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THE IMPACT OF BIOCHAR ON NITROUS OXIDE EMISSIONS AND NITROGEN CYCLING IN TEMPERATE CROPLAND SOILS

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presented by

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Summary

Biochar, a carbon-rich, porous pyrolysis product of organic residues is discussed as an option to tackle some of the major problems the global world food system is challenged with. Applied to soil, biochar would sequester carbon dioxide but could also have beneficial effects on nitrogen retention, hence crop yields and nitrous oxide (N_2O) emissions. There is little understanding about the corresponding mechanisms but many experiment have shown decreased N_2O emissions, increased yields and manifold changes in N transformation, indicating the potential of biochar in increasing N use efficiency. Whereas biochar effects on tropical soils in extensive agriculture are often positive, its impact in temperate fertile soils with intensive fertilisation is less unidirectional. Furthermore, especially for soil N_2O experiments, most studies were carried out in the laboratory in highly artificial conditions. We tested the effect of biochar on N use efficiency, crop yields and N_2O emissions in two field experiments; firstly on a lysimeter system with two different soils (sandy loam and silt loam) in a winter wheat - cover crop - sorghum rotation and secondly in a field trial with conventional maize cropping (on a Eutric Mollic Gleysol). On the lysimeter experiment fertiliser N was traced by 10 atom% ^{15}N labeled fertilizer to investigate biochar effects on nitrogen use efficiency and the partitioning between the different nitrogen pools. In the other field experiment, N_2O fluxes were measured with an automated system and an additional limestone treatment was introduced to check whether the same effect as with biochar is achieved only by a soil pH increase. Results showed positive effects of biochar on N_2O emissions with reductions of 15-50 %. Nitrogen use efficiency and yields were, however, not significantly altered by biochar. Mechanisms of how biochar acts in soil remain largely speculative. Nevertheless, biochar is considered as a save option to use in agricultural systems with minor effects in temperate soils. Further research is needed to understand the mechanisms of biochar in soils, especially with its ageing in mid- and long-term field experiments.

Key words: biochar, nitrogen use efficiency, nitrous oxide emissions, crop yields, temperate climate soils

Summary

Zusammenfassung

Biochar (Pflanzenkohle) ist ein kohlenstoffreiches, poröses Produkt aus einer Pyrolyse von organischem Material und wird als Option zur Lösung einiger der grossen Probleme des globalen Ernährungssystems gehandelt. Wird Biochar in den Boden eingebracht, könnte damit Kohlenstoff aus der Atmosphäre sequestriert werden mit zudem positiven Auswirkungen auf den Stickstoffhaushalt, landwirtschaftliche Erträge und Lachgas (N_2O) Emissionen. Obwohl die zugrundeliegenden Mechanismen noch wenig verstanden sind, haben verschiedene Experimente verminderte N_2O-Emissionen, erhöhte Erträge und vielfältige Veränderungen im Stickstoffkreislauf gezeigt. Damit bestünde ein Potential für eine erhöhte Stickstoffeffizienz, was ein wichtiges Ziel einer nachhaltigen Landwirtschaft ist. Während die Auswirkungen von Biochar auf tropische Böden in extensiver Landwirtschaft meist positiv sind, ist der Einfluss auf fruchtbare Böden mit intensiver Düngung in temperierten Klimazonen weniger klar. Zudem wurden die meisten Experimente zu N_2O-Emissionen unter sehr künstlichen Bedingungen im Labor durchgeführt. In meiner Arbeit habe ich den Effekt von Biochar auf die Stickstoffeffizienz, landwirtschaftliche Erträge und N_2O-Emissionen in zwei Feldexperimenten getestet. Im ersten Experiment wurde eine Winterweizen-Zwischenfrucht-Hirsen Fruchtfolge auf einem Lysimetersystem mit zwei verschiedenen Böden (sandiger Lehm und schluffiger Lehm) angebaut. Das zweite Experiment fand im freien Feld auf einem "eutric mollic gleysol" mit konventionellem Maisanbau statt. Im Lysimeterexperiment wurde der Dünger mit 10 % ^{15}N markiertem Stickstoff verfolgt um Veränderungen der Stickstoffeffizienz und der Stickstoffspeicherung im Boden zu testen. Im anderen Feldexperiment wurden die N_2O-Emissionen mit einem automatischen Messsystem gemessen. Neben "Biochar" und "Kontrolle" wurde ein weiteres Verfahren eingeführt um zu testen, ob die Effekte von Biochar auch unter Zugabe von Kalkstein erzielt werden, was auf einen reinen pH Effekt hindeuten würde. Die Resultate zeigen einen positiven Effekt von Biochar auf N_2O-Emissionen mit einer Reduktion von 15-50 %. Die Stickstoffeffizienz und die Erträge wurden jedoch unter Zugabe von Biochar nicht signifikant verändert. Die Mechanismen wie Biochar im Boden wirkt, bleiben mehrheitlich spekulativ. Trotzdem kann Biochar als sichere Massnahme in landwirtschaftlichen Systemen betrachtet werden, bei der nur geringe Effekte auf temperierte Böden zu erwarten sind. Weitere Forschung ist nötig um die Mechanismen von Biochar im Boden zu verstehen, vor allem unter Berücksichtigung von Alterung der Pflanzenkohle in mittel- und

Zusammenfassung

langfristigen Feldexperimenten.

Stichwörter: Pflanzenkohle, Stickstoffeffizienz, Lachgasemissionen, Biochar, landwirtschaftliche Erträge, temperierte Böden

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Introduction

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1.1 Global challenges of the food system

Humankind imposes an increasing pressure on the planet's ecosystems that, at the same time, is the basis for any form of life. As efficient as it might be in some ways, industrial agriculture is a major threat to natural resources our society is depended on. On the one hand, the green revolution has substantially increased yields but at the cost of an increasing use of fossil energy per joule of food (Smith and Gregory, 2013). Services provided by natural systems are replaced by energy intensive chemicals and technology. Especially the soil with its vivid biota is the most important resource for the food system, which services are undervalued due to a lack of understanding (Bender et al., 2016). Contrastingly, if managed properly, soils could function as greenhouse gas (GHG) sink while still being used for agriculture (Smith et al., 2008).

Furthermore, climate change will impose more risks on the food system as climate variability, water scarcity and temperatures increase (IPCC, 2014). Agriculture as a large emitter of greenhouse gases (roughly 30 %) will be strongly impacted by global change. By 2050, global yields are expected to decrease by about 17 % due to climate change (Nelson et al., 2014). During the same time, the population will globally increase to around 9 billion, hence food demand steadily increases (Godfray et al., 2010). In order to supply enough food in the future, sustainable intensification is proposed to close the global yield gaps between the currently realised and potentially achievable yields (Tilman et al., 2011). Still this would require further expansion of agricultural land if no food-demand side measures are taken into account (Bajželj et al., 2014). Namely, a key driver of the unsustainable pathway the world food system follows, is the animal based diet (Eshel and Martin, 2006). Increasing consumption of meat and dairy products require larger areas for the production of feed which is then lost through inefficient animal husbandry (Stehfest et al., 2009). Therewith not only vast amounts of resources (fossil energy and land) are consumed but also excess nutrients are polluting the environment and ethical misdoing to the animal's dignity add to humankind's grievance. If in the European Union, 50 % of animal-derived foods would be replaced with plant-based foods, nitrogen (N) emissions would be reduced by 40 %, GHG by 25-40 % and 23 % per capita less use of cropland for food production (Westhoek et al., 2014). Nevertheless, the vision of "climate-smart" soils offers a set of climate friendly practices on the production side that can significantly help to mitigate GHG emissions from agriculture (Paustian et al., 2016) . Biochar is one of these management options that offer a potential for decentralised, agricultural-based

GHG mitigation while possibly increasing yields to ensure future food supply (Smith, 2016).

1.2 Biochar – A vision for a more sustainable agriculture

1.2.1 Characterisation and origin of biochar

Biochar is carbonised material from incomplete combustion of organic residues (Lehmann et al., 2006). Technically speaking, biochar is produced by thermal decomposition in low-oxygen environment, called pyrolysis. It was found that pyrogenic material is associated with high levels of soil organic matter and available nutrients in anthropogenic soils of the Brazilian Amazon basin, called Terra Preta do Indio (Glaser et al., 2002). These Terra Preta soils have an impressive soil fertility and resilience compared to many other tropical soils. The high input of charcoal by the ancient settlements of pre-Columbian Indios and the deposition of other residues (i.e. fish bones) are responsible for the very long term (centuries) improvement of soil fertility. This raised the idea of applying such stable carbon amendment also in modern agriculture, in both tropical and temperate regions (Lehmann, 2007; Woolf et al., 2010). The expression "biochar" was created for the group of black carbon materials (also charcoal or pyrogenic organic matter) when used nowadays intentionally for soil application in order to increase soil fertility, carbon sequestration or restore degraded soils (see International Biochar Initiative (IBI)). Because in general, this is a very heterogeneous group of materials, different interest groups have constrained the properties of biochar to a typical range in order to assure the quality of biochar-based products. For example, the European Biochar Certificate (EBC, 2012) requires a carbon content of at least 50 %, molar H/Corg < 0.7, molar O/Corg < 0.4 or PAH content < 12 mg/kg DM. Besides the chemistry, especially the carbonaceous structure itself provides biochar its multitude of functions in soil. The aromatic rings are rather stable against decomposition (Maestrini et al., 2014), offering the potential to sequester carbon in soil. Different functional groups allow for chemical adsorption and the large number of pores from different sizes (nano to micro) provide room for air, water and microorganisms (Lehmann and Joseph, 2015). Biochar effects on soil physical properties are summarized in a recent meta-analyses by Omondi et al. (2016). They report a reduced soil bulk density by 7.6 %, increased porosity by 8.4 %, increased aggregate stability by 8.2 % and available water holding capacity was increased by 15.1 %. The multifaceted effects of biochar on soil biota are summarized in Lehmann et al. (2011), where they point out the low level of understanding about the mechanisms. However, in most cases microbial biomass increased with biochar and no direct negative effects on roots have been detected. Hence, biochar potentially alters soil functioning in the physical, chemical and biological sphere. Our methods of measuring, analysing and thinking about biochar effects on soil are heavily challenged due to its multitude of interactions between and within the environmental spheres (and time). Additionally the effects can vary between different biochars as its properties are heterogeneous depending on the type of pyrolysis process, highest treatment temperature, feedstock of organic residues and post processing (Atkinson et al., 2010). The new book "biochar for environmental management (Science, Technology and implementation)" edited by Johannes Lehmann and Stephen Joseph gives a great overview on the disciplinary knowns and unknowns about biochar (Lehmann and Joseph, 2015).
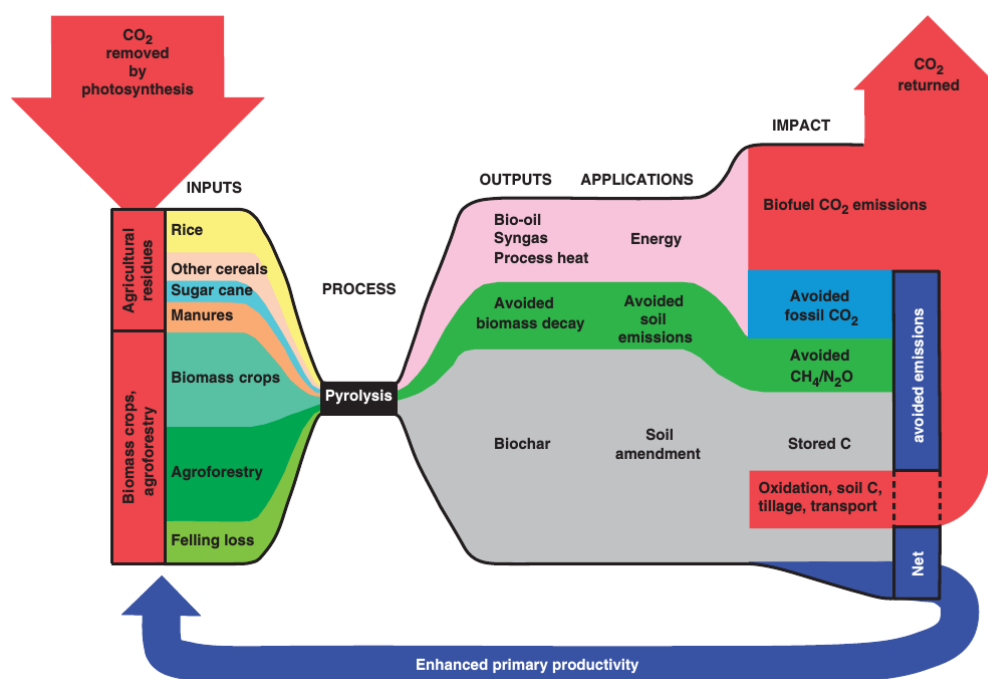
Figure 1.1 – An overview of the sustainable biochar concept. Inputs, processes and impacts on global climate are shown in approximated width of the relative proportions. Biochar offers the potential to avoid direct and indirect greenhouse gas emissions while enhancing primary productivity. Carbon inputs can both be provided from agricultural residues or biomass crops from agroforestry. (source: Woolf et al. (2010))

1.2.2 Biochars effect on soil nitrous oxide emissions

Nitrous oxide (N_2O) is a very potent greenhouse gas due to its long atmospheric lifetime of 114 years and its infrared absorption properties, hence having a 300-fold greater global warming potential than carbon dioxide (CO_2) (Thomson et al., 2012). In the stratosphere, N_2O will be the dominating ozone depleting substance in the future (Ravishankara et al., 2009). Although the emissions are microbial-mediated in natural soils, industrial agriculture is responsible for the significant boost of its emissions by adding large amounts of reactive N to the system. Reducing N_2O emissions from agriculture is a key goal for the transformation to sustainable agriculture (Decock et al., 2015). Biochar was proposed to have the potential to reduce N_2O emissions from agricultural fields (Van Zwieten et al., 2015). Within my master's thesis we found reductions of 40-98 % of N_2O emissions by biochar in an artificial laboratory experiment (Felber et al., 2012). There are many published laboratory experiments that show large reductions, but real field studies are more scarce and show transient effects. A meta analyses by Cayuela et al. (2015) has found a reduction of N_2O emissions of 54 ± 2 % in controlled laboratory studies and 28 ± 16 % in field experiments. Commonly the reduction potential in the field is much smaller than the effects in laboratory experiments. Furthermore, field experiments can show increased emissions (Verhoeven and Six, 2014) and numerous experiments could not show a significant change in N_2O emissions with biochar in the field (Karhu et al., 2011; Scheer et al., 2011; Suddick and Six, 2013; Anderson et al., 2014; Angst et al.,

2014). Still, in general there are many field experiments that show decreased N_2O emissions after the addition of biochar to soil (van Zwieten et al., 2010; Zhang et al., 2010; Taghizadeh-Toosi et al., 2011; Felber et al., 2013; Case et al., 2014).

