third experiment due to inconstancies of the fermentation procedures are at least one reason for discrepancies in mineralisation between first and third experiment.

3.5.3 Priming

Measurements of $\delta^{13}C$ showed that no priming had occurred for the M_3 amended soil with washed AER and without AER in the third experiment (Figure 3.9). According to our calculations of the portion of emitted C related to added C (Eq. 3.7, Figure 3.4), digestate M_1 with non-washed AER may have caused priming in experiment 1. The portion of emitted C related to added C exceeded 100 % and thus probably included soil-derived C, provided that C_{em} of the resins in amended soils was similar to C_{em} of the resins in the unamended control. The other way round, digestate SEW_1 with non-washed AER probably did not cause priming in the first experiment but SEW_3 with washed AER may have caused priming in the third (Figure 3.4). The whole pattern reminds of the discrepancies observed for the mineralisation curves in Figures 3.3 and 3.8. This means that priming went along with great mineralisation

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activity. Since great mineralisation activity can be a result of great amounts of readily available C, we assume that the addition of mineral N by digestates may not be sufficient for priming in soils, but should also include a certain amount of readily available C. This amount of readily available C in combination with high amounts of mineral N may stimulate microorganisms to accelerated activity and growth. After the readily degradable compounds have been depleted, the grown population of microorganisms may try to prolong their activity and produce extracellular enzymes to hydrolyse more complex and less available OM. This process is often accompanied by co-metabolism of SOM, i.e. priming (Blagodatskaya and Kuzyakov 2008).

It is important to bear in mind that our priming results gained by the calculation of *F* have to be interpreted with caution. Values for *F* can only be seen as valid if values for concentrations of emitted C by the resins in amended and unamended soils are assumed to be similar. Based on this precondition, the portions of emitted C related to added C by SEW_3 and M_1 exceeded those found for digestates in the literature so far (Alburquerque 2012a, Bernal and Kirchmann 1992). We assume that the presence of AER in our cases stimulated microbial activity and therefore favoured priming. Kuzyakov (2010) already emphasised the importance of plant roots creating “hotspots” by the release of exudates, for instance, which stimulate microbial activity and promote priming. We assume that the pH enhancing effect of plant roots should also be taken into account when investigating priming in incubation experiments.

3.6 Conclusions

The mineralisation of digestates in soils and their potential to promote priming can vary greatly, even though they stem from the same biogas plants, but sampled at different times.

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The degree of digestate stability may be the main reason and can differ over time to an extent that a single sample taking and incubation may not give reliable information about the mineralisation of digestates and whether priming is induced or not. Our results indicate that priming was not solely promoted by the addition of mineral N in digestates, but also by the addition of easily available OM, which may at first increase microbial growth and finally SOM co-metabolisation after depletion of the easily available compounds. Further research could deal with the hypothesis that neither fully digested OM promotes priming (since microorganisms need a sufficient amount of readily available C), nor little digested OM promotes priming (since microorganisms would preferentially degrade the added substrate). Instead, moderately digested OM, i.e. a digestate with a sufficient but not too high amount of readily available OM, may induce priming in soils.

Storage of digestates – even in closed containers and at low temperatures – can alter the magnitude of digestate mineralisation in soils. Therefore, studies about the mineralisation of digestates in soils should be conducted with digestates at the end of the storage process that often follows anaerobic digestion. A too early sampling from the anaerobic digester would overestimate the extent of digestate mineralisation in soils.

3.7 Acknowledgements

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4 P mineralization and transport in the vicinity of an anion sink – experiments and modeling[3]

4.1 Abstract

Digestates from biogas plants provide valuable crop nutrients, such as phosphorus (P). Plant uptake of anions like phosphate (PO_4^{3-}) causes an alkalinisation of the rhizosphere due to release of hydrogen carbonate (HCO_3^-). We investigated the transport of P to an anion sink simulating the anion exchanging activity of a plant root in digestate amended soils and hypothesised that the HCO_3^- source induces enhanced mineralisation of organic P. We expected this effect to strengthen with decreasing distance from the HCO_3^- source. Anion exchange resins (AER) saturated with HCO_3^- were used in an incubation experiment. Available PO_4-P was measured in 10 slices of the soil-digestate-mixtures (1 mm each) after 2, 8 and 32 days, and in the resin and total uncut samples after 2, 4, 8, 16, 32 and 64 days of incubation. Anion exchange resins increased pH by 2-3 units. Concentrations of available PO_4-P in uncut samples increased within 4 days, which we attribute to enhanced microbial P mineralisation activity. Afterwards, PO_4-P concentrations decreased within 4 days, which we attribute to precipitation of calcium phosphate due to further increasing pH. Concentrations of PO_4-P were lowest near the AER and showed a peak migrating away from the resin with time. A new model linking diffusive transport and mineralisation intensity of organic matter as a function of distance from AER could well describe the observed spatial PO_4-P distribution and accumulation in the resin. However, further studies are needed to account for

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mechanisms like precipitation in models for P transport.

4.2 Introduction

The increasing number of biogas plants has also increased the amount of biogas residues, the digestates. After fermentation, these digestates, rich in nutrients such as nitrogen (N) and phosphorus (P), can be applied as a fertiliser for agricultural production (Garg et al. 2005, Abubaker et al. 2013, Clements et al. 2012, Haraldsen et al. 2011), provided that they are non-hazardous for soils and the environment in general. Phosphorus limitation in agricultural soils necessitates P amendment, the demand of which is predominantly met through the use of phosphate rock (Cordell et al. 2009). Due to contamination with cadmium and uranium (Rothbaum et al. 1986, Kratz and Schnug 2006), the increasing scarcity of easily available stocks (Cordell et al. 2011), and resulting dependency on exporters (Schröder et al. 2010), there is a need to find a replacement for phosphate rock fertiliser. Reuse of all available and harmless P sources will also avoid unnecessary waste streams.

Both organic and inorganic P reserves are contained in soils. The hydrolisation of complex organic P compounds by various enzymes (phosphatases) produced by soil microorganisms (Tao et al. 2008), or plant roots and fungi (Wang et al. 2013) is necessary for the uptake of P by plant roots. The activity of phosphatases is pH dependent. Phosphomonoesterase, mostly delivered by roots or fungi, has been observed and classified in acidic to alkaline pH conditions, while the phosphodiesterase activity was higher under neutral to alkaline conditions, and was associated with bacteria and actinomycetes (Eivazi and Tabatabai 1977, Turner and Haygarth 2005).

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Plant roots extrude ions to maintain electrical neutrality during uptake of charged nutrients. The uptake of cations such as NH_4^+ or K^+ results in pH decreases in the rhizosphere due to the extrusion of H^+ ions. Uptake of anions such as NO_3^- or PO_4^{3-} results in HCO_3^- or OH^- release, and the increase of pH (Marschner 1995, Dakora and Phillips 2002). As a result the mineralisation of organic matter (OM) usually increases as well and this could lead to an enhanced P availability.

As described by Barber et al. (1963), nutrient transport to plant roots depends on mass flow and diffusion in the soil solution. In fact, diffusion may contribute more than 90 % of phosphate transport to plant roots (Barber 1995). Hitherto existing models do not consider additional P supply due to mineralisation of OM.

The aim of our study was the investigation of phosphate-dynamics in digestate amended soils influenced by an anion sink simulating the anion exchanging activity of plant roots. We hypothesised that the presence of an anion sink (HCO_3^- source) and the consequentially increasing pH induces enhanced mineralisation of organic P and that this effect increases with decreasing distance from the anion sink. Thus in our model conception, a term considering phosphate release as a result of location dependant OM mineralisation has been combined with a diffusion model.

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4.3 Materials and Methods

4.3.1 Experiment

We incubated a topsoil taken from an arable field of the Leibniz Centre for Agricultural Landscape Research (ZALF) near Prenzlau, Germany with two different digestates. The sieved soil (<5 mm) was mixed with liquid digestate from a) sewage sludge, fat and mash and b) pig slurry and food residues. The digestates were taken from full scale biogas plants in Germany. The material characteristics are summed up in Table 4.1.