When it comes to mechanisms that should explain the effect of biochar on N_2O emissions, there are numerous mechanisms proposed within the different spheres of natural science (Van Zwieten et al., 2015).

chemical soil functioning

- biochar could reduce N availability in soil
- biochar changes bioavailable C in soil,
- bioavailable C in biochar is oxidized by N_2O
- biochar increases soil pH (Obia et al., 2015)
- the role of biochar in altering redox reactions (Cayuela et al., 2013)

physical soil functioning

- biochar affects gas diffusivity and soil aeration (van Zwieten et al., 2010)
- sorption of N_2O onto biochar (Cornelissen et al., 2013)

soil biota

- microbial inhibitors in biochar (Spokas, 2013)
- adsorption of bacterial communication molecules (Masiello et al., 2013)
- microbial N immobilisation due to increased C/N ratio by biochar (Nelissen et al., 2015)
- indirect microbial reactions to biochar mediated changes in physical or chemical conditions

agricultural effects

- enhanced plant uptake (Crane-Droesch et al., 2013)

For all these mechanisms there are studies that support or contradict some of the hypothesised options. The testing of these hypotheses are especially difficult because all these plausible mechanisms interact with each other and hence it is not easy to test them separately. If one still tries to do so, the new artificial system may not be comparable to the actual net of interactions biochar provokes in soil. In addition to the examples in the biochar book (Van Zwieten et al., 2015) Ameloot et al. (2016) rejected the hypothesis that biochar exerts an individual physical control over soil denitrification and that biochar creates denitrification hotspots in soil enhancing complete reduction of N_2O to N_2. Most prominent biochar induced N_2O emission reduction was to be found in manipulations that promoted labile C availability (Ameloot et al., 2016). This is also in agreement with the findings of my master's thesis (Hüppi, 2011). Cayuela et al. (2013) proposed an interesting but speculative mechanism, that biochar function as an electron shuttle, facilitating the transfer of electrons to soil denitrifying microorganisms that promote the reduction of N_2O to N_2. This idea was further supported by Klüpfel et al. (2014), who showed that biochar pyrolyzed at an intermediate temperature (400-500°C) has the highest capacity to both donate and accept electrons in its carbonaceous structure. Later on Cayuela et al. (2015) also identified the H/Corg ratio of biochar being a key factor in mitigating N_2O emissions from soil. The H/Corg ratio is also an indicator for the degree of polymerisation and aromaticity of biochar, hence the structures that are known to allow for long-distance electronic energy transfer (Winiger et al., 2014).

In contrast, there are also mechanisms and situations that lead to increased N_2O emissions

with biochar. Sánchez-García et al. (2014) showed that biochar increased N_2O emissions during nitrification. There is a number of studies that have suggested the biochar induced increase in soil pH could be responsible for reduced N_2O emissions (van Zwieten et al., 2010; Zheng et al., 2012). A corresponding mechanism to the pH effect has already been described by Stevens et al. (1998) showing the control of soil pH on the $N_2O : N_2$ ratio. Bakken et al. (2012) showed that low pH impedes the synthesis of a functional N_2O reductase enzyme. This effect was detected in biochar-soil slurries when N_2 emissions were increased with biochar while N_2O emissions decreased at the same time (Obia et al., 2015). The pH hypothesis is further discussed in chapter 4 (Hüppi et al., 2015).

Limited N availability can be a result of different factors how biochar impacts soil N dynamics (Clough et al., 2013) and can lead to N immobilisation (Nelissen et al., 2015). Sorption of N on biochar can also play a role while ammonium was rather sorbed than nitrate (Hale et al., 2013). In manuscript 2 effects of biochar on N cycling is discussed in more depth (see chapter 4). Changes in gas diffusivity can impact N_2O emissions as well by the influence biochar obviously has on soil physical properties (Omondi et al., 2016). Together with changes in surface area and soil pore volume of different sizes, N_2O emissions were significantly decreased with biochar (Mukherjee et al., 2014).

1.2.3 Effect of biochar on yields

For practical applications in agriculture, the most important argument to use biochar, is the increase of soil fertility. Increased yields were reported in many field experiments and summarised in meta analyses (Jeffery et al., 2011; Crane-Droesch et al., 2013). They report a grand mean yield increase of about 10 %, but with large variability and dependence on many factors (crop type, soil, biochar type, application rate, etc.). Crane-Droesch et al. (2013) showed that soil cation exchange capacity and soil organic carbon content are the strongest predictors for positive yield response with biochar. Hence, tropical soils that are low in these fertility parameters, may profit the most from biochar application whereas temperate soils with high carbon content can be less improved by the addition of biochar. The observation of relevant properties in meta analyses itself does not yet provide the mechanism for the yield effect of biochar. Similar to the mechanisms for the N_2O reduction, for biochar effects on yield, various mechanisms are proposed, partly confirmed and falsified. The biochar book chapter by Jeffery et al. (2015) lists known direct mechanisms (induced systemic resistance to pests, reduction of Al toxicity and introduction of nutrients) and known indirect mechanisms (nutrient availability effects, pH effects and reduced N losses by N leaching or N_2O emissions). Furthermore, there are hypothesised mechanisms for positive yield effects (biotic interaction including mycorrhizal fungi or biological N-fixers and increased water availability) and negative hypothesized mechanism (N immobilisation, pH increases in high pH soils, phytotoxicity, reduced efficacy of pesticides, sulphur content and salinity issues) (Lehmann and Joseph, 2015). A better understanding of such processes would aid the end users choice of the most suitable and sustainable biochar for their production system.

1.2.4 Effect of biochar on N cycling and N use efficiency

Relevant for both yield effects and N_2O emissions from soils are biochar effects on N cycling. There is again a multitude of possibilities where and how biochar can impact the numerous processes in soil N cycling (Clough and Condron, 2010). From an agronomic perspective an

increase in N use efficiency would be a key outcome for a sustainable biochar use. Increased yields at a constant input of N is one effect that has already been discussed above. A substantial reduction of N losses could be achieved by reducing N leaching or reduce gaseous N losses. Most of the experiments published showed a decrease in nitrate and/or ammonium leaching (Güereña et al., 2013; Dempster et al., 2012; Yao et al., 2012; Ventura et al., 2013). Concerning leaching of nutrients in general, again, the biochar book chapter by Laird and Rogovska (2015) offers a number of mechanisms that allow for reduced leaching: Physical retention of water and nutrients, retention of nutrients by cation exchange, retention of nutrients by anion exchange, liming value of biochar changes soil pH which influences nutrient solubility, adsorption of biogenic organic compounds containing nutrients and microbial activity influences nutrient cycling and co-precipitation of nutrients. None of these mechanisms could be proven to be the one responsible for the observed effects. Regarding N_2 emissions, there are hardly any studies that have looked at the impact of biochar. Obia et al. (2015) showed in anoxic slurries with biochar, that N_2O and NO emissions were decreased, while N_2 production increased. Also reports about increased nosZ activity (Harter et al., 2014; Van Zwieten et al., 2014) show that biochar may not reduce denitrification losses but rather suppress N_2O emissions by fostering the total reduction to N_2.

There are many other steps in the N cycling that were investigated with respect to biochar. Prommer et al. (2014) found reduced organic N transformation rates with biochar. Others found accelerated gross nitrification (Nelissen et al., 2012; Anderson et al., 2011). Also increased soil N immobilisation has often been observed (Nelissen et al., 2015; Bruun et al., 2012). For soil N dynamics and stabilisation, aggregate formation plays a key role (Six et al., 2004). Several authors observed increased aggregate stability, water retention and porosity after biochar application (Obia et al., 2016; Sun and Lu, 2014). Still, these observation cannot explain in more detail how biochar actually acts in soil and which properties are relevant. There are effects and interactions on many disciplinary fields within soil science but most studies do not connect the different aspects and hence cannot explain the complex interactions.

Objectives

There is a need for major improvements in agricultural practices to meet the global challenges pointed out in the introduction. Biochar is proposed and advocated as significant contribution to these needs. However it remains unclear whether the variety of positive effects are seen, especially in temperate soils. Furthermore the lack of understanding concerning the mechanisms of biochar in soil, hampers the well designed use of biochar for its specific purpose (i.e. reduced N_2O emissions, improved crop fertility, carbon sequestration).

The objective of this thesis was to test the impact of biochar within agricultural field trials in temperate soils. The main focus was on how biochar changes N_2O emissions from soil, yield and fertiliser use efficiency. The mechanisms of possible effects need to be elucidated in order to explain biochars impact in such a system. For this reason, N flows were traced with ^{15}N labelled fertiliser on an open air lysimeter (Fig. 2.1). In this first experiment, N use efficiency, fertiliser leaching, N_2O production from fertiliser, plant fertiliser uptake and soil fertiliser N retention are tested for the effect of biochar. In the second experiment on a maize field, N_2O emissions are continuously measured with an automated static chamber system (Fig. 2.2). An additional pH treatment was introduced that increased the soil pH to the same level with limestone as the biochar had increased the pH. If the limestone would result in a similar N_2O effect, this would indicate, that the mechanism of biochar maybe only due to the changed chemical properties through liming.

In addition to the biochar related questions, technical issues about static chamber measurements are addressed in a separate chapter. Because in both experiments, static chamber measurements are the method chosen for the N_2O measurements, unsolved issues within the greenhouse gas chamber flux methodology are addressed. Specifically the use of non-linear flux calculation schemes increases the uncertainty of the flux estimate dramatically. On the other hand, linear interpretation leads a systematic underestimation of the flux. Therefore, commonly used calculation scripts were evaluated and an improved selection procedure between the options is suggested for available flux calculation scripts.

In particular the following research questions are addressed with a open air lysimeter experiment, a maize field trial and a R-based flux simulation:

1. Is there a significant effect of biochar addition on peak and cumulative N_2O and CO_2
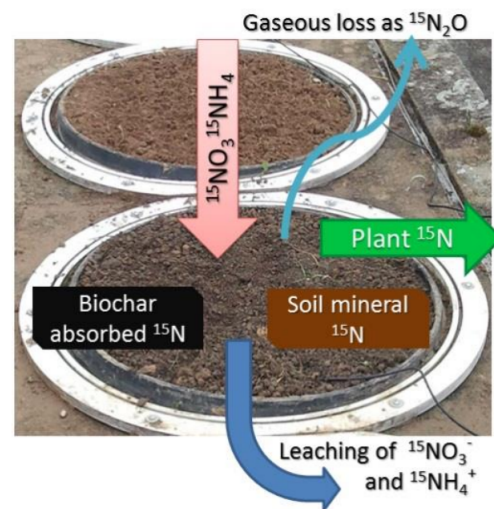
Figure 2.1 – Schematic of the fertiliser tracer experiment on the lysimeter system

Figure 2.2 – Picture of the automatic N_2O chamber measurement system in the field before maize was planted

fluxes for annual crops under field conditions? – Lysimeter and field trial

2. Are there yield effects (i.e. increased N uptake) by biochar on various common crops of the temperate region? – Lysimeter and field study
3. Does biochar effect the origin of N taken up by plants? – Lysimeter study
4. Does the biochar effect on N cycling depend on soil type? – Lysimeter study
5. Is there a significant difference between the effect of biochar and the effect of increasing soil pH by lime on soil N_2O emissions? - Field trial
6. Is biochar capable of reducing N loss through leaching? - Lysimeter study
7. How can the static gas flux chamber data be automatically evaluated for its suitability for non-linear flux models, depending on the sampling setup, flux size and measurement precision? - R simulation

Synthesis

3.1 Results and Discussion

3.1.1 Finding 1 – Effect of biochar on nitrous oxide fluxes

We found a 52 % reduction in N_2O soil emissions from biochar compared to control treatment in the maize field trial. Although we have measured N_2O with a very high time resolution, the triplicated field experiment did not show significant treatment effects due to a large variability in the lime treatment that we introduced in addition to the biochar and control treatment. If we would simply follow the judgement of the p-value being higher than 0.05, we had to admit, there is no effect of our treatment on N_2O emissions, hence also no biochar effect. But this is a good example for the ASA's statement on p-values (Wasserstein and Lazar, 2016) reminding the scientific community that this simple understanding of statistics is not sufficient for a proper scientific discussion. P-values are uncertain, especially with a low number of replicates (3 is the very minimum) and the p-value does also not reflect any effect size. In our case, the power of the anova and hence also of the p-value was very low. The chance to get a significant effect on the 5 % alpha level would only be 23 %. Only with at least 10 replicates, the power within this underlying dataset would exceed 80 %. Most of the variance in the nine cumulative N_2O emissions from the different chambers is due to one replicate in the liming treatment that had the highest emissions. If one would remove this chamber on the "N_2O hotspot", the interpretation would change radically. This has not been done in the publication for SOIL (see chapter 4) because there were no scientific explanations for the hotspot and the two remaining replicates in the liming would not be sufficient for the analysis. However, if disregarding the hotspot, the liming treatment would have similar cumulative emissions as the biochar treatment and the biochar effect would be very significant. But leaving out the lowest cumulative lime replicate would lead to a significant difference to the control. Therefore our conclusion towards decreasing N_2O emissions by biochar (notable by 50 %) is much stronger than conclusions about the pH effect. Without having an understanding of how the hotspots are driven (mouse holes, local excess of N or high water contents etc.) we have to expect them in any treatment. By chance, this could have happened within a biochar plot, which would have masked any reduction effect.

In the lysimeter experiment, we measured N_2O manually and found significant reductions with biochar of 15 %. The technical challenges with the semi-automated gas sampling and

the low precision of the in-house GC caused an unfortunate loss of data in the beginning. In the second year during sorghum cropping, the emissions were rather low and the difference between the treatments too. Together with the large reductions in the maize field, this shows that in temperate intensive cropping systems under real field conditions, N_2O emissions can be reduced by biochar. There was no effect of biochar on CO_2 emissions, but the measurements were not well suited for soil emissions because plants grew within the chamber. This data is not published but was used as quality control for the N_2O measurements. The results from N_2O emissions are in agreement with other world-wide biochar experiments published (Cayuela et al., 2015). Mechanistically our experiments cannot support a specific hypothesis. However, we can point out, that biochar has not restricted N supply for denitrification according to the soil mineral N measurements. But these measurements may not reflect the N supply situation of soil biota being involved in N_2O production. Pore size distribution was certainly changed by biochar application, what was also indicated by changed water contents. Not only more pores for water are provided by biochar but also the oxygen content was probably higher reducing favourable conditions for N_2O production. Further speculative explanations are changed redox properties in soil with the indication that our biochars had a rather small H/Corg ratio. However, it is barely understood how improved electron transport along biochar polymer would impact microorganisms being relevant for N_2O processing (Kappler et al., 2014). Another interesting field of explanations is the reduced ability of some bacteria to communicate with each other because biochar absorbs those signalling molecules (Masiello et al., 2013).

3.1.2 Finding 2 – Yield effect of biochar

We have not seen any yield effects in any of our experiments nor for any of the crops (maize, winter wheat, green rye, sorghum) due to biochar application. This supports the finding also from meta-analyses Crane-Droesch et al. (2013) that yield effects are small in temperate, fertile soils. High N supply from fertiliser application did probably also relieve N limitation for plants and microorganisms as well as possible N immobilisation by biochar. There was no increase in plant N uptake. Our results also show that plant growth was probably not limited by factors that were affected by biochar, i.e. pH and base saturation. There are a number of other factors that were possibly driving the yields in our experiments. Droughts limited the growth of both the winter wheat and maize cropping, which hampered the plant's full potential to take up available N in soil. In the lysimeter system this leads to a very low uptake of the fertilised N, leading to a low level of N use efficiency. In contrast to other studies (Karer et al., 2013), I did not find a reduction in water stress in drought situations by holding additional water in the pores of biochar.

Large variability in field experiments are common, especially due to the heterogeneity of soil. When biochar is irregularly applied to soil, spatial heterogeneity in the soil biochar content can significantly affect soil quality and wheat growth (Olmo et al., 2016). The rototiller used in the maize field and the manual mixing in the lysimeter may have reduced this risk in my experiments. However, at smaller scales (1-10 cm) one could often observe biochar hotspots that could be relevant for the soil sampling and the placement of the static chamber. Additionally external factors did increase the variability of our yields, namely birds largely consumed the winter wheat (roughly two thirds) and sorghum (95 %) whereas the maize field was sometimes in-homogeneously dominated by weeds. These and other management

problems can lead to a significant confounding in agricultural field trials, especially if a PhD student does not have the experience needed. Nevertheless, a significant increase in plant N uptake by the biochar can hardly be expected in this temperate fertile soils. Meta analysis on biochars crop yield response point out the lack of data from temperate regions (Atkinson et al., 2010; Jeffery et al., 2011). Globally an effect of plus 10 % is found by different studies, but richer soils appear to be less likely to benefit from biochar (Crane-Droesch et al., 2013).

3.1.3   Finding 3 – Origin of N taken up by plants

Plants took up N either from the labelled fertilizer or from the soil N-pool. We have used ^{15}N labelled fertiliser in the lysimeter system to track mineral N flows in the cropping system. Fertiliser derived aboveground plant N was not different with biochar compared to control plots. We conclude that N availability for plants of either fertiliser or soil N was not affected by biochar. However, the N availability may not have been the limiting factor to plant N uptake as N supply was high and growth was limited by other factors (heat and water supply, see finding 2). Steiner et al. (2008) demonstrated an enhanced fertiliser N uptake in sorghum by biochar with ^{15}N tracer in a Central Amazon soil. They explained these results with improved N retention in soil due to increased cation exchange capacity or to increased microbial N immobilisation. In my case the cation exchange capacity was not increased (see chapter 5), neither is an increased microbial N immobilisation expected due to high fertilisation rates. Both effects may be less important in temperate soils compared to tropical ones.
Fertiliser N use efficiency was not increased in my experiment as a result of unchanged plant N uptake and N losses. The minor differences in decreased losses of N_2O are not significant, neither could the N_2 loss be measured. Other studies have reported increased N use efficiency by biochar (van Zwieten et al., 2009; Chan et al., 2007). But as pointed out above, without increased plant N uptake and no significant change in the major quantitative N fluxes of the system, I cannot confirm an increased N use efficiency by biochar in our temperate, intensive cropping system.

3.1.4   Finding 4 – Biochar effects on N cycling with respect to soil type

There was no biochar effect on yields, N use efficiency and plant N uptake. N_2O emissions were significantly reduced in both soils, but the reduction was not dependent on soil type. In a laboratory experiment Singh et al. (2010) looked at N_2O emissions and inorganic N leaching in two different soils with four different biochars. Although their difference between the two soils (Alfisol and Vertisol) were much larger than our silt and sandy loam, they did not see very different effects between the soils, i.e. decreased ammonium leaching and N_2O emissions but no reductions in nitrate leaching. But interestingly their effects increased with time, which they explained with increased sorption capacity of biochars through oxidative reaction on its surfaces with ageing. Effects of leaching in the lysimeter experiment are hence both expected to further increase with time because of ageing and the delay of the N signal through the rather deep soil column.

3.1.5   Finding 5 – Nitrous oxide reduction effect related to pH changes

There is no evidence that the N_2O emission reduction with biochar, relative to control, is solely induced by a biochar-induced increase in soil pH. The pH hypothesis is thus not supported

by our data. But as pointed out in finding 1 this is a very weak indication because of the large variability within the lime treatment. If the highest or lowest replicate was removed, the picture would dramatically change. However, in the lysimeter experiment the pH significantly increased with biochar over at least nine months, whereas also the N_2O emissions were significantly decreased. Also in the literature decreased N_2O emissions are sometimes related to pH effects from biochar (van Zwieten et al., 2010; Zheng et al., 2012). But also if increased pH would lead to lower N_2O emissions, this would still not directly explain a mechanism in soil. Increased soil pH would probably increase total denitrification and hence act as microbial effect (Bergaust et al., 2010). This was shown in biochar-soil slurries by Obia et al. (2015).

3.1.6 Finding 6 – Biochars effects on N leaching

Over the two years of the lysimeter field study, there was no significant effect of biochar on leaching. However, with time, N amounts in leachate from biochar treated plots decreased leading to a significant, but still small, effect in the last year. With this large and undisturbed lysimeter columns leaching needs to be monitored over longer time. Also with ageing the ability of biochar to impact leaching may significantly change with time (also see finding 4). Fertiliser N added in the first year could be distinguished from soil N by the ^{15}N label in the leachate. The ^{15}N in leachate was however only measured in bulk from the freeze dried residues of the collected water. A separated extraction of the leachate nitrate and ammonium could provide a more detailed data of leaching within this system. The few points in time measured, did not show any difference between treatments, neither in fertiliser nor soil N. Reduced N leaching from soil is often reported in experiments with biochar (Laird et al., 2010; Güereña et al., 2013; Ventura et al., 2013). These studies, however, mostly use laboratory experiments or small scale lysimeters. Our system does more closely reflect field conditions but suffer from increased variability and response time.

3.1.7 Finding 7 – Optimised flux calculation scheme for static chambers

The new proposed decision function and flux simulation environment offers great opportunities to understand a specific measurement system and decrease uncertainty and bias of non-steady state greenhouse gas chamber measurements. The simulations also offers a set of indicators for model uncertainty and bias that should be given with the publication of static chamber data (bias, uncertainty, detection limit). Furthermore it improves the nonlinear HMR decision script (Pedersen et al., 2010) or other procedures (Leiber-Sauheitl et al., 2014) by significantly reducing the uncertainty of the estimate without a large increase in bias. In the current literature on static chamber derived GHG measurements, often rather crude assumptions are used to model the increase in concentration during closure time (Venterea et al., 2009).

3.2 Conclusions and Implications

Biochar did not change fertilizer use efficiency or N partitioning among the different ecosystem compartments of the agricultural system (soil, plant or leachate) over the course of my 2 year lysimeter study. Biochar treatment caused a decrease in N_2O emissions but no change in the source of N for N_2O production. Soil pH might play a role in reducing N_2O emissions but there is a multitude of other processes that can have a relevant impact. Although the

observed effects due to biochar application (i.e. reduced N_2O emissions and leaching) are small compared to the overall N balance, they are important to understand biochar functioning in agricultural systems and to manage them more sustainable. Especially reduced N_2O emissions have a large relevance for climate mitigation and the overall biochar GHG balance. Biochar application in Swiss agricultural soils has the potential to significantly reduce N_2O emissions and possibly reduce N leaching without impairing crop yields. The high supply of nutrients, especially N, may mask the beneficial effect that biochar could have on plant growth. However, a comprehensive life cycle assessment is needed to verify if these improvements can counterbalance possible adverse effects from biochar production (e.g., competition for biomass as a resource) and other adverse effects (e.g., introduction of organic and inorganic pollutants to soil). In any case, biochar has the potential to increase soil carbon content and reduce GHG emissions by stored C and avoided CO_2 and N_2O (Woolf et al., 2010).

New insights into mechanisms of biochar functioning are rather scarce. We have not seen effects in targeted mechanisms that the experiments were focused on, i.e. fertiliser N partitioning and pH. It is not expected that individual mechanisms could be the single most relevant factor for biochar in the agricultural system. Rather, it is the case that biochar changes a multitude of processes in an interconnected way. To understand interactions, each possible factor needs to be controlled and sufficiently replicated. Additionally, the variability in field experiments is large due to several contributions: inhomogeneities in soil, unequal management effects, natural variability or the precision of measurement methods. The limited number of replication is not able to overcome these uncontrolled sources of variability, especially when only the conventional p-value threshold of 0.05 is considered. Furthermore, the interaction between many factors imposes major scientific challenges that have to be addressed in an interdisciplinary soil science framework (i.e. soil chemistry, biology and physics). Meta-analysis can gain additional insights from integrating many individual studies, but they cannot elucidate mechanistic links between empirical observations and actual processes in soil. This might be a reason why the reduction in N_2O emissions by biochar application still cannot be explained. However, several of the proposed mechanisms in the introduction are plausible. Among others, changed redox properties, increased soil aeration through increased pore volume and microbial effects on N availability are plausible but yet unproven mechanisms.

My field experiments revealed the behaviour of biochar in real climate field situation over roughly two years. This has more relevance than many artificial laboratory experiments with respect to the agricultural and natural systems (plants, seasons, fluxes, soil biota). However time scales matters a lot in mechanisms of how biochar is proposed to act in soil. The short term effects are prone to vanish within months and could also be reversed as biochar properties change with ageing in soil (Nelissen et al., 2015). It would be very important to observe all the effects and fluxes for longer time period. I.e. sorption or pH effects are rather short term, whereas soil structure changes in longer time scales. Biochar also adsorbs soil nutrients and increases the C/N ratio when applied fresh, which implies a short term negative effect on nutrient supply but a potential slow release later on. The application in combination with compost is highly recommended by the practitioners within the biochar community. However, this introduces much more variability and uncertainties, hence would require even more field replicates, more precise and integral methods. Mechanisms of how biochar should be

loaded with nutrients and supply them later on to plants could not be revealed in detail yet. The number of unknowns and swampy explanations implies the risk for biochar research to keep focusing on individual disciplines without significant progress in process understanding and mechanisms. Soil science itself struggles with a very complex matrix to study. If the complexity of the charosphere is added to soil, its network of interactions seem to be too complex for a single disciplinary approach. Especially biochar interactions with soil biota are poorly understood (Lehmann et al., 2011).

In manuscript 3 (chapter 6) a simple approach to optimally balance the bias and uncertainty in static GHG chamber measurements is developed. Although this sounds like a simple and obvious goal, it has not sufficiently been solved. Users of low frequency measurement systems (gas chromatography) are prone to either introduce a bias or large uncertainties when interpreting their concentration measurements. Although this is a rather old measurement technique, there are still no straightforward solutions that guide the users to an optimal use of nonlinear vs. linear flux calculation schemes. My new approach is a sophisticated step forward to reliable flux measurement from automated flux calculation scripts. It leads to a more objective treatment of the data, with less expert knowledge needed.

3.3 Perspectives

Biochar research needs to overcome its standoff with some new ideas. Innovative and conventional scientific methods from the different disciplines in natural science need to target the many unknowns of the system. But maybe only a holistic approach can answer the questions that remain. To me it looks like the separation of the individual components hinders interactive mechanisms to show the effect of the entity.

There is a set of data that was collected from the field experiments, that could not yet be analysed in detail. For example the data on nitrifier and denitrifier gene abundance (nifH, amoA, nirK, nirS and nosZ) from the 2nd field experiment will eventually be evaluated and published by Hans-Martin Krause (Fibl). This could reveal a possible microbial pathway for reduced N_2O emissions (Harter et al., 2014). If the enhanced nosZ expression on either DNA or RNA level are confirmed, a pyrosequencing of the same samples should be considered. This might show biochar-induced shifts in the taxonomic composition and structure of the soil bacterial community in the field being relevant for the reduction of N_2O emissions (Harter et al., 2016). Alternatively NanoSIMS could provide more insights to in situ processes of microorganisms on biochar that are potentially relevant for N transformation (Behrens et al., 2012). In parallel to the sampling campaign for the functional genes, a ^{15}N label on nitrate was applied in the ammonium nitrate fertiliser. Data from soil and N_2O samples could show changes in nitrate transformation pathways by biochar and liming, but the samples are not measured yet. Furthermore, data on soil physical properties (pore size distribution, water retention curves) are collected and await to be published by Thomas Keller (Agroscope). Changes in soil gas diffusion properties by biochar seem to impact soil N_2O emissions (Berisso et al., 2013). These datasets will allow further insights into mechanisms from different disciplines. Both examples are expected to show differences between biochar and control and hence indicate relevant pathways for decreased N_2O emissions.

Biochars electron donating and accepting capacity (Klüpfel et al., 2014) could play an important role for its N_2O reduction potential. Possibly the combination of electron donating

and accepting capacities of the biochar may be relevant to N_2O reduction (Cayuela et al., 2013). Biochar samples from different field experiments were given to Michael Sander (ETH) to measure redox properties. To my knowledge this data is not ready yet. To study the connection between biochars redox properties and N_2O reduction in soil, one could gather a set of biochars with different ratios of electron donor and acceptor capacities (determined by the pyrolysis heat treatment temperature) and then test them in a laboratory experiment for their effect on N_2O reduction. This could provide an indication on how to design biochar for the purpose of reducing N_2O emissions from the field.

More data could be analysed regarding the downward transport of biochar and fertiliser N in the lysimeter soil column. Differential Scanning Calorimetry (DSC) data could show how fast biochar is transported within the soil column (Rumpel et al., 2015). Furthermore, by separating the biochar particles one could test if there is a larger ^{15}N fertiliser concentration in the charosphere compared to the bulk soil.

If the driving mechanisms of biochar functioning in soil in general and for reducing N_2O emissions in particular can be found, biochar could precisely be designed for a specific purpose. Researchers should find out, what kind of biochar can be applied to which kind of soil in which agricultural system and management. Also the longevity of biochar effects need to be understood in detail. There are both perspectives for a short term effect of biochar (related to labile C or N in biochar, pH increase, changed N transformation rates or sorption) as well as that effects can be enhanced with the ageing of biochar (surface area and number of binding sites are increased, soil biota populates biochar etc.). Only if these questions are resolved, the relevant long-term potential of biochar can be elucidated.

New field experiments with biochar should involve more specialists from different fields in soil science (i.e. soil physics, soil C and N, soil biota, system perspective etc.). The effects of different organic residues applied to agricultural fields could be compared (i.e. fresh plant material, composted organic matter, residues from biogas plants, biochar). This system would need to be monitored on a longer time scale, up to 10 years. That is what is planned for a new long-term field experiment by Benjamin Seitz (Fibl and Agroscope).

Future research towards a sustainable food system with the support of biochar will, besides the environmental aspects, also involve social and economic perspectives. How large and complex should biochar production plants be in order to be economically feasible and to have enough organic feedstock from residues within short transport distances? The pyrolysis reactor should also be able to deliver high quality energy, if possible electricity or at least heat. Lifecycle assessments may show in the future which systems are appropriate for specific farming systems.

In steady state chamber measurements, the suggested flux calculation scheme can be safely applied by any user. The established gas flux calculation script from Roland Fuss (Fuss, 2015) could be updated with more appropriate decision rules and equipped with the simulation and visualisation tool I have developed. Eventually the script with all its gas flux functions can be published as R package on CRAN. Thereby a user can not only calculate his fluxes based on a solid reference but also increase the understanding of his measurement system. It can

be further explored how the simulations performs when a more complex diffusion model is applied instead of HMR (Venterea, 2013).