Table 4.1: Characteristics of soil and digestates used in the study (dm: dry matter, C_{org}: organic carbon, N_{tot}: total nitrogen, P_{tot}: total phosphorus)

	pH	dm [%]	C_{org} [g kg^{-1} dm]	texture	N_{tot} [g kg^{-1} dm]	P_{tot} [g kg^{-1} dm]	abbreviation
soil	5.8	-	6.4	silty sand	1.1	0.2	-
digested sewage sludge, fat and mash	7.4	2.6	240	-	57	33	SEW
digested pig slurry and food residues	7.8	3.2	328	-	48	22	SL

We applied 42.5 g SEW fresh matter (fm) and 38.4 g SL fm to 1 kg dm soil. The application rate corresponds to a nitrogen application of 170 kg ha^{-1}. For the incubation the soil was mixed with the digestates and if necessary filled up with deionised water to 63.5 % of the water holding capacity of the soil. Each sample containing 50 g of the homogenised mixture was filled in a jar lined with aluminium foil and compacted to a bulk density of 1.35 g cm^{-3} (soil column diameter: 4.6 cm, hight: 2 cm). The jar was placed in a polypropylene beaker and closed by a screw cap.

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Six replicates of each digestate-soil-mixture (SDM) were incubated: three with and three without addition of a layer of 4 g anion exchange resin in the HCO_3^- form (Merck Ion exchanger III, strongly basic) at the bottom of the jar beneath the SDM. The resin has not been washed after saturation with HCO_3^- and thus represents an “extreme scenario” concerning HCO_3^- release. A glass fibre filter (Pall, Type A/D, 3 µm pore diameter, thickness 254 µm) separated the resins from the SDM.

The mixtures of soil and digestate were incubated in a respirometer at 20 °C (water basin) for 64 days. Additionally the whole set of samples was repeated six times in parallel, placed in a cup board at a constant air temperature of 20 °C and sampled destructively after 1, 2, 4, 8, 16, or 32 days. A preliminary test showed that samples in the respirometer emitted about 1.7 % less CO_2 than samples in the cupboard. We considered this difference as negligible and assumed similar conditions and processes for respirometer and cupboard. After the corresponding incubation period the SDM samples were homogenised by hand and air dried. Calcium-acetate-lactate (CAL) extractable P (Schüller 1969) was determined by ICP-OES (iCAP 6000 Series, Thermo Scientific). Soil pH-values were measured in 0.01 M $CaCl_2$ solution (sample:solution ratio of 1:2.5) using a Knick pH-meter (761 Calimatic).

As shown in Table 4.2 another two replicates at days 2, 8 and 32 were frozen by liquid nitrogen. After removing the resin and glass fibre filter, the frozen columns were stuck onto a carrier plate. The first centimetre of the frozen soil columns which formerly directly adjoined the filter and the resin was cut into 1 mm slices with a Cryomikrotom (Microm HM 500M) at -25 °C. For columns without resin the first, fifth and ninth millimeters were cut out exemplarily.

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Table 4.2: Replicates for each soil-digestate-mixture (SDM) used in the incubation experiment

	with resin		without resin	
	m	f	m	f
1 day in cubboard	3	-	3	-
2 days in cubboard	3	2	3	2
4 days in cubboard	3	-	3	-
8 days in cubboard	3	2	3	2
16 days in cubboard	3	-	3	-
32 days in cubboard	3	2	3	2
64 days in respirometer	3	-	3	-

m: mixture of the whole sample and subsequent CAL-extraction

f: freezing by liquid nitrogen, cutting into slices and subsequent CAL-extraction

We analysed CAL extractable P (Schüller 1969) with a Continuous Flow Analyser (880 nm wavelength, Skalar San plus system 5120) in 1 g of each air-dried soil layer. In case not a whole gram could be gained from the soil layers, corresponding smaller amounts of soil layer and CAL solution were used.

Phosphate sorbed to the resins was extracted by horizontally shaking six times with 100 ml 0.1 M NaCl solution for columns from days 1 and 2 (presumably still lower PO_4^{3-} concentrations) and with 200 ml 0.1 M NaCl solution for columns from days 4, 8, 16, 32 and 64 (presumably already higher PO_4^{3-} concentrations). Shaking duration was 1 hour and shaking speed 200 rpm. Afterwards, P or PO_4^{3-} were measured by ICP-OES or CFA, respectively.

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4.3.2 Theory

4.3.2.1 Model

Our experiment did not allow water flow. Thus, we assumed the water content to be constant over space and time. Decay of organically bound P was assumed to follow a first order reaction and to be highest in the vicinity of the resin and exponentially decrease with increasing distance from the resin. These assumptions lead to the following coupled one-dimensional partial differential equations (see chapter 4.7):

$$\frac{\partial P_{\mathrm{m}}^{\mathrm{tot}}}{\partial t} = D * \frac{\partial^2 P_{\mathrm{m}}^{\mathrm{tot}}}{\partial z^2} + k(z) P_{\mathrm{o}} \qquad \text{(Eq. 4.1)}$$

and

$$\frac{\partial P_{\mathrm{o}}}{\partial t} = -k(z) P_{\mathrm{o}} \qquad \text{(Eq. 4.2)}$$

where t [d] is time, z [cm] is the spatial coordinate, $P_{\mathrm{m}}^{\mathrm{tot}}$ [mg cm^{-3}] is the total concentration in the mineral form, given by liquid and sorbed phase concentration, P_{o} [mg cm^{-3}] is the concentration of organically bound P, $D^* = D/R$, where D [cm^2 d^{-1}] is the diffusion coefficient and R [-] is the retardation factor for a linear sorption isotherm and $k(z)$ [d^{-1}] is the location dependent decay parameter. Note that $P_{\mathrm{m}}^{\mathrm{tot}}$ and P_{o} are related to bulk volume. The location dependency of k is simply given by an exponential function:

$$k(z) = (k_0 - k_\infty) e^{-\alpha z} + k_\infty \qquad \text{(Eq. 4.3)}$$

where k_0 [d^{-1}] is the maximum value for k located at z = 0, which is the boundary between soil and filter-covered resin, k_∞ [d^{-1}] is the minimum value for k at a distance from the resin, where the influence of the resin diminishes, and α [cm^{-1}] is a shape parameter representing the relative location dependency. Note that setting $\alpha = 0$ leads to a uniform distribution of

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$k(z)$ with $k(z) = k_0$ and setting $k_0 = 0$ leads to a pure diffusion model without production.

To solve equations 4.1 and 4.2 they need initial and boundary conditions. The initial conditions for both equations are set to uniform distributions:

$$P_{\mathrm{m}}^{\mathrm{tot}}(z, t=0) = P_{\mathrm{m},0}^{\mathrm{tot}} \qquad \text{(Eq. 4.4)}$$

and

$$P_{\mathrm{o}}(z, t=0) = P_{\mathrm{o},0} \qquad \text{(Eq. 4.5)}$$

where $P_{\mathrm{m},0}^{\mathrm{tot}}$ [mg cm^{-3}] and $P_{\mathrm{o},0}$ [mg cm^{-3}] are the initial P-concentrations in total mineral and organically bound form, respectively. The simulation domain was 2 cm with $z = 0$ at the filter-covered resin surface and z = 2 cm at the column end. The boundary conditions for equation 4.1 where set to a no-flux condition at the end of the column and to a Dirichlet boundary condition with $P_{\mathrm{m}}^{\mathrm{tot}}(z = 0, t) = 0$ at the filter-covered resin surface. The latter accounts for a very high sorption capacity of the resin. The boundary conditions for equation 4.2 were simply set to no-flux conditions.