Science still desperately sticks to arbitrary p-values hence producing a lot of results that cannot be replicated (Wasserstein and Lazar, 2016). This and other issues of Desperate Science must be addressed by all individuals and institutions working in research. One specific deficit that bothers me personally is the lack of openness (Open Access, Open Review, Open Source etc.) in public funded research. In this thesis, all publications will be published open access. Unless the system doesn't increase its efficiency, progress will slow down continuously. An important step forward against the Desperate Science phenomena is awareness; about the challenges and needs of the society but also within the scientific community itself.

Manuscripts Part II

4

Effect of biochar and liming on soil nitrous oxide emissions from a temperate maize cropping system

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Abstract

Biochar, a carbon-rich, porous pyrolysis product of organic residues may positively affect plant yield and can, owing to its inherent stability, promote soil carbon sequestration when amended to agricultural soils. Another possible effect of biochar is the reduction in emissions of nitrous oxide (N_2O). A number of laboratory incubations have shown significantly reduced N_2O emissions from soil when mixed with biochar. Emission measurements under field conditions however are more scarce and show weaker or no reductions, or even increases in N_2O emissions. One of the hypothesised mechanisms for reduced N_2O emissions from soil is owing to the increase in soil pH following the application of alkaline biochar. To test the effect of biochar on N_2O emissions in a temperate maize cropping system, we set up a field trial with a $20\,t\,ha^{-1}$ biochar treatment, a limestone treatment adjusted to the same pH as the biochar treatment (pH 6.5), and a control treatment without any addition (pH 6.1). An automated static chamber system measured N_2O emissions for each replicate plot ($n = 3$) every 3.6 h over the course of 8 months. The field was conventionally fertilised at a rate of

$160\,kg\,N\,ha^{-1}$ in three applications of 40, 80 and $40\,kg\,N\,ha^{-1}$ as ammonium nitrate.

Cumulative N_2O emissions were 52 % smaller in the biochar compared to the control treatment. However, the effect of the treatments overall was not statistically significant ($p = 0.27$) because of the large variability in the data set. Limed soils emitted similar mean cumulative amounts of N_2O as the control. There is no evidence that reduced N_2O emissions with biochar relative to the control is solely caused by a higher soil pH.

4.1 Introduction

Agriculture faces major challenges regarding world food security because of climate change, continued population growth and resource-depleting practises (IAASTD et al., 2009). Accounting for roughly 12 % of anthropogenic greenhouse gas (GHG) emissions per year, agriculture is a sector with a considerable mitigation potential and, at the same time, is highly vulnerable to the consequences of a changing climate (IPCC, 2014). With its 300-fold warming potential compared to CO_2, nitrous oxide (N_2O) from soil is a downside of the large productivity increase in agriculture, mostly due to synthetic nitrogen fertiliser application. Reducing agricultural N_2O emissions would reduce the GHG induced radiative forcing (IPCC, 2014), improve the stability of the stratospheric ozone layer (Ravishankara et al., 2009) and reduce agriculture's energy intensity when achieved with a lower nitrogen fertiliser use (IAASTD et al., 2009).

Biochar is produced by thermal decomposition of organic material in a low-oxygen environment, called pyrolysis. This stable charcoal-like material has the potential to contribute to the mitigation of climate change by increasing soil carbon (C) (Lehmann, 2007; Woolf et al., 2010; Lal et al., 2011). In addition, biochar can increase crop yields (Jeffery et al., 2011; Biederman and Harpole, 2013; Crane-Droesch et al., 2013) and reduce water stress, which helps to adapt to climate change (Mulcahy et al., 2013). Its application to soils that have a small cation exchange capacity and low organic carbon content is associated with higher crop yields (Crane-Droesch et al., 2013), with an overall mean response of 10 % (Jeffery et al., 2011). For fertile, temperate soils improvement of soil quality is not key to biochar application. Rather, biochar effects on soil-borne GHG emissions, N_2O in particular, have become a strong argument for its amendment.

Biochar also controls nitrogen (N) cycling (Clough et al., 2013). Biochar can reduce N leaching (Steiner et al., 2008; Güereña et al., 2013) and soil-borne N-containing GHG (Van Zwieten et al., 2015). Especially N_2O emissions from soil are reduced on average by 54 % in lab studies and 28 % in field measurements (Cayuela et al., 2015). In field situations, N_2O reduction effects are typically difficult to verify because of less uniform conditions and a large spatial and temporal variability of fluxes (Felber et al., 2013; Schimmelpfennig et al., 2014). A few field experiments indicated an increase in N_2O (e.g., Verhoeven and Six, 2014; Liu et al., 2014), many showed no significant effects (Karhu et al., 2011; Scheer et al., 2011; Suddick and Six, 2013; Anderson et al., 2014; Angst et al., 2014) while other studies indicated decreasing N_2O emissions (e.g., van Zwieten et al., 2010; Zhang et al., 2010; Taghizadeh-Toosi et al., 2011; Felber et al., 2013; Case et al., 2014). Only few studies with biochar have looked at N_2O emissions beyond 120 days (Verhoeven and Six, 2014), hence there is a large uncertainty about longer term effects of biochar addition.

Biochars are often alkaline and therefore increase soil pH after application (Joseph et al., 2010). Denitrifying bacterial communities have the potential to increase their N_2O-reducing activity

with increasing pH, which may reduce N_2O emissions from soils (Cavigelli and Robertson, 2001; Simek and Cooper, 2002; Čuhel et al., 2010). Low pH possibly impedes the synthesis of a functional N_2O reductase enzyme (Bakken et al., 2012). Some authors suggest that the elevated soil pH is responsible for reduced N_2O emissions following biochar application through increased activity of N_2O reducing bacteria (van Zwieten et al., 2010; Zheng et al., 2012). In contrast, Yanai et al. (2007) argue that the suppression of N_2O emissions by biochar is not through increased N_2O reduction activity because biochar ash also increases soil pH but does not reduce N_2O emissions. Cayuela et al. (2013) showed that biochar's acid buffer capacity was a more important factor in denitrification than the pH shift in soil. There are indications that biochar enhances nosZ expression, the gene responsible for the transcription of the N_2O reductase in denitrifying microorganisms (Harter et al., 2014; Van Zwieten et al., 2014). This could be a mechanistic link to the observed reduction in N_2O emissions through biochar increasing soil pH and microbial activity. In contrast, under conditions favouring nitrification and not being as sensitive to pH as total denitrification, biochar addition increased N_2O emissions in the lab (Sánchez-García et al., 2014) and possibly in the field (Verhoeven and Six, 2014).

In this study, we test (i) whether N_2O emissions are reduced following the application of biochar to soil of a temperate maize cropping system and (ii) whether this possible reduction in N_2O emissions is similar when soil pH is increased by other means. The latter was tested by a treatment where limestone was added to increase soil pH to the same level as that from the addition of $20\,t\,ha^{-1}$ biochar. N_2O emissions and maize yield were quantified during one growing season in the field.

4.2 Method

4.2.1 Field site

The experiment was established on a cropland field near the Agroscope research station in Zurich, Switzerland (47.427° N, 8.522° E, 437 m a.s.l.). The climate is temperate with a mean annual air temperature of 9.4 °C and mean annual rainfall of 1054 mm (Climate data 1981–2010, (Meteoswiss, 2013) from the MeteoSwiss station Zurich Affoltern 500 m from the experimental site). The field was under conventional management with maize in 2013, the year prior to the experiment.

The soil is a clay loam with a particle size distribution of 37 % sand, 27 % silt and 36 % clay. The soil is a Eutric Mollic Gleysol (Drainic) (IUSS Working Group, 2014). The untreated soil has a pH of 6.3 in water ($1:2.5\ w/v$), total organic carbon content of $26.2\,g\,kg^{-1}$, total N of $2.9\,g\,kg^{-1}$ and bulk density of $1.3\,g\,cm^{-3}$.

4.2.2 Biochar

Several biochars were screened in advance to pick one with a high liming capacity and with properties in agreement to the guidelines for contents of polycyclic aromatic hydrocarbons (PAHs), C and N of the European Biochar Certificate (EBC, 2012). The chosen biochar was produced in a Pyreg reactor (Pyreg GmbH, Dörth, Germany) by Verora in Edlibach ZG, Switzerland in late 2013 (see chapter 30, case study 2 in Lehmann and Joseph, 2015). Pyreg reactors use slow pyrolysis in a continuous system with an average residence time of circa 25 min and a

peak temperature of approximately 650 °C. The feedstock was green waste mainly from tree pruning. The biochar has the following properties: 64.9 % total C; 62.1 % C_{org}, pH 9.8 (1 : 10 in water); liming capacity 17.2 % $CaCO_3$, 148 m^2 g^{-1} BET surface area and ash content 20 %. Elemental ratios are 0.11 O / C and 0.33 H / C molar and 94 C / N by mass. Moisture content at the time of application was 12 %. Biochar was sieved < 3 mm shortly before it was spread on the field.

4.2.3   Experimental setup

Three different treatments were introduced: 20 t ha^{-1} biochar, control without additions and a limestone treatment to increase the soil pH to the same level as with biochar. The field was split into 3 × 3 plots with a size of 2 by 3 m (6 m^2 per plot and three replicates for each treatment). One metre buffer zones were established between plots on all sides. The three different treatments were arranged in a randomised complete block design with the 3 × 3 grid accounting for spatial variability. The whole field, including the buffer zones, were planted with maize (Zea mays). Initial pH values were not different among treatment plots (see pH measurement from January 2014 in Fig. 4.2).

4.2.4   Field management

The field was ploughed in autumn 2013 after the maize harvest. In January 2014, 20 t ha^{-1} biochar and 2 t ha^{-1} limestone, respectively, were spread on the wet, ploughed field surface. Freshly applied biochar was gently mixed with the first 1–3 cm of soil by hand at the same time. In mid-February 2014, the automated GHG chamber system was installed and in March the field was harrowed by a rototiller to a depth of circa 15 cm. The chamber frames were reset into the soil again and Decagon TE5 temperature and humidity sensors (Decagon Devices Inc., Pullman, WA, USA) were placed at a depth of 8 cm in the centre of each plot. The TE5 sensor measures the volumetric water content in soil by time domain reflectometry (TDR) at 70 MHz.

In May, potassium (K) and phosphorus (P) fertiliser was applied at a rate of 41.4 kg P ha^{-1} and 132 kg K ha^{-1}. Nitrogen was applied in three portions of 40, 80 and 40 kg N ha^{-1} on the 26 May, 16 June and 16 July, respectively, as ammonium nitrate (LONZA-Ammonsalpeter 27.5 % N). At each event fertiliser was spread on each plot of 6 m^2 and chamber frame of 0.03 m^2 separately to ensure equal distribution. On the 5 May, two of the three lime replicates were treated with another 1 t ha^{-1} of limestone because the pH was not in the same range as the biochar plots. Maize (Padrino from KWS SAAT AG, Einbeck, Germany) was sown on the 8 May with 0.14 m distance within rows that were 0.6 m apart from each other. For plant protection only one herbicide application was conducted on the 19 June with 1 L ha^{-1} Dasul (Syngenta, Basel, Switzerland), 1 L ha^{-1} Mikado (Bayer CropScience, Germany) and 1 kg ha^{-1} Andil (Omya AG, Switzerland). Despite manual weeding and herbicides a considerable amount of weeds emerged. Plots were harvested on the 13 October.

4.2.5   Nitrous oxide measurement

N_2O and CO_2 emissions were measured with static chambers of a fully automated measurement system (Flechard et al., 2005; Felber et al., 2013) consisting of nine stainless steel chambers (30 × 30 × 25 cm). These chambers were placed on PVC frames inserted 3 cm deep

into soil. The frame positions were moved three times during the growing season to obtain a better spatial representation of each plot. After maize had been sown, the chamber positions were between rows and no vegetation was grown within the chamber frame. Each of the nine chamber lids was automatically closed and opened sequentially (over a period of 3.6 h) allowing N_2O and CO_2 to accumulate in the chamber headspace for 15 min. Chamber headspace air was circulated (1 L min^{-1} air flow) through an inlet and outlet line from each chamber through polyamide tubes (4 mm I.D.) to the analytical system and back to the chamber headspace after sample analysis. The analytical and chamber control instruments were installed in a nearby field cabin under temperature-controlled air conditioning. N_2O concentrations were continuously measured and stored every minute using a gas filter correlation analyser (TEI Model 46c, Thermo Fisher Scientific, Waltham, MA, USA). The gas stream is exposed to infrared light from specific bands (filtered), both from N_2O absorbing and non-absorbing bands. From this difference a gas specific and concentration-sensitive signal is retrieved. The instruments linearity is described with $\pm 2\%$ with negligible interference of H_2O, CO_2 or CO. CO_2 was measured with an infrared sensor from Liston Scientific Corp. (Irvine, CA, USA). The system was calibrated every 11 h with three different concentrations from certified gas standards (Carbagas, Rümlang, Switzerland). The N_2O analyser showed a drift with room temperature variations that the air conditioning could not avoid completely. Hence a temperature correction factor was applied to the raw data from a regression of the device temperature with data during calibrations in May. The temperature correction factor used was about 9.1 ppm per °C temperature change from the 37 °C device reference temperature. The mean N_2O analyser device temperature in June–July was 37.4 ± 2 °C (± 1 SD).

N_2O and CO_2 fluxes from soil were calculated from the continuous concentration measurement (resolution 1 per min) when chamber lids were closed. Data from the first 3 min of the total 15 min closure time were omitted from the flux calculation to remove signal noise due to gas exchange from the system during chamber switching and closing (Felber et al., 2013). The same flux estimation procedure (R-script by R. Fuss on bitbucket.org, see Fuss, 2015) was used as in Leiber-Sauheitl et al. (2014). It is a modification of the HMR package (Pedersen et al., 2010) that chooses between exponential curvature for non-linear chamber behaviour (Hutchinson-Mosier regression) and robust linear regression (Huber and Ronchetti, 1981). The exponential HMR scheme considers non-linear concentration increase in the chamber due to a possibly decreasing concentration gradient, chamber leakage and lateral gas transport. Robust linear regressions provide a more reliable flux estimate for low fluxes when there is a lot of variation due to limited measurement precision and outliers. Following the flux script's recommendation, non-linear HMR was used for 1034 fluxes, whereas for all the other 13 034 fluxes the robust linear regression was chosen. The resulting flux estimates from this procedure were then filtered for implausible large N_2O uptake by soil (i.e. when the ambient N_2O concentration suddenly drops with increased mixing in the boundary layer). N_2O fluxes smaller than -10 ng N_2O m^{-2} s^{-1} (Neftel et al., 2010) were removed as well as data associated with a likely invalid chamber functioning (i.e. frozen lids) indicated by CO_2 fluxes < -0.5 μmol m^{-2} s^{-1} (Felber et al., 2013). From the entire data set of 14 068 fluxes, 302 were rejected due to the CO_2 flux threshold and an additional 49 fluxes due to N_2O (2.5 % of the total number of fluxes removed). Considering a cumulative sum of all fluxes in the data set, filtering reduced this number by 0.61 %.
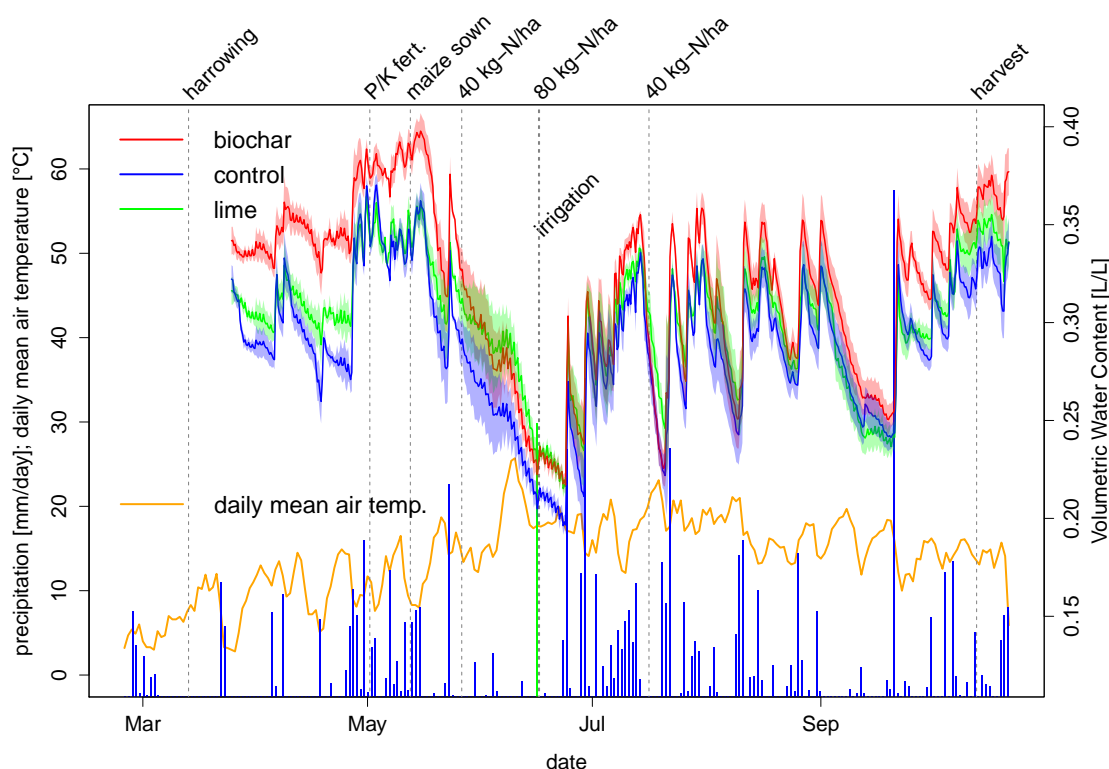
Figure 4.1 – Soil moisture means for each treatment are shown in red, blue and green solid lines with 1 SE as shaded area. Blue bars show the rainfall in $mm\,d^{-1}$ and the orange line is daily mean air temperature. The green bar indicates the irrigation of 33 mm with the second N fertilisation.

4.2.6 Yield

The harvest (above-ground biomass) was separated into grain yield (kernels) and by-product (stems, leaves). Cobs were threshed and dried whereas the by-product was weighed freshly on the field, chaffed and a sub-sample was then dried to measure water content and for further plant nutrient analysis. From both grains and by-product, dry matter total N, P, K, Ca and Mg content were measured (FAL, 1996). For yield based N_2O emissions, cumulative N_2O emissions in $kg\,N_2O\text{-}N\,ha^{-1}$ were related to total above-ground biomass dry matter (DM) yield in $t\,ha^{-1}$ (see Table 4.3) and to total above-ground N uptake in $kg\,N\,ha^{-1}$ (see discussion).

4.2.7 Soil sampling and analysis

Soil samples for pH, ammonium (NH_4^+) and nitrate (NO_3^-) measurements were taken on the 31 January, 31 March, 26 May, 16 June and 4 September 2014. At each sampling, five randomly distributed soil cores per plot were taken (0–10 cm) and pooled. Soil pH was determined in moist soil samples using water at a ratio of $1:2.5\ w/v$ and measured with a PH100 ExStik pH meter (Extech Instruments Corp., Nashua, NH, USA). Soil bulk density was measured on the 27 June at a depth of 3–8 cm using $100\,cm^3$ steel cores, 3 per plot.

For soil NO_3^- and NH_4^+ concentrations, 20 g of moist soil were mixed with 100 mL 0.01 M $CaCl_2$

solution. The suspension was shaken for 30 min, filtered and then analysed by segmented flow injection analysis on a SKALAR SANplus analyser (Skalar Analytical B.V., Breda, Netherlands).

4.2.8 Statistical analysis

The obtained fluxes from the automated chamber system were aggregated to 6 h means producing a regular, smoothed data set. The system was able to measure each chamber three times for every 11 h calibration cycle during regular operations, hence on average 1.6 measurements for each chamber were included in each 6 h mean. Still missing values after this aggregation step were linearly interpolated for each chamber. Treatment averages and standard errors were calculated from the three chambers on the replicated plots. If not indicated otherwise, treatment means are shown with ±1 standard error (SE).

Statistical analyses were performed with R (version 3.0.1, The R Project, 2014). Significance level was chosen at $p < 0.05$ for all procedures, unless indicated otherwise. Significant treatment effects for cumulated fluxes were determined using ANOVA from rbase package (treatments: control, biochar and lime; $n = 3$). Bartlett test of homogeneity of variances showed conflicting ANOVA assumptions for the cumulative fluxes. This could be improved by log transformation of the flux data.

4.3 Results

4.3.1 Environmental field conditions

The year started with above-average temperatures and low rainfall (Fig. 4.1). End of May to June was dry with high temperatures being on average for Switzerland 1.5 °C above the 1981–2010 norm (Meteoswiss, 2015). The soil's volumetric water content fell to circa 20 %, inducing high water stress on the young maize seedlings. The lack of soil moisture presumably hampered the dilution of the first application of 40 kg N ha^{-1} in the soil solution. Along with the 2nd N fertilisation the field was therefore irrigated with 33 mm water (shown as green bar in Fig. 4.1). The summer months July and August were rather cold and wet with daily mean air temperatures below 20 °C (Meteoswiss, 2015).

Soil volumetric water content tended to be higher in biochar plots (Fig. 4.1) with 37 out of 423 (8.7 %) half-daily means showing a statistically significant treatment effect.

4.3.2 Soil pH and nitrogen

Soil pH increased with limestone and biochar addition by circa 0.4 pH units (Fig. 4.2). During the time with major emissions in June, the pH between control and biochar/lime soils significantly ($p < 0.001$) increased from 6.1 to 6.5 with. The initial soil pH was on average 6.3 and not different among treatments. Following biochar application soil pH increased to up to 7.4 whereas with addition of limestone soil pH increased to up to 6.9 (averages across replicates). The pH sharply decreased after the initial peak, especially in those two liming plots, which were treated subsequently with another 1 t ha^{-1} in May. Soil pH of biochar and lime treatments were not significantly different at any sampling time, whereas soil pH of the control treatment was systematically below that of the amended soils.

Mean soil bulk density was not statistically different between treatments (1.31 ± 0.03 g cm^{-3} in
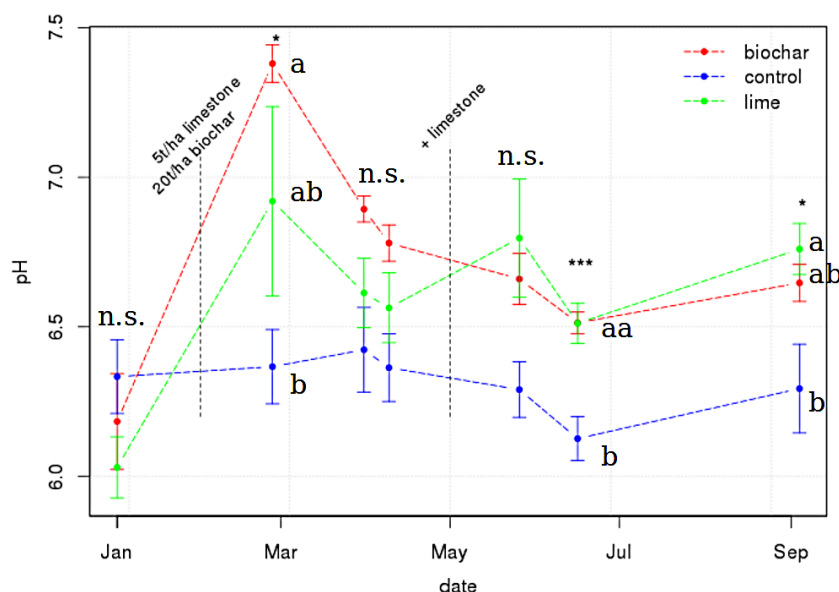
Figure 4.2 – Soil pH (mean with 1 SE bars) during the time of the experiment. Significant differences ($p < 0.05$) are indicated with stars according ANOVA test and Tukey Honest Significant Differences (TukeyHSD) are indicated by different letters, n.s. = not significant.

Table 4.1 – Nitrate content ($mg\,NO_3^-$-$N\,kg^{-1}$) in soil during the experiment. Standard error is indicated in brackets.

Date	Biochar	Control	Lime
2014-01-31	2.77 (0.41)	2.92 (0.13)	3.12 (0.25)
2014-03-31	6.26 (0.98)	8.57 (0.77)	8.40 (0.76)
2014-05-26	3.13 (0.36)	7.54 (1.18)	5.86 (1.45)
2014-06-16	9.19 (1.66)	9.38 (3.69)	11.65 (1.24)
2014-09-04	1.30 (0.15)	1.09 (0.21)	1.33 (0.26)

the control, $1.29 \pm 0.07\,g\,cm^{-3}$ in biochar and $1.36 \pm 0.04\,g\,cm^{-3}$ in the liming treatment).

Soil mineral N was not statistically different between treatments at any sampling date (Tables 4.1 and 4.2).

4.3.3 N_2O **fluxes**

Emissions were characterized by peak events, particularly in summer, and by background emissions in spring and autumn (Fig. 4.3). Main emissions occurred after the second fertilisation event with the highest application rate around early August. Afterwards, there were only emissions from one of the lime plots but almost none until the end of October from all the other plots. This also corresponds to the low amounts of available soil N, indicating that the plants had taken up most of it. All treatments revealed similar temporal N_2O emission dynamics but the height of the peaks differed. During peak events emissions from the biochar treatment were often lower than those from the other treatments, especially compared to the control. This resulted in an increasing difference in cumulative fluxes (Fig. 4.4) between

Table 4.2 – Ammonium content (mg NH_4^+-N kg^{-1}) in soil during the experiment. Standard error is indicated in brackets.

Date	Biochar	Control	Lime
2014-01-31	1.11 (0.07)	1.00 (0.12)	0.68 (0.05)
2014-03-31	0.42 (0.24)	0.36 (0.21)	0.25 (0.21)
2014-05-26	0.11 (0.08)	0.12 (0.07)	0.47 (0.40)
2014-06-16	0.45 (0.13)	2.48 (1.80)	1.67 (0.36)
2014-09-04	0.38 (0.33)	0.39 (0.14)	0.16 (0.06)

Table 4.3 – Cumulated N_2O emission per area, above-ground dry matter yield and above-ground (ab.) plant N uptake for each plot (grains, stems and leaves).

Treatment	Block	N_2O per area [kg N_2O-N ha^{-1}]	N_2O per dry matter yield [kg N_2O-N $(t\text{-}DM)^{-1}$]	ab. plant N uptake [kg-N ha^{-1}]
biochar	1	1.63	0.112	162
biochar	2	1.99	0.145	142
biochar	3	1.48	0.126	123
control	1	3.06	0.255	143
control	2	3.39	0.325	109
control	3	4.26	0.378	118
lime	1	6.76	0.591	121
lime	2	1.24	0.097	135
lime	3	2.80	0.230	131

control and biochar. Mean cumulative emissions for the entire growing season were 170 ± 16.5, 353 ± 31.7 and 359 ± 164 mg N_2O-N m^{-2} for biochar, control and lime treatments, respectively (see Table 4.3 for plotwise results). Relative to the control, mean cumulative N_2O emissions were 52 % smaller in the biochar treatment. The whole treatment effect was, however, not statistically significant ($p = 0.26$) due to the large variability in the data set. Emission means from control and lime were very similar. With lime, N_2O emissions were highly variable and this treatment included both the chamber with the highest and also the one with the lowest cumulative emission. Alternatively, we also calculated p values comparing only biochar and control treatments with a Welch Two-Sample t test. This resulted in a significant difference ($p = 0.022$). All p values have to be treated with caution because they were produced with a minimal number of replicates. Furthermore, a large treatment effect size is not reflected in the p value.

N_2O emissions per unit N applied calculated from the 160 kg N ha^{-1} with the mean cumulative emissions during the growing season, resulted in 1.06 % for biochar, 2.21 % for control and 2.25 % for the lime treatment. Like cumulated emissions, these values were not significantly different among treatments and have the same variance and p values.
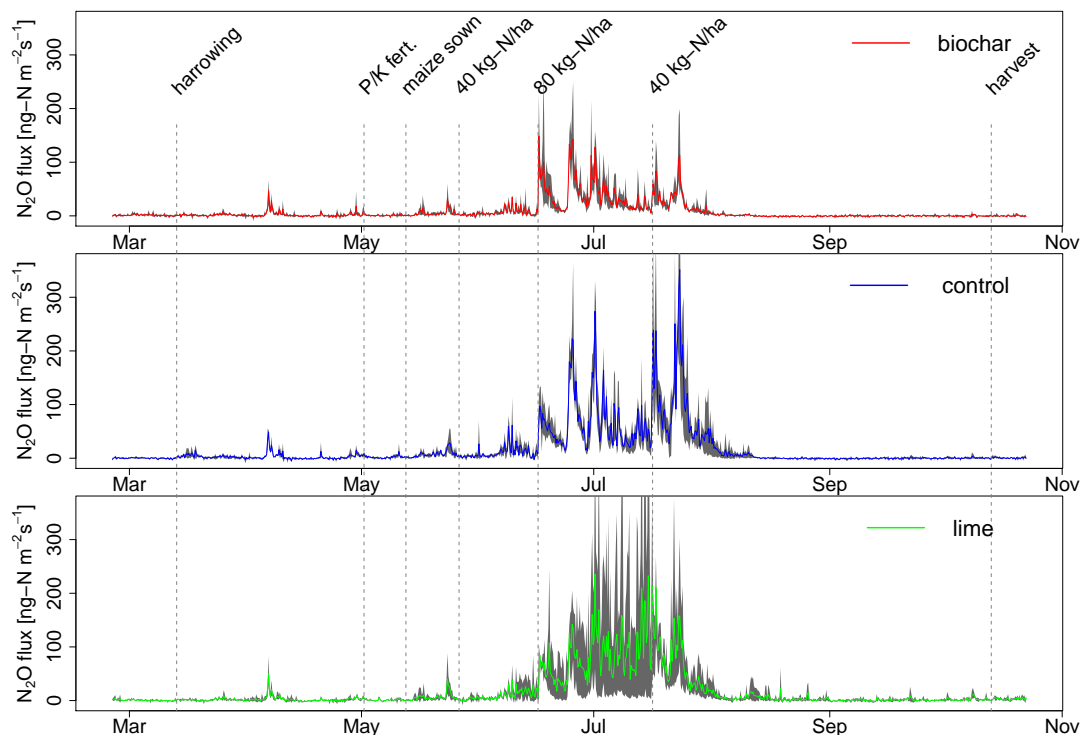
Figure 4.3 – Mean N_2O emissions for each treatment (coloured line) with highest and lowest replicate in grey.