4.3.2.2 Parameter estimation

In order to solve the model combination outlined above, a total of six parameters have to be identified, namely $P_{\mathrm{m},0}^{\mathrm{tot}}$, $P_{\mathrm{o},0}$, D^*, k_0, k_∞ and α. As $P_{\mathrm{m},0}^{\mathrm{tot}}$ is unlikely to be largely changed at z = 0.7 to 1.0 cm after two days, $P_{\mathrm{m},0}^{\mathrm{tot}}$ was set to the mean of the measured values at these distances and day 2. Parameter k_∞, which resembles the decay parameter in the bulk soil unaffected by the resin, was taken from respiration experiments with the same mixtures without resin and fitting a simple one pool decay model to the CO_2 production data (data not shown). Thus, the simplified assumption is that decay of organic P resembles decay of

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organic C. The remaining four parameters were estimated by means of inverse modelling (Hopmans et al. 2002), minimising the weighted sum of squared residuals between observed and model predicted values for cumulative P release into the resin as well as P concentrations in the soil column:

$$\Phi(b) = \omega_{\mathrm{C}} \sum_{i=1}^{r} [P_{\mathrm{m}_i}^{\mathrm{tot}} - \hat{P}_{\mathrm{m}_i}^{\mathrm{tot}}(b)]^2 + \omega_{\mathrm{F}} \sum_{i=1}^{k} [F_i - \hat{F}_i(b)]^2 \qquad \text{(Eq. 4.6)}$$

where $P_{\mathrm{m}_i}^{\mathrm{tot}}$ and F_i are single measured concentrations and cumulative outflow into the resin, $\hat{P}_{\mathrm{m}_i}^{\mathrm{tot}}$ and $\hat{F}_i$ are model predicted values, r and k are the number of measured concentrations and outflow values, ω_{C} and ω_{F} are the weights for the two data types and b is the parameter vector containing the four adjustable parameters: $b = (P_{\mathrm{o},0}; D^*; k_0; \alpha)$.

To account for the different data types we followed the suggestion of Peters and Durner (2008a) by calculating the weights for the data classes by $\omega_{\mathrm{C}} = 1/(P_{\max} - P_{\min})$ and $\omega_{\mathrm{F}} = 1/(F_{\max} - F_{\min})$, where $P_{\max}$, $P_{\min}$, $F_{\max}$ and $F_{\min}$ are the maximum and minimum values of the measured data sets.

4.3.2.3 Diagnostic variables

A descriptive measure giving the mean deviation between model and data is the root mean square error (RMSE):

$$\mathrm{RMSE} = \sqrt{\frac{1}{r} \sum_{i=1}^{r} [y_i - \hat{y}_i]} \qquad \text{(Eq. 4.7)}$$

where y_i and $\hat{y}_i$ are measured and model predicted quantities for either concentrations or

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cumulative fluxes.

The coefficient of determination (R^2) is given by:

$$R^2 = \left(\frac{\sum_{i=1}^{r} (y_i - \bar{y})(\hat{y}_i - \bar{\hat{y}})}{\sqrt{\sum_{i=1}^{r} (y_i - \bar{y})^2 \sum_{i=1}^{r} (\hat{y}_i - \bar{\hat{y}})^2}} \right)^2 \qquad \text{(Eq. 4.8)}$$

where $\bar{y}$ and $\bar{\hat{y}}$ are the means of the measured and model predicted quantities.

In order to compare the models, we used a modification of Akaike's information criterion (Akaike 1974) which was suggested by Hurvich and Tsai (1989):

$$\mathrm{AICc} = n \ln(\Phi_{\min}/n) + 2k + \frac{2k(k+1)}{(n-k-1)} \qquad \text{(Eq. 4.9)}$$

where $\Phi_{\min}$ is the sum of weighted squared residuals between fitted model and data, n is the number of data points and k is the number of freely adjustable parameters. The first term penalises a poor fit, the second term the number of parameters and the third term is the correction term for small values of n/k. The smaller the AICc, the better is the corresponding model.

4.4 Results and discussion

4.4.1 Change of pH-value

The temporal course of pH during respiration, as an average of the whole soil column, is demonstrated in Figure 4.1. The pH of samples without resin ranged from 5 to 5.5 throughout

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the whole experiment. Adding the resin caused pH-values of 6 to 6.4 for the first day. This leads us to the assumption, that hydrogen carbonate diffusion from the resin and its surrounding solution to the soil was fast and caused a pH increase of one unit within one day. In the following week the SEW amended soil showed a pH increase up to 7-7.5, which we assume to be mainly caused by a further exchange of anions like nitrate or phosphate from the SDM and HCO_3^- from the resin. A stronger increase of the pH (up to 8.5) was obvious in the SL amended soil. In some studies a higher pH-value was measured under laboratory conditions during drying compared to in situ measurements (Elberling and Jakobson 2000, Elberling and Matthiesen 2007). The authors assumed that mineralisation of OM led to an accumulation of H_2CO_3/CO_2, which was equilibrated in soil solution. When soil solution came in contact with the lower CO_2 partial pressure in the atmosphere, the equilibrium shifted, resulting in degassing CO_2, lowered H^+-activity and therefore increased pH-values. A strengthened mineralisation of OM in SL-samples compared to SEW-samples has been found in an independent incubation experiment (data not shown). Thus we assume a corresponding influence on the pH-value during sample preparation (drying).

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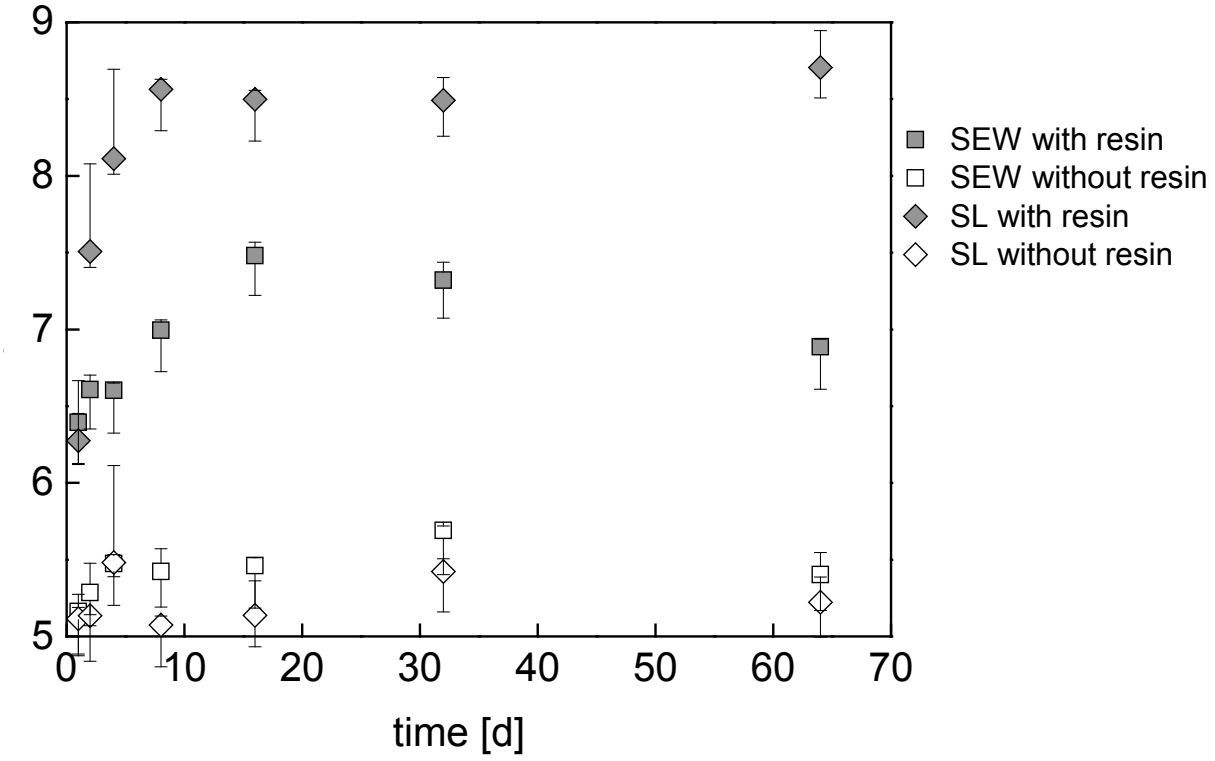

Figure 4.1: Mean pH of soil-digestate-mixtures (SDM) during incubation.