4.3.4 Maize yields and plant growth

Maize yields were not significantly different between treatments, for both grains (product) and by-product (i.e. stems, leaves) (Fig. 4.5). Nitrogen uptake by maize did not differ among treatments (Fig. 4.6, Table 4.3). Table 4.3 shows cumulated N_2O emissions for each plot and per area as well as per DM yield. Yield based emissions (Table 4.3) resulted in 0.128 ± 0.010 kg N_2O-N per t-DM, for biochar, 0.319 ± 0.036 kg N_2O-N per t-DM for control and 0.306 ± 0.148 kg N_2O-N per t-DM for the liming treatment. Although the yield based emission with biochar is 60 % lower compared to the control, overall there is no significant treatment effect ($p = 0.19$). There was no difference between treatments for any of the measured nutrients in the yield (data not shown).

4.4 Discussion

4.4.1 N_2O emissions

Our high-frequency automated N_2O chamber measurements give a detailed picture of the emissions from a biochar and lime field trial. Neither soil NO_3^- nor NH_4^+ concentrations can explain N_2O emission patterns at any point in time. Although cumulative N_2O emissions were not significantly different among the three treatments, emissions with added biochar were 52 % below the control treatment. The magnitude of reduction is in agreement with the
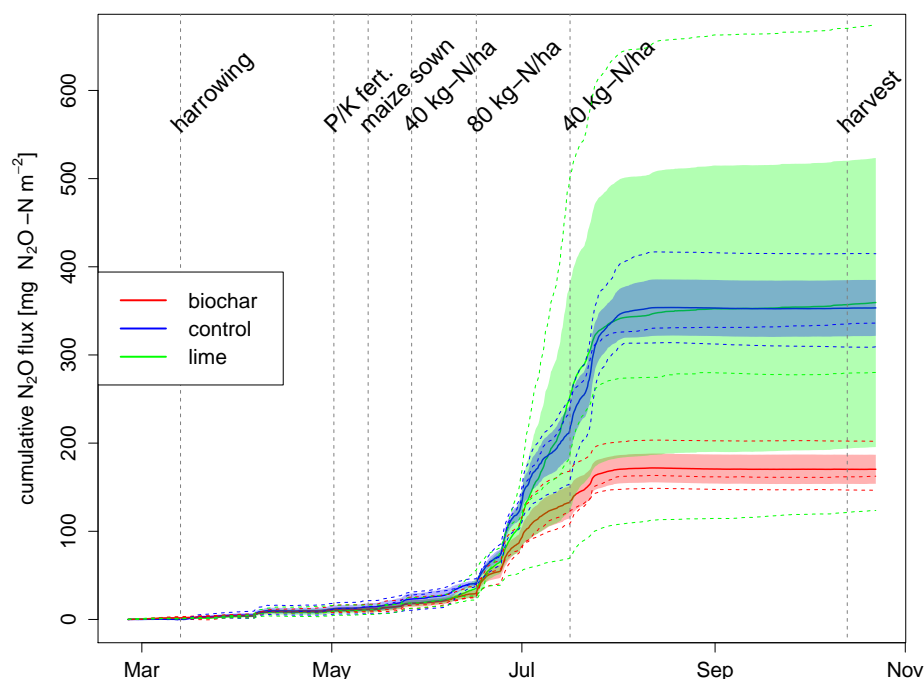
Figure 4.4 – Mean cumulative N_2O fluxes as solid lines. Shaded areas represent the standard error of the mean from the three replicates (dashed lines) per treatment.

meta-analysis of Cayuela et al. (2015), who showed a general reduction of N_2O emissions by biochar of $49 \pm 5\,\%$ (lab and field experiments) but it is larger than the reduction found by the same authors under field conditions ($28 \pm 16\,\%$). In our maize field in the temperate zone, N_2O emissions can thus decrease with biochar addition as much as they have been shown to be reduced under controlled lab conditions.

Our results show no decrease in N_2O emissions when limestone is used to increase the soil pH to the same level as that with biochar. This finding does not support the hypothesis that biochar's N_2O reduction effect is similar to a geochemical adjustment of soil pH. However, it must be considered that the large variability among the three replicates hampers the power of this conclusion. A post-hoc power analysis showed a $23.4\,\%$ probability of accepting a true alternative hypothesis considering the obtained results in cumulative N_2O emission. To have at least a power of $80\,\%$ we would have needed 10 replicates for each treatment. The high variability solely in the liming treatment might be due to additional lime application to the field in May 2014 and the high spatial-temporal variability of that soil property in general. The two replicates that received additional limestone were the ones that emitted more N_2O than the other plot. Hence, instead of reducing emissions by increasing the pH, the additional limestone application could have provoked local arbitrary disturbance to soil chemistry leading to emission hotspots. To determine the biochar effect on N_2O emissions, we therefore also compared only the biochar and control treatments (see results); according to the analysis of that reduced data set, the cumulative emissions in the biochar amended plots were significantly lower (by $52\,\%$) than in the control treatment.
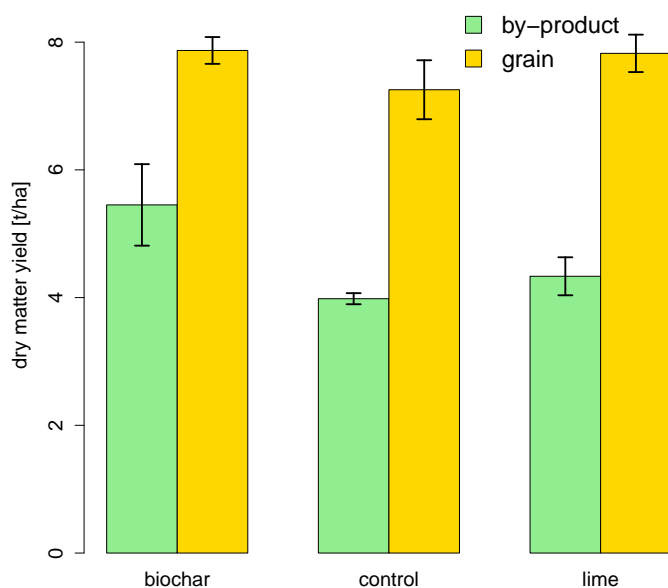
Figure 4.5 – Grain yield and by-product biomass production (dry matter yield). Error bars show 1 SE ($n = 3$).

Using the same measurement technique, application rate and similar biochar properties Felber et al. (2013) also reported N_2O emission reductions, but smaller as compared to the difference we saw between biochar and control. In line with our results other field studies have shown significant reductions in N_2O emissions following biochar amendment (Taghizadeh-Toosi et al., 2011; Liu et al., 2012). A number of studies found no significant effect of biochar addition in the field (Schimmelpfennig et al., 2014; Angst et al., 2014; Scheer et al., 2011; Karhu et al., 2011; Anderson et al., 2014). Often the much higher variability in the field and the low number of replications make it difficult to reproduce reduction effects observed in laboratory studies. In particular, Angst et al. (2014) found no significant difference but there was a tendency for lower emissions with biochar addition, suggesting that the variability in the field was too high to get significant effects. However there are also studies that showed increased emissions from biochar application in the field (Verhoeven and Six, 2014; Shen et al., 2014). There is a large variability of biochar properties and effect size between these field studies. Since the driving mechanism of how biochar reduces N_2O emissions from soil are still unknown, it can only be shown by meta-analysis that a low $H : C_{org}$ ratio seems to beneficial for N_2O suppression (Cayuela et al., 2015).

Biochar has been shown to increase water-holding capacity and reduce bulk density (Peake et al., 2014). Mukherjee et al. (2014) showed that 92 % decrease in N_2O emissions by biochar coincided with reduced bulk density by 13 % and increased soil nanopore surface area by 12 % relative to the control. In our experiment there are some situations where increased volumetric water content with biochar coincide with reduced N_2O emissions (Fig. 1 and 2). Although there was no significant difference in bulk density, supposed increase in nanopore
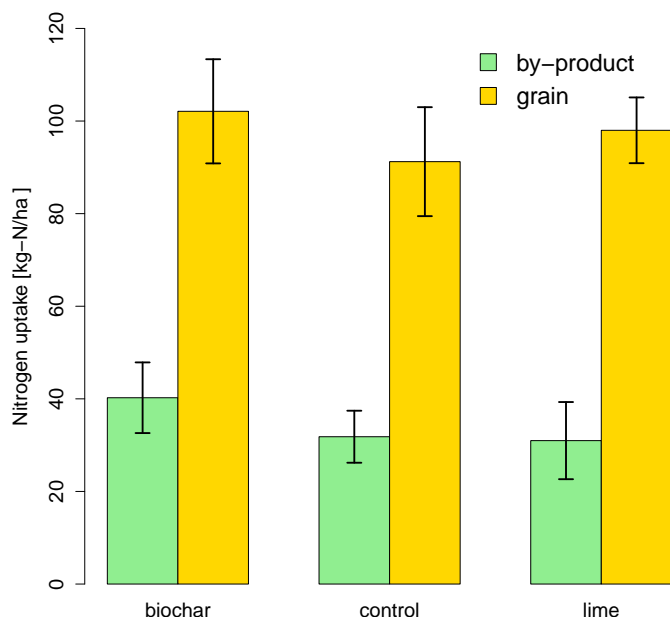
Figure 4.6 – N uptake by grains and by-product (stem, leaves). Error bars show 1 SE ($n = 3$).

surface provides both suitable pores for water retention and oxygen air. Overall the improved soil aeration by biochar dominates the effect of increased water content and hence does not favour denitrification (van Zwieten et al., 2010).

Sánchez-García et al. (2014) found that biochar increases soil N_2O emissions produced by nitrification-mediated pathways. In our study, the water content (Fig. 4.1) was high during periods of high emissions, suggesting that during periods of high water content denitrification dominates the N_2O production in soil. The high emissions were thus often triggered by large precipitation events. There are many indications from lab experiments that biochar can reduce N_2O emissions in denitrifying conditions at high water content (Yanai et al., 2007; Singh et al., 2010; Felber et al., 2013; Harter et al., 2014). Under denitrification conditions, the pH between 6 and 8 exerts control over the $N_2O : N_2$ ratio (Stevens et al., 1998), especially with a pH of the soil below 7, when the reduction of N_2O to N_2 is inhibited by acid conditions (Simek and Cooper, 2002). Various studies have suggested that an elevated soil pH is responsible for reduced N_2O emissions following biochar application through increased activity of N_2O reducing bacteria (van Zwieten et al., 2010; Zheng et al., 2012). In contrast, Yanai et al. (2007) argued that the suppression of N_2O emissions by charcoal is not due to increased N_2O reduction activity with increased soil pH because biochar ash increased the pH to the same degree as biochar, but did not reduce N_2O emissions. In the lab, Cayuela et al. (2013) found no N_2O mitigation when soil pH was increased to the same level as biochar did but with $CaCO_3$ addition. They also showed that biochar's buffer capacity but not biochar pH was highly correlated with lower N_2O emissions compared to pH-adjusted biochars (Cayuela et al., 2013). In our case, we used a biochar with rather high liming capacity (17.2 % $CaCO_3$) and pH (9.8). We can confirm that with this kind of biochar N_2O emissions can effectively be reduced also in real field conditions,

although the high variability in the limed treatments does not allow us to reject the hypothesis of soil pH being the major driver of N_2O emission reductions.

More recent studies show that biochar enhances nosZ abundance in soil bacteria, which can lead to lower N_2O emissions (Harter et al., 2014; Van Zwieten et al., 2014). Some authors relate this enhancement of N_2O reducing bacteria to biochar's redox activity that facilitates electron shuttling for the sensitive process of N_2O reduction (Kappler et al., 2014; Cayuela et al., 2013). This shuttling might be the connection between reduced N_2O emissions and low $H : C_{org}$ ratios (Cayuela et al., 2015) in biochar that refers to condensed aromatic structures and its quinone/hydroquinone moieties being electro-active by allowing electron transfer across conjugated pi-electron systems (Klüpfel et al., 2014). Such high electro-catalytic activity has also been shown in N-doped C nanotube arrays (Gong et al., 2009). Hence, in contrast to a promotion of microbial N_2O reduction, there is also the possibility that biochar abiotically reduces N_2O through its electrocatalytic abilities represented by a high aromaticity with low $H : C_{org}$ ratios. Indeed, this is one of the various abiotic mechanisms that reduce N_2O emissions suggested by Van Zwieten et al. (2015).

4.4.2   Yield and nutrients

In our experiment, grain and by-product biomass production was not increased by biochar application to soil. There is large uncertainty around the yield effect of biochar but meta-analyses reported an average increase of 10 % (Jeffery et al., 2011; Liu et al., 2013). Crane-Droesch et al. (2013) described a more detailed global response of biochar on yields. They identified a substantial and specific agroecological niche for biochar in soils with low organic C content and low cation exchange capacity, typical for highly-weathered tropical or sandy soils. Given these findings, we would not expect a large increase in productivity at our site which is rich in soil C and clay. Positive yield response could however increase with time (Crane-Droesch et al., 2013) and might not show clear effects within the first year of the biochar application yet. Our data are also in agreement with Jay et al. (2015), who showed that biochar had no effect on the yield of different crops after a single rotational application (20 and 50 t ha^{-1}) in a sandy loam under intensive management.

Nitrogen uptake was not changed by biochar or liming. Although there was no significant difference in P uptake between the treatments, by-product material from biochar-treated plots tended to have higher P uptake than the control (+100 % increase, data not shown). Vanek and Lehmann (2014) showed significant increase in P availability through enhanced interactions between biochar and arbuscular mycorrhizas.

Liu et al. (2012) reported a biochar application rate dependent decrease in emission intensity per yield, from 0.17 kg N_2O-N t^{-1} in the control to 0.10 and 0.07 kg N_2O-N t^{-1} with 20 and 40 t ha^{-1} biochar applied. For an agronomic assessment of N_2O emissions it is most relevant to relate the cumulative emissions to the yield (Van Groenigen et al., 2010). Emissions of the control per above-ground N uptake (29.6 kg N_2O-N (kg N)$^{-1}$) are much higher than the reported mean of 8.4 kg N_2O-N (kg N)$^{-1}$ at fertilisation rates between 180 and 190 kg N ha^{-1} (Van Groenigen et al., 2010). With biochar however, this number decreases by 60 %, whereas with lime it remains at the level of the control ($p = 0.21$). Yield-based emissions are a good way to express biochars effects both on N_2O emissions and yield, but the experimental uncertainties of each data set are also cumulated within this number.