4.4.2 Effect of AER on P availability

Figure 4.2 shows the concentration of CAL extractable PO_4-P of the SEW and SL amended soils. The concentrations of PO_4-P in the samples prepared with AER were consistently higher compared to the PO_4-P concentrations in samples without resin. This can be explained by the more alkaline conditions in samples with resin compared to samples without resin (Figure 4.1). On the one hand, a higher pH mostly leads to a higher microbial activity and thus to an increased mineralisation of organic matter (Curtin et al. 1998, Aciego Pietri and Brookes 2008) and it is known that the release of P is affected by the mineralisation of organic matter (Barber 1995). As described above, higher phosphodiesterase activity under neutral to alkaline conditions was demonstrated by Turner and Haygarth (2005). Accordingly

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the authors found a high availability for the labile phosphodiesters. On the other hand, phosphate may have derived from inorganic sources since a higher pH also facilitates the solubility of iron (Fe) or aluminium (Al) minerals (Lindsay 1979). Hydrogencarbonate present in alkaline soils can also have supported desorption. Nagarajah et al. (1968) showed that HCO_3^- can exchange phosphate ligands from mineral surfaces.

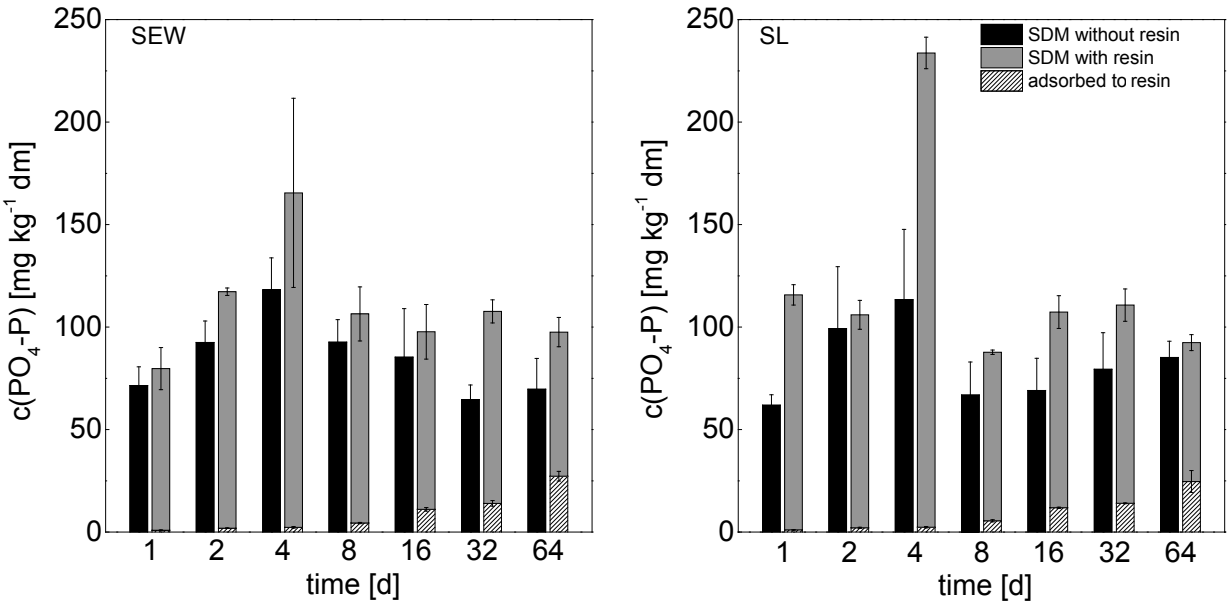

Figure 4.2: Concentration of PO_4-P as a mean of the whole soil-digestate-mixture (SDM) with and without anion exchange resin

4.4.3 Mean PO_4-P availability over time

The PO_4-P concentrations of all treatments followed a similar trend (Figure 4.2). CAL extractable PO_4-P increased for 4 days and then decreased back to the level which was found at the beginning of the experiment. Investigating P sorption kinetics of mineral P amended sterile and unsterile soils, Sinegani and Sedri (2011) observed similar time trends for the phosphate concentration of unsterile soils. Phosphate concentrations increased in both investigated soils and decreased again after approximately 30 days. The authors attribute the increase of phosphate availability to the phosphate solubilising activities of

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special microorganisms, namely the release of organic acids. These processes are also imaginable for our SDM without resins. Additionally, the supply of organic matter by digestates and – in the case of samples with resin – the increased pH may have enhanced mineralisation of organic P by microorganisms and thus led to a release of mineral P, as already described above. Sterile soils did not reveal increasing phosphate concentrations in the study of Sinegani and Sedri (2011). After a fast decrease at the beginning phosphate concentrations remained quite constant for the rest of the incubation time. This supports the importance of microorganisms for P mineralisation. Decreasing PO_4-P concentrations can be explained by a microbial P immobilisation (Bünemann et al. 2012). Furthermore, the precipitation of calcium phosphate most likely occurred in samples with pH values above 7 (Lindsay 1979) and led to lower CAL extractable PO_4-P in samples with resin.

Besides changing pH and PO_4-P availability in the SDM, we observed a slow increase of PO_4-P in the resins (Figure 4.2). It has been shown that P supply to plants is basically diffusion-dependent (Barber 1995), although P is rather immobile because of chemical reactions such as precipitation or sorption to the soil matrix. Nevertheless the portion of PO_4-P diffused to the resins increased from less than 1 % at day 1 to nearly 30 % of the whole CAL extractable PO_4-P within 64 days of incubation. The SEW and SL treatments did not show notable differences in PO_4-P accumulation in resins.

Figure 4.3 highlights P release from digestates calculated as $P_d = 100(P_e - P_c)/P_a$, where P_e is PO_4-P extracted from the SDM, P_a is total P added by the digestates and P_c is PO_4-P extracted from the soils without digestate. Samples with resins show increasing P release from digestates during the first four days. It is obvious that the huge proportions of ~ 250 % for SEW and ~ 650 % for SL were only possible, if P from organic or inorganic soil P pools was released in addition to digestate P. These results may indicate P-priming.

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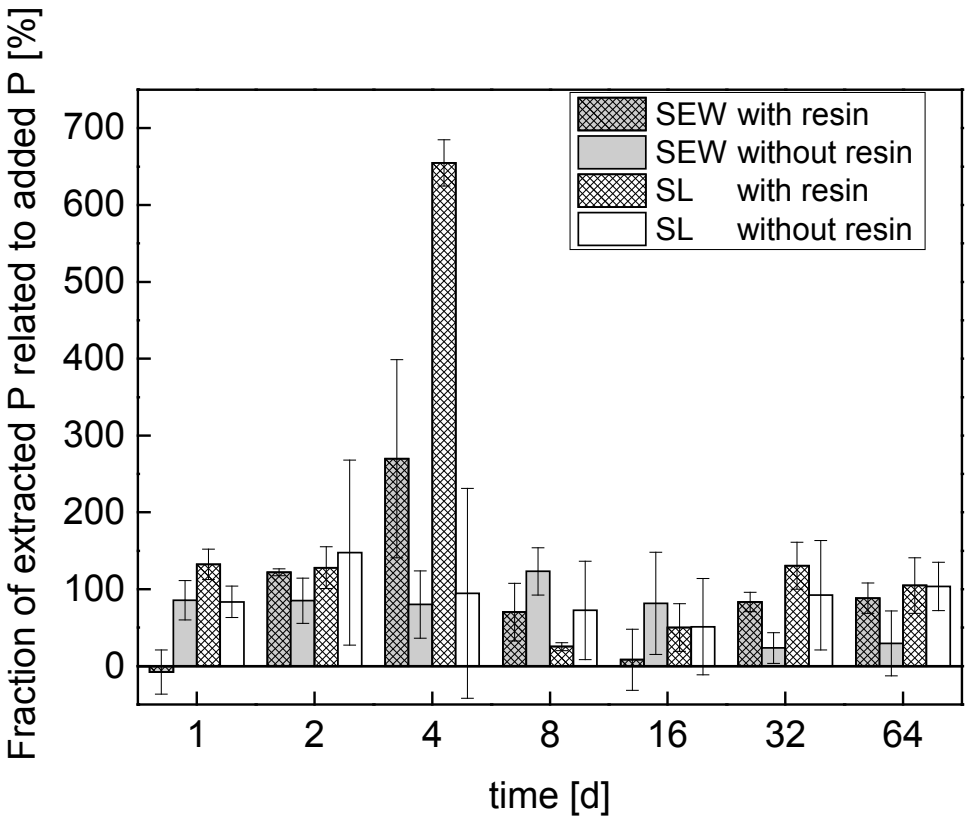

Figure 4.3: Portion of extracted P related to P added with digestates. $P_d = 100(P_e-P_c)/P_a$, where P_e is PO_4-P extracted from the SDM, P_a is total P added by the digestates and P_c is PO_4-P extracted from the soils without digestate.