4.5 Conclusions

We found a 52 % reduction in N_2O soil emissions from biochar compared to control treatment in a maize field trial. This shows that also in temperate, intensive maize cropping systems under real field conditions, N_2O emissions can be reduced substantially by biochar. There is no evidence that the reduction with biochar, relative to control, is solely induced by a higher soil pH. The pH hypothesis is thus not supported by our data.

N use efficiencies and N$_2$O emissions in two contrasting, biochar amended soils under winter wheat – cover crop – sorghum rotation

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Abstract

Biochar, a carbon-rich, porous pyrolysis product of organic residues, is evaluated as an option to tackle major problems the global food system is facing. Applied to soil, biochar can sequester carbon dioxide but also have beneficial effects on nitrogen (N) cycling and therefore enhance crop yields and reduce nitrous oxide (N$_2$O) emissions. There is little understanding of the underlying mechanisms, but many experiments have shown increased yields and manifold changes in N transformation, indicating a potential to increase N use efficiency. Whereas biochar's effects on tropical soils in extensive agriculture can be positive, less is known about its use in temperate fertile soils with intensive fertilisation. We tested the effect of biochar on N use efficiency, crop yields and N$_2$O emissions in a lysimeter system with 2 types of soil (sandy loam and silt loam) in a winter wheat - cover crop - sorghum rotation. ^{15}N-labelled ammonium nitrate fertiliser (170 $kg\,N\,ha^{-1}$ in 3 doses, 10 % ^{15}N) was applied to the first crop to monitor its fate in 3 ecosystem components (plants, soil, leachate). Green rye was used as cover crop to keep the first year's fertiliser N for the second year's sorghum crop (fertilised

with 110 $kg\,N\,ha^{-1}$ in 2 doses and natural abundance ^{15}N content). We observed no effects of biochar on N fertiliser use efficiency, yield or N uptake for any crop. Biochar reduced leaching (by 43 ± 19 %) only towards the end of the experiment with leaching losses being generally low. N_2O emissions were reduced by 15 ± 4 % compared with the control treatments in both soil types. Our results indicate that biochar application can produce environmental benefits in terms of N_2O emission and N leaching but does not substantially affect the overall N cycle and hence crop performance in the analysed temperate crop rotation.

Highlights:

- No effect of biochar on nitrogen fertiliser use efficiency
- No effect of biochar on yield and nitrogen uptake by plants
- Minor effects of biochar on leaching
- Significantly reduced N_2O emissions (15 %) by biochar
- Small potential of biochar for environmental benefits without compromising yields

5.1   Introduction

Global food production increases the demand for agricultural land, water and fossil energy and leads to high greenhouse gas (GHG) emissions and resource depletion (IAASTD et al., 2009). These challenges for agricultural research are reinforced by climate change (IPCC, 2014) and an increasingly animal-based diet at a global scale (Stehfest et al., 2009; Westhoek et al., 2014). Increasing nitrogen (N) use efficiency is an important factor for reducing inputs and lessening harmful impacts on the environment (Decock et al., 2015; Zhang et al., 2015). The application of biochar to agricultural soils is discussed as an option to tackle several of these challenges agriculture faces today (Lehmann, 2007). Biochar is produced by thermochemical transformation of organic residues and can be mixed with compost or be applied directly to soil (Schmidt et al., 2014). Further, sequestration of carbon dioxide (CO_2) from the atmosphere via biochar application might help to mitigate climate change (Woolf et al., 2010). Reduced fertiliser demand through biochar application can reduce fossil energy consumption (Woolf et al., 2014), and increasing yields (Crane-Droesch et al., 2013) would reduce GHG emissions caused by land-use change for additional agricultural land (Fearnside, 2000). Biochar application to soils is likely to impact soil N dynamics (Clough et al., 2013) with a potential to reduce nitrous oxide (N_2O) emissions from agricultural fields as recently demonstrated (Cayuela et al., 2015; Hüppi et al., 2015) but also contradicted (Verhoeven and Six, 2014; Angst et al., 2014). Hence, an improved knowledge of biochar's influence on soil N processes in an agricultural context is still needed.

A positive response of crop yields after biochar application to soil is a major potential biochar benefit. Jeffery et al. (2011) found a significantly higher mean crop productivity (+10 %) in biochar-amended versus control soils in a meta-analysis (>60 studies). The yield response was highly variable and specific to soil and biochar properties. There are indications that positive yield effects are associated with the ability of biochar to reduce water stress in drought situations (Karer et al., 2013). Increased maize yield after biochar application was also attributed to the enhanced availability of calcium (Ca) and magnesium (Mg) in soil (Major et al., 2010). Furthermore, positive yield response with biochar was found in acidic and sandy textured soils (Liu et al., 2013). This shows that yield response to biochar strongly depends on soil conditions. A meta-analysis by Crane-Droesch et al. (2013) found both soil cation

exchange capacity and soil organic carbon (C) content to be strong predictors for positive yield responses with biochar.

Most relevant from an agronomic viewpoint is that biochar may also modulate plant N uptake and yield through its influence on N dynamics in the soil. Biochar can affect the agricultural N cycle by i) reducing organic N transformation rates (Prommer et al., 2014), ii) accelerating gross nitrification (Nelissen et al., 2012; Anderson et al., 2011) and iii) increasing soil N immobilisation (Nelissen et al., 2015; Bruun et al., 2012; Zheng et al., 2012). Thus, yield effects after biochar application may be driven by changes in the soil's N cycle, e.g. via an increased N use efficiency. Further, many studies have shown that biochar may help to prevent N leaching (Güereña et al., 2013; Ventura et al., 2013; Laird et al., 2010). However, there are currently no studies that tried to trace the fate of fertiliser N in the plant-soil system after biochar application.

Many of the above mechanisms how biochar might change N cycling in agricultural fields may also affect gaseous N emissions from soils, namely N_2O, nitrogen gas (N_2), nitric oxide (NO) and ammonia (NH_3). Biochar may increase N loss from NH_3 volatilisation (Taghizadeh-Toosi et al., 2011) but may reduce N_2O emissions from soil (Cayuela et al., 2015). Decreased N_2O emissions can be related to reduced inorganic N availability, caused by increased microbial N immobilisation or a decrease in nitrification rates (Wang et al., 2015; Cayuela et al., 2013). Moreover, elevated soil pH after alkaline biochar application could enhance N_2 formation by enhancing N_2O reductase activity (Harter et al., 2014; Van Zwieten et al., 2014; Obia et al., 2015). An increase in soil aeration, owing to the highly porous structure of biochar, may reduce anoxic sites for N_2O production in soil (Yanai et al., 2007; Rogovska et al., 2011). Finally, there is evidence that abiotic redox reactions on biochar play an important role for reduced N_2O emissions from soil (Quin et al., 2015).

Together, there is still little direct evidence showing how biochar may control the efficiency of N uptake by crops and how it changes the fate of fertiliser N in the soil-plant system. In this study, we traced fertiliser N in an open-air lysimeter system, filled with 2 types of soil, over 2 years with various crops. In the first year, winter wheat was fertilised with ^{15}N-labelled ammonium nitrate. The partitioning of the inserted ^{15}N label into the 2 N pools allowed for differences to be detected in plant N uptake, soil N content, N leaching and N_2O emissions. During consecutive planting of a cover crop during winter and sorghum in the subsequent year, the pathways and fate of the ^{15}N label were traced through the crop rotation to study mid-term effects of biochar on soil N cycling in an open-air environment. In particular, we wanted to test whether biochar application in 2 types of soil;

- increases aboveground plant N uptake and plant yield,
- affects sources of N (fertiliser vs. soil-derived),
- decreases N leaching and N_2O emissions and
- increases retention of fertiliser N in soil.

5.2 Methods

5.2.1 Lysimeter system

The lysimeter system at the Agroscope research facility Reckenholz Zurich (47.43°N, 8.52°E) contained 16 pots of 0.6 m diameter (area of 0.28 m^2) and 0.6 m soil depth built in a concrete block in the open air. The lysimeter pots were arranged in a single line in this concrete block,

each pot equipped with an outlet for the leachate at 70 cm depth. The 10 cm below the soils were filled with gravel and stones. The pots had been filled with 2 types of soil (8 pots each) in 1988; these soils differed in texture (sandy loam soil: 19 % clay, 25 % silt, 57 % sand; and silt loam soil: 19 % clay, 54 % silt, 28 % sand) and soil organic C content (0.7 % and 1.7 %, respectively). Since the initiation of the lysimeter system, pots have been cropped with various field crops. The climate at the study site was temperate, with a mean annual air temperature of 9.4 °C and mean annual precipitation of 1054 mm (climate data 1981-2010 from the 50 m nearby MeteoSwiss station Affoltern (Meteoswiss, 2013)).

5.2.2 Biochar

The biochar was produced at the PYREG reactor of Swiss Biochar GmBH in Lausanne in early 2012. The feedstock was wood chip residues after sieving from a compost production plant. The organic elemental composition of the biochar was 0.68 % N, 67.8 % C, 1.1 % hydrogen (H) and 8.3 % oxygen (O), resulting in a C/N ratio of 99.5 by mass, O/C of 0.092 and H/C of 0.20 (molar ratios). The specific surface area measured by N_2 adsorption was 226 $m^2\,g^{-1}$, the pH (1:5 biochar to 0.01 M calcium chloride ($CaCl_2$)) was 10.1 and the liming capacity corresponded to 15.4 % calcium carbonate ($CaCO_3$). Differential scanning calorimetry (at increments of 10 °C min^{-1}) under air revealed a 50 % burnoff temperature of 468.1 °C and a biochar peak temperature of 486.6 °C (for method details see Leifeld (2007)).

5.2.3 Experimental management

Preparation and biochar application

Soils in the 16 lysimeter pots were manually turned and mixed on the 10th of October 2012 down to 20 cm depth. The soil was sampled to analyse total C and total N contents, pH and mineral N content. Soil C and N contents, pH and the Lolium perenne yield in 2012 were used to assign biochar and control treatments to the lysimeters, in order to minimise pre-experimental differences between the treatments. Half of the lysimeters of each soil type were treated with 20 t ha^{-1} (0.566 kg biochar per 0.28 m^2 lysimeter pot) wood chip biochar on the 24th of October 2012. The first 10-15 cm of soil were taken out of the lysimeters and mixed with biochar by hand in multiple steps to evenly distribute biochar in the soil. The control plots were treated the same way but without biochar amendment. Each of the 4 replicates per treatment was equipped with a Decagon TE5 temperature and soil moisture probe at 6-9 cm depth, logging at a 30 min interval.

First year: winter wheat (Triticum aestivum)

One day after biochar application (24th of October 2012) and seedbed preparation, ammonium nitrate (LONZA-Ammonsalpeter 27.5 % N, no ^{15}N enrichment), phosphorus (Landor, Tripelsuperphosphat 46 % P), potassium (potash salt granulated 60 % K) and Mg (Landor, Granumag 29 % Mg + sulphur [S]) were applied to each plot at a rate of 43 kg N, 86 kg P, 114 kg K and 21 kg Mg ha^{-1}. One hundred and twenty seeds of winter wheat (breed: Siala) were sown in 5 lines approximately 3 cm deep.
^{15}N fertiliser was applied to all lysimeters in 3 applications with 10 % ^{15}N double-labelled ammonium nitrate ($^{15}NO_3^{15}NH_4$). The first fertiliser application took place on the 23rd of

April 2013 with 70 $kg\,N\,ha^{-1}$ diluted in 1.5 l of water per lysimeter, with another 1.5 l water added after fertilisation (equal to 10.8 mm of rain for each plot). The second N application was performed on the 15th of May (50 $kg\,N\,ha^{-1}$) and a third N fertilisation on the 14th of June (50 $kg\,N\,ha^{-1}$; always with the same amount of water). Winter wheat was harvested on the 16th of July. For the N balance calculations, the winter wheat grain yield was corrected for losses due to bird feeding. Details about the loss estimation can be found in the supplemental material.

Winter cover crop: green rye

On the 25th of September 2013, the soil in the lysimeters was turned and mixed by hand and green rye was sown as a winter cover crop. Plant material was sampled on the 23rd of December 2013 and 27th of March 2014 to determine ^{15}N uptake, and green rye was harvested on the 10th of April 2014. The amount of recovered N from the fertiliser applied in the previous year was calculated by the ^{15}N content (aboveground biomass only). On the 14th of April 2014, the cover crop harvest was fully returned to the soil and mixed via manual tillage.

Second year: sorghum (proso millet)

After cover crop incorporation, sorghum (proso millet, breed: Quartet) was sown at a rate of 200 seeds per lysimeter on the 6th of May 2014. At the same time, unlabelled ground ammonium nitrate fertiliser was added to the seeding rows at a rate of 30 $kg\,N\,ha^{-1}$. On the 12th of June, 50 $kg\,N\,ha^{-1}$ were spread with 1.5 l water, and another 30 $kg\,N\,ha^{-1}$ were applied on the 21st of July. LONZA-Ammonsalpeter fertiliser was used without ^{15}N enrichment ($\delta^{15}N$ -6.14 ‰). Plant material was first sampled on the 2nd of July and again with the harvest on the 17th of September 2014. The sorghum yield was quantified as combined straw and grain yield.

5.2.4 Soil sampling and analysis

Soil (0-10 cm) and crops were sampled before each fertilisation event and at harvest. Soil pH was measured shortly after sampling, and an aliquot of 10 g was dried, ground and used for bulk ^{15}N measurement. The pH was measured in a 1:2.5 moist soil:water suspension, quickly shaken and equilibrated for at least 10 hours and then measured using a PH100 ExStik pH meter (Extech Instruments Corp., Nashua, NH, USA). For ammonium and nitrate measurements (soil mineral N content; N(min)), N was extracted from 20 g field-moist soil (stored frozen) with a 2 M potassium chloride (KCl) solution and filtered. The filtrate was analysed by segmented flow injection analysis with a SKALAR SANplus analyser (Skalar Analytical B.V., Breda, The Netherlands). For the elemental analyses, soil samples were dried at 105 °C, sieved <2 mm and ground in a ball mill at a frequency of 25 s^{-1} for 5 min.

At the end of the experiment, on the 22nd of October 2014, all lysimeters were destructively sampled by taking 2 soil cores per lysimeter, each of 7.7 cm diameter and 60 cm length. Bulk density was calculated for each 10 cm segment from these soil cores. To calculate the soil total ^{15}N content, an aliquot of each segment was taken, dried and ground for ^{15}N analysis.

5.2.5 ^{15}N measurement

The amount of ^{15}N in bulk samples was quantified by elemental analysis isotope ratio mass spectrometry (EA-IRMS) on an Integra2 instrument (Sercon, UK) at the University of Basel. Briefly, sample material was combusted in the presence of O_2 in an oxidation column at 1'030 °C, combustion gases were passed through a reduction column (650 °C), and produced N_2 gas was purified (separated from CO_2) and transferred to the IRMS for online isotope measurements. The atom % ^{15}N of the samples was then calculated from 28 N_2, 29 N_2 and 30 N_2 peak heights according to Drury et al. (1987).

5.2.6 Lysimeter leachate

The leachate from the lysimeters was sampled irregularly depending on the outflow (roughly after 20 l from each pot). The volume was measured and an aliquot was taken for further analysis. Ammonium and nitrate concentrations were measured on the same SKALAR SANplus analyser as for the soil samples. The ^{15}N content of the dissolved N residues was determined by EA-IRMS following freeze-drying of a leachate subsample.