4.4.4 Spatial distribution of available phosphate

Figure 4.4 shows the spatial distribution of PO_4-P concentrations for the SEW treatments. The PO_4-P concentrations vary between 100 and 150 mg kg^{-1} dm at day two for both replicates, reflecting a relatively large heterogeneity of the material. In both replicates a strong decrease of PO_4-P concentration within the first 3 to 4 mm adjoining the resin is visible after eight days of incubation. At larger distances, no distinct concentration decrease is identifiable. The influence of the resin was enlarged to 6-7 mm after 32 days of incubation. This decrease of PO_4-P concentration in the vicinity of the resin is accompanied by an increase of PO_4-P in the resin, which can be explained by a simple diffusion and sorption

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process. Moreover, the concentrations at larger distances from the resin increase with time, with the peak concentration migrating away from the resin with time. This pattern is well pronounced for SEW and can be observed by trend for SL, as well (Figure 4.5). Thus, simultaneously to the mere diffusion and sorption process, a production process occurs. The production might be explained by degradation of organically bound P. The moving peak concentration indicates that degradation is most pronounced in the vicinity of the resin.

For the SL mixture, the decrease of PO_4-P concentration in the vicinity of the resin is already visible after two and after 32 days of incubation (Figure 4.5). The temporal and spatial courses of PO_4-P concentration are similar to the courses in the SEW treatments, although the production and peak migration is less pronounced. At the eighth day a higher variation between the PO_4-P concentrations of the two replicates can be observed, which possibly masks the proposed processes of degradation and diffusion/retardation. At day four the SL mixture showed an enormous increase of PO_4-P concentration, followed by a clear decrease (Figure 4.2). The underlying processes at the days around the fourth cannot be attributed to mere diffusion/sorption and continuous production. Causing mechanistic explanations are still open.

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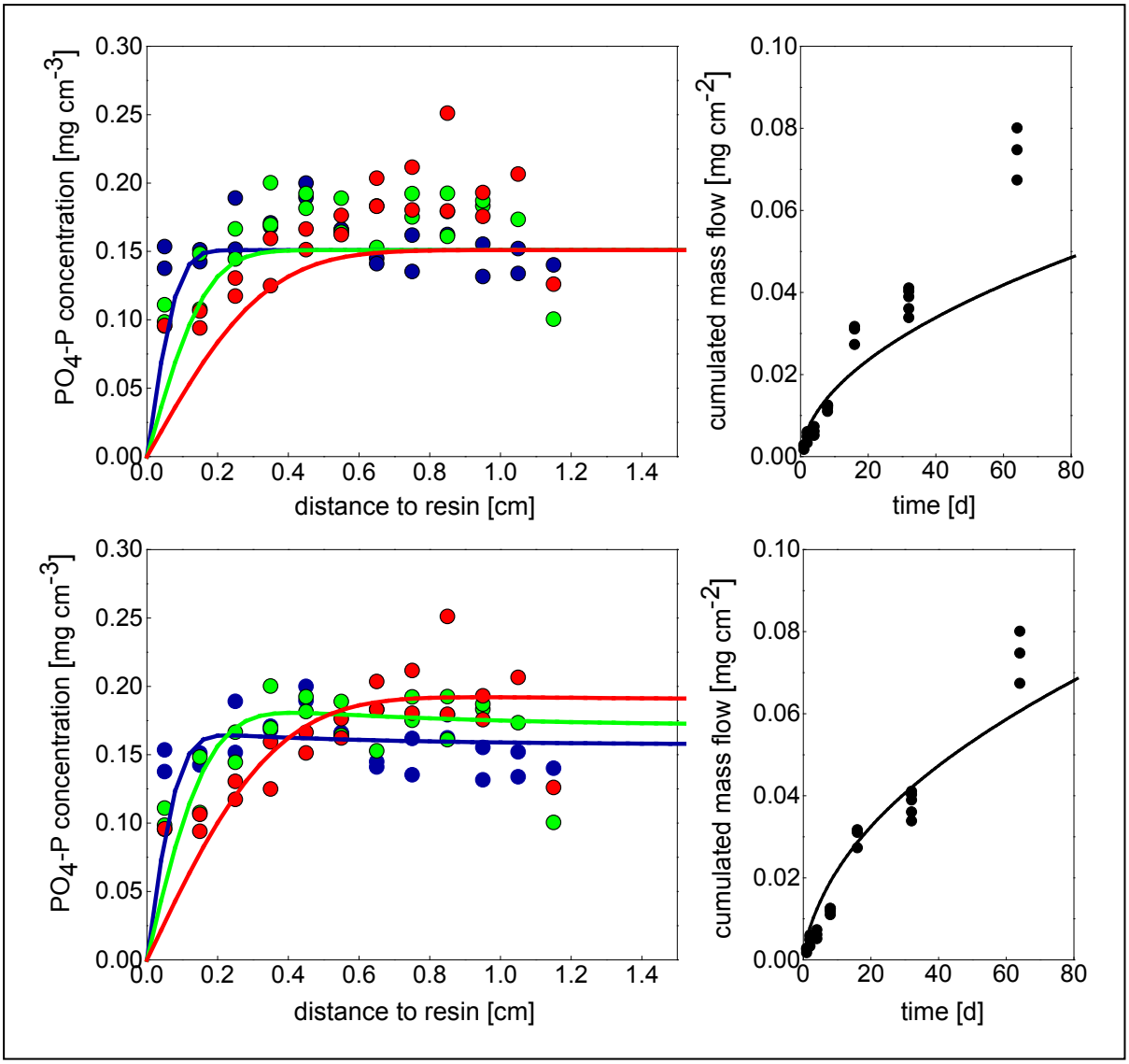

Figure 4.4: Measured data and fitted model for SEW amended soil. Left: Spatial distribution of PO_4-P concentrations at the three depicted times; Right: Amount of PO_4-P cumulated in anion exchange resins as function of time. Upper row: Simple diffusion model; Lower row: Diffusion + decomposition model.

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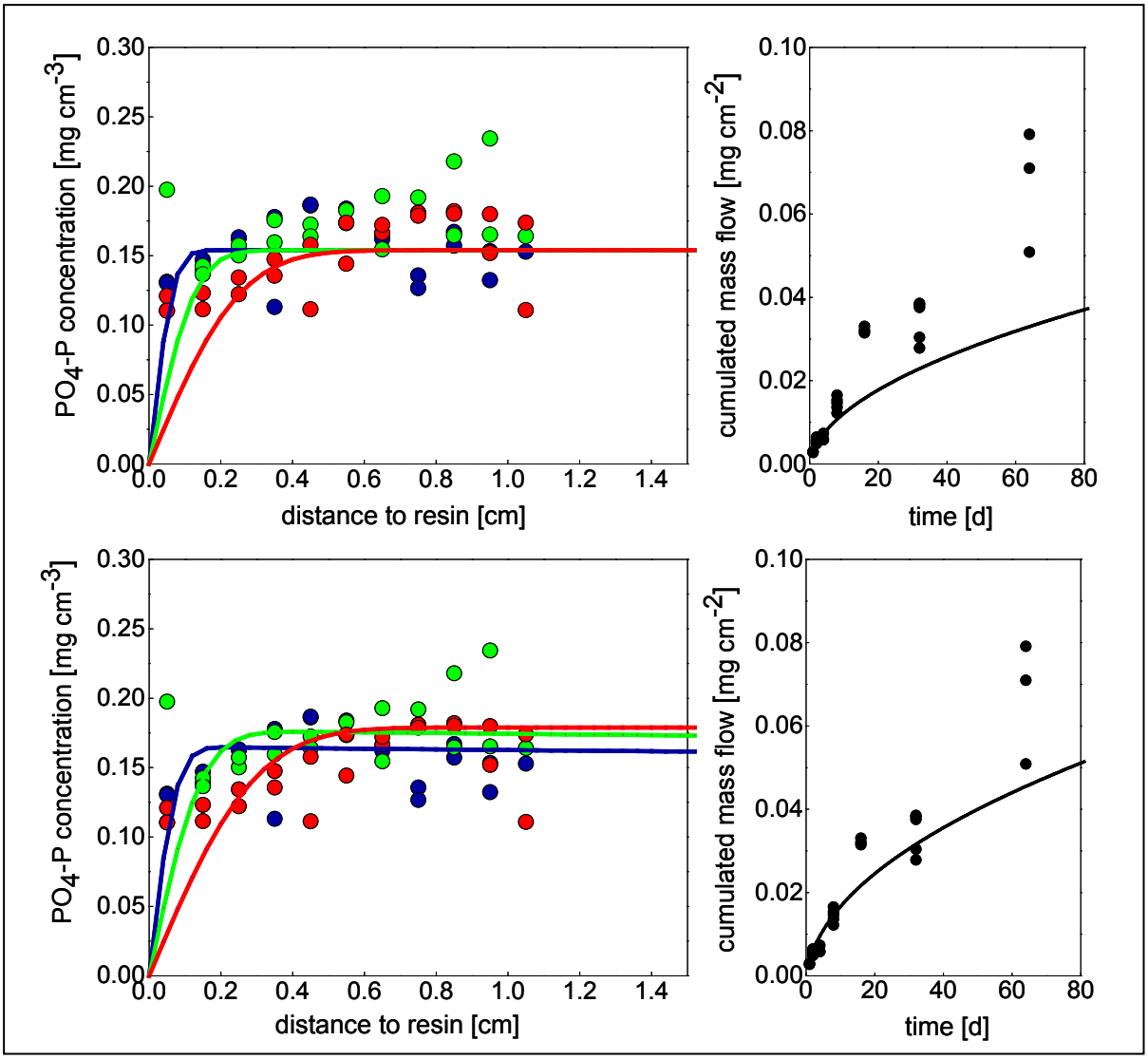

Figure 4.5: Measured data and fitted model for SL amended soil. Left: Spatial distribution of PO_4-P concentrations at the three depicted times; Right: Amount of PO_4-P cumulated in anion exchange resins as function of time. Upper row: Simple diffusion model; Lower row: Diffusion + decomposition model.

4.4.5 Model results

The pure diffusion/sorption model and the model with additional location dependent degradation of organically bound P were fitted to the data (Figures 4.4 and 4.5). As already discussed above, the mere diffusion and sorption model, which was suggested by Barber (1995) cannot adequately describe our data (Figures 4.4 and 4.5, upper rows).

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Implementation of the mineralisation process leads to far better description of the data (Figures 4.4 and 4.5, lower rows). Especially for the SEW data the concentration peak migrates with time, which is well in accordance with our model considering location dependent mineralisation of organic P. The superiority of the proposed model is also shown by the diagnostic variables (Table 4.3). The RMSE and R^2 are better for the more complex model in both treatments for both data types. Moreover, the AICc, which allows comparison of models with different numbers of adjustable parameters, shows that the new model should be selected for data description. If the difference between the AICc is larger than 10, the model with the higher AICc (inferior model) should be disregarded as it has no support in describing the measured data (see Burnham and Anderson 2004, Peters and Durner 2008b). Note that the difference between absolute, not relative, values for AICc must be compared.

Table 4.3: Diagnostic variables for the two models and both treatments. $F(t)$ is cumulated P in resin; $P(z,t)$ is spatial concentration distribution at the three times; n_{Para} is number of adjusted parameters.

	model		RMSE		R^2		AICc
		n_{Para}	$P(z,t)$	$F(t)$	$P(z,t)$	$F(t)$	
SEW	diffusion	2	0.035	0.012	0.486	0.952	-859.4
	diffusion + production	4	0.026	0.007	0.564	0.952	-918.6
SL	diffusion	2	0.032	0.014	0.267	0.882	-838.9
	diffusion + production	4	0.030	0.009	0.305	0.911	-859.3

The effective diffusion coefficient (D^*) was in the same order of magnitude for both SDM (Table 4.4). Thus diffusion and sorption are assumed to be similar. Compared to SL (Figure 4.5), the graph for SEW shows a higher PO_4-P release due to a higher estimated initial organic P source ($P_{o,0}$). However, the estimated decay parameter k_0 for SEW was lower and decreased more than double as fast with increasing distance from the anion sink as indicated by the larger value for α. According to our model the amount of released organic P after 32 days was higher for SEW than for SL. This led to clearly developed concentration maxima in

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this fit. Parameter α for SL amended soil showed a more uniform distribution of the depletion rate, resulting in weaker maxima. Correlation between estimated parameters reached a maximum of 0.83 and usually remained below 0.4.

Table 4.4: Parameters estimated for the two different models and both treatments.

	model	$P_{o,0}$ [mg cm^{-3}]	D^* [cm^2 d^{-1}]	k_0 [d^{-1}]	α [cm^{-1}]
SEW	diffusion	0.277	$1.079 \cdot 10^{-3}$	-	-
	diffusion + production	0.042	$1.298 \cdot 10^{-3}$	0.254	2.051
SL	diffusion	0.181	$0.616 \cdot 10^{-3}$	-	-
	diffusion + production	0.025	$0.855 \cdot 10^{-3}$	0.300	0.435

Both the spatial distribution of PO_4-P concentration at different times as well as accumulation of PO_4-P in the resin are well described by our simple model approach, which effectively describes the complex processes mentioned above with only 4 adjustable parameters.

Amounts of PO_4-P cumulated in the AER were slightly overestimated in the beginning and underestimated in the end of incubation for both treatments (Figures 4.4 and 4.5, lower rows, right). One possible explanation is the connection of PO_4-P release to the depletion of OM, which we expected to increase with rising pH. The pH effect is only spatially differentiated in our model concept, whereas the pH effect of the resins depends on the diffusive transport of HCO_3^- in soil solution, which would spread out with time. Thus the weaker pH effect in the beginning of the experiment may have led to less PO_4-P released than estimated and to less cumulated amounts. The other way around the pH has a stronger effect than estimated in the end of the experiment. Additionally, organic substance consists of C pools with different stability. Our one pool model only describes the easy degradable pool. If organically bound P was lacking in the easily degradable pool, the P release would have started delayed.

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Our model predicts an increase of PO_4-P concentration over time, but the PO_4-P concentrations of the average samples of the whole resin-affected columns did not confirm this prediction (Figure 4.2). Nonetheless, we could detect indications for phosphate production due to OM mineralisation in the first centimeter of the column for both SDM. These contradictory observations can be attributed to the heterogeneity in the soil columns containing zones which were less affected by the anion sink due to a higher distance from it than others.

4.5 Conclusions

Our results indicate that the presence of an anion sink simulating the anion exchanging activity of a plant root and the resulting higher pH lead to enhanced mineralisation of organic P which is most pronounced in the vicinity of the anion sink (HCO_3^- source). The increase of PO_4-P availability was accompanied by remarkable P priming. Since hitherto existing studies mainly focus on C and N priming it is necessary to investigate P priming more intensively and to understand and distinguish underlying processes. Furthermore, experiments should be conducted extending the simulation of the properties of plant roots by processes like release of cations and exudates.

Our simple model integrating PO_4-P transport by diffusion and P release by mineralisation of SOM with location-dependent decay parameter is able to describe P dynamics in the investigated digestate-amended soils. However, our results indicate that other processes e.g. solution-precipitation reactions or microbial immobilisation may have occurred. Further research and model development is needed to account for these processes. In addition, the P decay parameter of bulk soil, k_∞ should be determined directly by measuring P-

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mineralisation.

4.6 Acknowledgements

We thank the Federal Ministry for the Environment, Nature Conservation, Building and Nuclear Safety for funding and Ellen Kandeler and Holger Pagel for providing the Cryomicrotom and technical support with it.

4.7 Appendix

The general one-dimensional continuum equation for the element concentration in the mineral form is expressed by

$$\frac{\partial P_{\mathrm{m}}^{\mathrm{tot}}}{\partial t} = -\frac{\partial J}{\partial z} + r \qquad \text{(Eq. 4.10)}$$

where t [d] is time, z [cm] is the spatial coordinate; $P_{\mathrm{m}}^{\mathrm{tot}}$ [mg cm^{-3}] is the total element concentration in the mineral form, given by liquid and sorbed phase concentration, J [mg cm^{2}d^{-1}] is the solute flux density and r [mg cm^{-3}d^{-1}] is the source/sink term. Note that $P_{\mathrm{m}}^{\mathrm{tot}}$ is related to bulk volume. In our experiments, the solute flux density is solely given by diffusion in the liquid phase:

$$J = -\theta D \frac{\partial P_{\mathrm{m}}^{\mathrm{l}}}{\partial z} \qquad \text{(Eq. 4.11)}$$

where $P_{\mathrm{m}}^{\mathrm{l}}$ [mg cm^{-3}] is the liquid phase concentration, θ [-] is the volumetric water content and D [cm^{2}d^{-1}] is the diffusion coefficient.

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Inserting equation 4.11 into equation 4.10 and assuming θ and D to be constant leads to:

$$\frac{\partial P_{\mathrm{m}}^{\mathrm{tot}}}{\partial t} = \theta D \frac{\partial^2 P_{\mathrm{m}}^{\mathrm{l}}}{\partial z^2} + r \qquad \text{(Eq. 4.12)}$$

Total concentration in the mineral form is the weighted sum of liquid and solid phase concentrations and can be expressed as:

$$P_{\mathrm{m}}^{\mathrm{tot}} = \theta P_{\mathrm{m}}^{\mathrm{l}} + S\rho_{\mathrm{b}} \qquad \text{(Eq. 4.13)}$$

where ρ_{b} [g cm^{-3}] is the soil bulk density and S [mg g^{-1}] is the concentration of adsorbed element.

Assuming a linear sorption isotherm $\left(S = k_{\mathrm{d}} P_{\mathrm{m}}^{\mathrm{l}}\right)$ leads to

$$P_{\mathrm{m}}^{\mathrm{tot}} = \theta P_{\mathrm{m}}^{\mathrm{l}} + k_{\mathrm{d}} P_{\mathrm{m}}^{\mathrm{l}} \rho_{\mathrm{b}} = \left(\theta + k_{\mathrm{d}} \rho_{\mathrm{b}}\right) P_{\mathrm{m}}^{\mathrm{l}} \qquad \text{(Eq. 4.14)}$$

where k_{d} [cm^{3}g^{-1}] is the linear sorption coefficient. Rearranging equation 4.14 gives:

$$P_{\mathrm{m}}^{\mathrm{l}} = \frac{P_{\mathrm{m}}^{\mathrm{tot}}}{\theta R} \qquad \text{(Eq. 4.15)}$$

where $R = 1 + \frac{\rho_{\mathrm{b}}}{\theta} k_{\mathrm{d}}$ is the retardation coefficient.

Finally, inserting equation 4.15 into equation 4.12 with constant R and θ yields:

$$\frac{\partial P_{\mathrm{m}}^{\mathrm{tot}}}{\partial t} = D^* \frac{\partial^2 P_{\mathrm{m}}^{\mathrm{tot}}}{\partial z^2} + r \qquad \text{(Eq. 4.16)}$$

where $D^* = D/R$.

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The source term r is given by decay of the concentration of the organically bound element (P_o [mg cm^{-3}], related to bulk volume) and described by a location dependent first order reaction:

$$\frac{\partial P_o}{\partial t} = -k(z)P_o = -r \qquad \text{(Eq. 4.17)}$$

where $k(z)$ [d^{-1}] is the location dependent decay parameter.

If no sorption takes place, equation 4.13 is given by

$$P_m^{tot} = \theta P_m^l \qquad \text{(Eq. 4.18)}$$

and $R = 1$. Note that P_m^{tot} and P_o are defined for total volume and not for single phase concentrations.

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5 Synthesis and conclusions

5.1 Synthesis of the results with regard to the objectives and hypotheses

The results of this thesis contribute to the basic knowledge about digestates and their fate in soils. The repeated monthly analyses of digestates over a one-year period revealed that the elemental composition of digestates varied greatly depending on the feedstock material. Sewage sludge-derived digestates exhibited the highest concentrations of P and heavy metals. In some cases, heavy metal concentrations even exceeded German legal threshold values for sewage sludge. Copper and Zn concentrations of slurry-derived digestates also exceeded the German legal threshold values for biowastes at some sampling dates, while digestates from maize do not pose a risk for the environment with regard to their elemental composition.

The first objective of this thesis was to contribute to a broader database about concentrations of diverse elements in digestates and differences between digestates from varying feedstock. This aim could be achieved. The results confirm the need for thorough analyses of digestates before application to arable land.

Another important lesson learned is that the composition of digestates can vary remarkably over time. The properties of digestates sampled on one day from a specific biogas plant may be very different from those of digestates sampled on another day from the same plant. This applies in particular for digestates from co-fermentation. In these digestates the variations

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between element concentrations over time were much higher than in digestates from mono-fermentation. Feeding co-fermentation plants with varying ingestates at varying proportions can explain these results. It can be concluded that a single sample taking is not sufficient to get representative information about the elemental composition of digestates.

This finding could be accounted for in the developed statistical method to identify element losses or accumulations during anaerobic digestion in case data about the amount and composition of biogas are not available. Several sampling dates for digestates and ingestates were needed for an element balancing in the biogas plant: two for the digestate from sewage sludge and fat, five for the digestate from sewage sludge, fat and mash, as well as for the digestate from maize, and ten for the digestate from slurry and food residues. With the help of these results, an element balancing for the four anaerobic digestion processes could be realised. Nitrogen was depleted in all fermenters which can be attributed to volatilisation of NH_3. Sulfur was probably emitted as H_2S, while Mg was depleted due to the precipitation of struvite and Cd and Zn due to the formation of sulfides in some cases. Iron was accumulated in two fermenters and Mn in one. Attrition of the stirrer apparatus is an imaginable reason for that. A significant accumulation of phytotoxic heavy metals during the fermentation processes could not be discovered. Thus, the pollution of the digestates with hazardous metals from stirring devices of biogas tanks may be ruled out. The only metals accumulated – Fe and Mn – do not pose a risk to plant health and the application of these elements to soils is uncritical.

These findings make clear that the elemental composition of digestates cannot be concluded from the feedstock material. The digestates have to be analysed *after* the anaerobic digestion and for a representative characterisation of a digestate, more than one sampling date may be required.

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After gaining this basic information about digestates and their elemental composition, the next step was the investigation of the mineralisation of digestates and their potential to promote carbon priming in soil, especially under conditions similar to those which develop when plant roots exchange anions. The incubation experiments which were designed to study these questions showed that the anion sink adsorbing nutrient anions and releasing HCO_3^- in return led to increased pH and thus increased mineralisation of OM. Thus, incubation experiments should account for conditions in soil that are generated by plant roots because they can have a profound effect on the mineralisation of OM and consequential CO_2 evolution.

The results further indicate that the presence of an anion sink increases the potential for priming in soils. Provided that C emission from the resins in amended and unamended soils is not varying remarkably, priming could be seen for the maize-derived digestate in the first incubation experiment and for the sewage sludge-derived digestate in the third experiment – both with integrated anion exchange resin. It cannot be excluded that microbial biomass turnover is part of the priming observed. According to Blagodatskaya and Kuzyakov (2008), apparent priming could partly have occurred. However, another perspective on this matter is imaginable. On the one hand, real priming is restricted to SOM turnover, thereby only referring to “dead” matter and not including microbial biomass turnover. The latter is classified as apparent priming. For process oriented investigations with the aim to identify CO_2 sources as specific as possible, this differentiation seems suitable. On the other hand, with regard to GHG emissions induced by priming, a differentiation between lifeless SOM decomposition (real priming) and soil microbial biomass decomposition (apparent priming) is not necessary. Whether solely dead SOM was decomposed and CO_2 was emitted, or partly soil microorganisms were decomposed and CO_2 was emitted, does not play an important role here since CO_2 from soil was emitted in any case. Therefore, priming that has occurred in the incubation experiments of this work – whether real or apparent or both – can be

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regarded as a process that has led to undesirable additional GHG emission.

The assumption that the addition of OM with low concentrations of bio-available C and high concentrations of available N leads to priming in soil cannot be confirmed. Indications for priming just occurred for digestates that also showed a high amount of mineralised C, i.e. the digestate must have contained rather easily available OM. This indicates that a sufficient amount of easily available C is mandatory for priming, even if high amounts of nutrients are available in the added substrate.

The incubation experiments confirm again that digestates can be immensely heterogeneous depending on their feedstock material but also over time. Digestates revealed pronounced discrepancies in mineralisation when sampled at different times from the corresponding biogas plant. A lot of parameters that might have led to varying mineralisation of one and the same digestate, such as pH, heavy metal or C content, could be excluded as possible explanations. Instead, the degree of stability of the digestates may be a very likely reason.

The ambition to get a high methane yield often requires a too low retention time of digestates in the fermenters and a too early feeding of fresh material. As a consequence, the biomass is incompletely digested and can thus lead to higher mineralisation in soils. Additionally, process failures can promote insufficient degrading of OM in the biogas reactor. Referring to the aims of this thesis, it can be learned that digestates may cause priming in soils when influenced by an anion sink and that their mineralisation rate and amount in soil can cover a wide range, both probably depending on their degree of stability.

With regard to digestates and their effects in soils when used as fertiliser, this thesis could also contribute basic new knowledge about nutrient availability of digestates in soil. On the

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one hand, analyses of the 1 mm layers of the incubated soil samples showed that phosphate availability was intermittently reduced, probably due to precipitation of calcium phosphate especially in samples with anion exchange resin and thus higher pH. However, the mineralisation of OM allowed a supplementary delivery of P and thus partly a compensation of the P immobilisation as calcium phosphate. The hypothesis that the presence of an anion sink (HCO_3^- source, simulating anion exchange of plant roots) would increase pH and thus the mineralisation of organic P could be supported. As expected, the additional supply of P also influenced the P concentration gradient with increasing distance from the anion sink. Phosphate concentrations in the 1 mm layers did not simply show a depletion zone in the near of the resin, as would have occurred, if only diffusion governed P transport to the resin. Instead, a peak in P concentration migrating away from the resin with time could be observed. This peak can be interpreted as sign for supplementary delivery of P due to OM mineralisation and could also be described by a new model, which combined a conventional diffusion model with a term for a first order decay of organic P. This term included a location dependent decay parameter, i.e. a parameter that accounts for OM mineralisation being highest close to the anion sink and decreasing with increasing distance from the anion sink.

In conclusion, models of P transport to plant roots in soils should also account for additional P supply due to the mineralisation of OM, which is increased by the alkalinising activity of plant roots. Another important factor influencing P transport to plant roots is the formation of P precipitates, which also depends on pH and thus on the activity of plant roots.

5.2 Relevance for practice

In order to recycle nutrients and protect valuable resources, digestates should always be used as fertilisers, if possible. The results show that the decision about the fertiliser value of

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digestates from biogas production should be based on analyses of the digested material because the feedstock material is not only altered due to the volatilisation of CH_4 and CO_2 but also due to gaseous losses of N and S and precipitation of minerals. The analyses should also be repeated in adequate intervals since the elemental composition of digestates can vary over time, especially in co-fermentation biogas plants. This probably also applies to the degree of stability of digestates which may strongly influence the mineralisation and potential for priming in soils. In life cycle assessments, the potential of digestates to promote priming and thus additional GHG emissions to the atmosphere should urgently be accounted for. The maximisation of biogas yield is essential for the economic feasibility of biogas plants but the original intention to reduce GHG emissions during the production of energy needs to be considered as well. When energy is produced in a less polluting way, but the resulting digestate causes additional GHG emissions after application to arable land, this original intention may be failed. Therefore, these two concerns – economic feasibility and positive life cycle assessment of biogas plants – need to be conciliated. Actually, this seems to be a great challenge in practice since the economic feasibility of biogas plants strongly depends on the sponsorship by the EEG, which has been revised in 2014 and reduces many of the benefits for plant operators now. The costs for the promotion of bioenergy and the expansion of maize monocultures, for example, have led to this revision and resulted in a recent slow-down of newly built biogas plants. While 1,270 biogas plants have been built in 2011 in Germany, only 340 and 335 have been established in 2012 and 2013, respectively (Fachverband Biogas 2014). This gives reason for early in-depth research in those areas so that misdirected investment and belated political corrections can be avoided.

5.3 Future research

The results make clear that simplified methods to estimate the composition of digestates or

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predict their behaviour in soil are not suitable. Digestates are too variable concerning their composition and stability so that overall and undifferentiated statements or classifications cannot be made, yet. However, analyses of every digestate applied to soils are expensive and time-consuming and cannot always be realised. Thus, further examinations are needed to find a suitable tool that allows simple and cost-efficient estimations of the composition and fate of digestates in soil and also accounts for the variability of digestates' characteristics.

The potential of digestates to promote priming and the triggering circumstances need further investigation, as well. This could probably be achieved by further incubation experiments which use digestates that have been produced with varying retention times in the biogas reactor. This would lead to varying ratios of stable and easily available OM in the digestates and could elucidate whether the degree of stability of the digestate OM really decides about the potential to promote priming in soil or not. To support this hypothesis, the experiments could also cover a broader range of soils.

Another important matter of future research is the extension of the plant root models in incubation studies. The case of predominant nutrient cation uptake by plants could be simulated by cation exchange resins. It could be investigated to what extent the high pH of digestates compensates the increased proton release by the model root and whether priming is still likely to occur. The use of mixed resins (anion and cation exchanging) would increase the similarity to plant roots and could also give further information about the nutrient availability of digestates, thereby saving time and costs for pot trials or field experiments. However, with regard to priming, the method of integrating anion exchange resins in incubation experiments has to be developed further. The amount of emitted C by the resins should be determined exactly to distinguish it from the amounts of soil- and digestate-derived C emissions. One possibility is the $\delta^{13}C$ method, as shown for digestate M in the third experiment. This could be extended for digestates from C3 plants by using soils with

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sufficiently different $\delta^{13}C$ signal. Additionally, the amount of C sorbed to the resin at the beginning and at the end of the incubation should be measured for all samples.

New studies can also deal with the extension of the model for phosphate transport to an anion sink, such as a plant root. In addition to diffusion and supplementary delivery of P due to the mineralisation of OM, the precipitation of calcium phosphate at higher pH or other immobilisation processes could also be accounted for. However, the sensibility of a more complex model with even further parameters could be discussed, as well.

Finally, the influence of P removal on additional P supply due to OM mineralisation could be examined. It is still an open question, whether the additional P supply due to OM mineralisation is solely a pH effect or whether microorganisms exploit OM because of P shortage after P removal.

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