5.2.7 Nitrogen fertiliser use efficiency calculation

The N use efficiency was defined as recovered ^{15}N in the yield of the ^{15}N-labelled amount of fertiliser applied for winter wheat. From each harvest (plant shoot and grain from winter wheat and total aboveground plant for sorghum), total dried matter was quantified. An aliquot was ground and measured for ^{15}N. Labelled fertiliser N was then calculated from the dry matter yield multiplied by the ^{15}N atom % (Drury et al., 1987). Natural abundance $^{15}N/^{14}N$ ratios in soil and plant material prior to ^{15}N fertiliser application was subtracted from the measured ^{15}N. Residual ^{15}N stocks were then related to the total 17 kg $^{15}N\ ha^{-1}$ applied (10 % ^{15}N in 170 $kg\ N\ ha^{-1}$ applied as NH_4NO_3 fertiliser) during winter wheat cropping in 2013. In the second year during sorghum cropping, no additional ^{15}N label was applied. Hence, the 2 year rotation was designed to focus on N use efficiency from the fertiliser applied to winter wheat.

5.2.8 Greenhouse gas static chamber measurement

Greenhouse gas samples were collected from static opaque polyvinyl chloride chambers that were manually put over the entire lysimeter column. Chamber height was 25 or 65 cm depending on crop height. Chamber diameter was slightly larger than the lysimeter soil column (68 cm vs. 60 cm) resulting in an effective chamber volume of 91.5 l and 238 l for the short and the tall chambers, respectively. For each measurement, the chambers were manually placed in a ring with rubber sealing inside. Four 20 ml glass vials with rubber septa were filled with chamber air during the 30-45 min closure time. Automatic gas samplers were built to pump chamber air via injection needles through the sample vial. An electronic device controlled electromagnetic valves to open and close the chambers at predefined time steps to sample the chamber air regularly. Hence, the vials were not pre-evacuated but flushed with approximately 100 ml/min for at least 5 min.

Chamber gas samples were analysed within 4 weeks of collection on a gas chromatograph (7890A, Agilent Technologies, Santa Clara, CA, USA). As pre- and analytical column, a HayeSep Q 80/100 (Restek Corp., Bellefonte, PA, USA) was used at a length of 1.83 m and 2.44 m, re-

spectively. The gas samples were loaded onto separate sample loops that were then carried to a flame ionisation detector (FID) via a methanizer with helium for CO_2 and to an electron capture detector (ECD) by N_2 for N_2O detection. Oven temperature was set to 100 °C.

The N_2O flux for each chamber was calculated with the flux estimation procedure (R-script by R. Fuss on bitbucket.org, see Fuss (2015)) as used in Leiber-Sauheitl et al. (2014). It is a modification of the HMR package (Pedersen et al., 2010) that chooses between exponential curvature for non-linear chamber behaviour (Hutchinson-Mosier regression) and robust linear regression. However, the non-linear model could never be fitted successfully, therefore 634 fluxes were calculated with the robust linear and 35 with simple linear regression.

The yearly N_2O emission budget was calculated using linear interpolation between days without flux measurement. Because the measurements did not cover the whole year regularly, we interpreted annual emissions with caution. We checked the reliability of this approach by comparing it with mean annual emissions. The latter showed the same order of magnitude of emissions and similar treatment effects from soil and biochar (see supplemental material).

5.2.9 $^{15}N_2O$ measurement

N_2O from 2 emission peaks was collected at the end of the chamber sampling in 180 ml glass bottles with rubber crimp caps. The total N_2O in each sample was purged with carrier helium directly into a gas bench modified according to McIlvin and Casciotti (2010) and analysed by continuous flow gas chromatography-IRMS (Thermo Finnigan DELTAplus XP). Even with strongly ^{15}N-enriched samples, atom % ^{15}N was calculated using the equations from Stevens and Laughlin (1994) based on mass 45/44 and 46/44 N_2O ratios.

From the ^{15}N content in the N_2O of the chamber air, the background atmospheric 15N_2O, with a concentration of 0.325 ppm and 0.3634 % ^{15}N, was subtracted because it was already present at the beginning of the chamber measurement. This allowed us to determine soil-derived 15N_2O emissions, which were then used to estimate the N source for N_2O production in soil:

$$soil\, N_2O\,[at\%^{15}N] = \frac{^{15}N(chamber) \cdot c(N_2O\,chamber) -^{15}N(atm) \cdot c(N_2O\,atm)}{c(N_2O\,increase\,in\,chamber)} \quad (5.1)$$

5.2.10 Statistical analysis

Statistical analyses were performed with R software (version 3.0.1, The R Project, 2015). The significance level was chosen at $p < 0.05$ for all procedures, unless indicated otherwise. Significant treatment effects on the N pools were determined using a 2-way ANOVA from the rbase package (factor soil: sandy loam, silt loam; factor treatment: biochar, control).

5.3 Results

5.3.1 Fertiliser balance from ^{15}N tracing

A large fraction (44 % for sandy loam and 35 % for silt loam) of the applied fertiliser from 2013 was still contained in soil at the end of 2014 (Figure 5.1; Table 5.1). There was neither a significant difference between the 2 types of soil ($p = 0.07$) nor for biochar vs. control
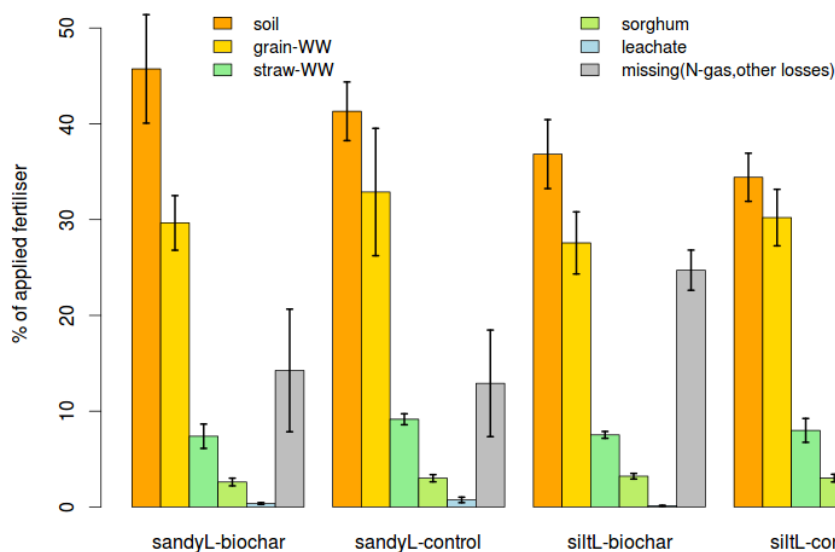
Figure 5.1 – Two-year fertiliser balance of 170 $kg\,N\,ha^{-1}$ with 10 % ^{15}N labelled (initial input: 17 kg ^{15}N). Error bars indicate the standard error from the 4 replicates.

treatments ($p = 0.4$) for fertiliser-derived soil ^{15}N. Winter wheat grains took up 30 % and straw 8 % of the ^{15}N-labelled fertilisers. The ^{15}N uptake was not affected by soil type or biochar application. The cover crop, green rye, took up 2.2 % of the applied ^{15}N fertiliser (Table 5.1); there were no differences between soil types ($p = 0.097$) or biochar treatments ($p = 0.57$). In the following year, after cover crop reincorporation, aboveground sorghum incorporated another 3 % of the previous year's fertiliser. Note that the cover crop ^{15}N was available in soil again for sorghum growth. The ^{15}N uptake by sorghum was not affected by soil type or biochar application.

Leaching of ^{15}N was minimal and the leachate contained only around 0.4 % of the labelled fertiliser after 1.5 years. Most of the ^{15}N label introduced by the fertiliser had not yet passed the soil column. Total leached ^{15}N over the experiment was not different between biochar and control treatments ($p = 0.18$), whereas there was a significant difference between soil types ($p = 0.026$); the sandy loam soil lost more N via leaching than the silt loam soil. Total N leaching in the second winter of the experiment was low, but biochar treatments had significantly lower leaching compared with the control ($p = 0.021$) during that time. Figure 5.2 shows the time series of NO_3^- and NH_4^+ leachate measurements during the experiment with the major peaks in winter (2012-13 and 2013-14). Whereas leached amounts in the first winter were in the expected range of roughly 35 $kg\,N\,ha^{-1}$, the leached N in 2013 accounted for only 5 $kg\,N\,ha^{-1}$. Water amounts leached through the soil columns were about the same in both winters (80-100 l per lysimeter equal to 285-360 mm, roughly one-third of annual rainfall).

The missing amount of ^{15}N fertiliser in Figure 5.1 refers to the difference between the applied amount of N fertiliser and the sum of ^{15}N recovered in soil, plants and leachate. The amount of missing fertiliser in the ^{15}N budget was 13.6 % in sandy loam and 24.5 % in silt loam. This missing fraction was not controlled by biochar application ($p = 0.84$) but differed between soil types ($p = 0.035$) (Figure 5.1). The silt loam had a larger fraction of missing fertiliser N
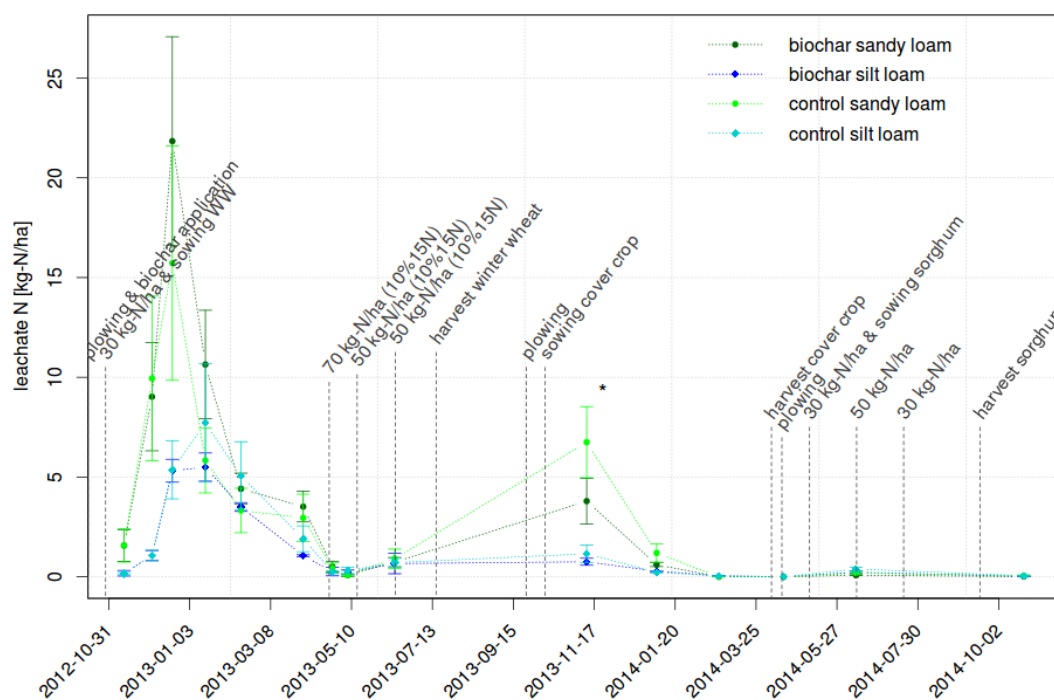
Figure 5.2 – Mean N leaching from the lysimeters by treatment over time. Measurement with significant treatment effect is indicated with an asterisk. Error bars indicate the standard error from the 4 replicates.

Table 5.1 – Fertiliser recovery (FR) as percentage of the applied (170 $kg\,N\,ha^{-1}$) fertiliser in the 3 ecosystem components (soil, plants, leachate) of the lysimeter system at the end of the experiment. Treatments; SandL = sandy loam, siltL = silt loam, bc=biochar, ctrl=control

FR in...	sandL-bc	sandL-ctrl	siltL-bc	siltL-ctrl	p-val. char	p-val. soil
soil	45.7 ± 5.7	41.3 ± 3.1	36.8 ± 3.6	34.4 ± 2.5	0.40	0.07
ww-grain	29.7 ± 2.9	32.9 ± 6.6	27.6 ± 3.2	30.2 ± 2.9	0.50	0.58
ww-straw	7.4 ± 1.3	9.2 ± 0.6	7.5 ± 0.4	8.0 ± 1.2	0.26	0.60
cover crop	2.5 ± 0.2	2.4 ± 0.1	2.1 ± 0.1	2.0 ± 0.4	0.57	0.10
sorghum	2.6 ± 0.4	3.0 ± 0.4	3.2 ± 0.3	3.0 ± 0.4	0.79	0.42
leachate	0.4 ± 0.1	0.7 ± 0.3	0.1 ± 0.04	0.2 ± 0.04	0.18	0.03
missing	14.3 ± 6.4	12.9 ± 5.6	24.7 ± 2.1	24.2 ± 2.7	0.84	0.04

compared with the sandy loam, but with a smaller variability.

In summary, biochar application did not induce significant changes across the 2 years in the quantity of fertiliser ^{15}N recovered in the plants, soil or leachate (Figure 5.1).

There was almost no difference in ^{15}N or total N uptake in any of the crops between the 2 types of soil or between the biochar treatments (Figure 5.3). Solely fertiliser uptake into winter wheat grain was slightly higher in the silt loam than in the sandy loam ($p = 0.013$).

The 2 types of soil had significantly different soil mineral N content (Nmin), pH, cation exchange capacity (CEC) and base saturation (Table 5.2). Biochar did not affect Nmin or soil CEC at any point in time. However, biochar application increased soil base saturation ($p < 0.001$) and pH ($p < 0.001$).

5.3.2 Nitrous oxide emissions

N_2O emissions were discontinuously measured. However, both background emissions and emission peaks were captured (Figure 5.4). Interpolated yearly N_2O emissions were around 2 $kg\,N\,ha^{-1}\,y^{-1}$ (Figure 5.4). An ANOVA of the mean N_2O flux over the measured time span resulted in a p-value of 0.026 for the biochar treatment and 0.039 for the soil types (Table 5.3). This analysis indicates significantly higher emissions in the sandy loam than silt loam and a significant reduction of N_2O emissions with biochar compared with the control by 11 and 21 %, respectively. Biochar plots tended to have lower emissions especially at peak events (Figure 5.4). Yearly mean N_2O emission estimates resulted in N_2O emission factors of around 1 %, being in the expected range from the IPCC (2014).

Although emissions were different between treatments when $^{15}N_2O$ was measured, we did not see any preferential N_2O release from labelled fertiliser (Table 5.3). Figures 5.6 and 5.7 show meteorological parameters from the winter wheat and cover crop-sorghum periods, respectively. The year 2013 started with relatively cold temperatures and 2 pronounced frost events that are reflected by below zero degree temperatures and low (liquid) volumetric water content (VWC) during soil frost (Figure 5.6). In July 2013, there was a dry period coinciding with the last fertilisation. Total precipitation for 2013 was 1'027 mm. The cropping period for green rye and sorghum (Figure 5.7) began with relatively warm winter temperatures and a dry
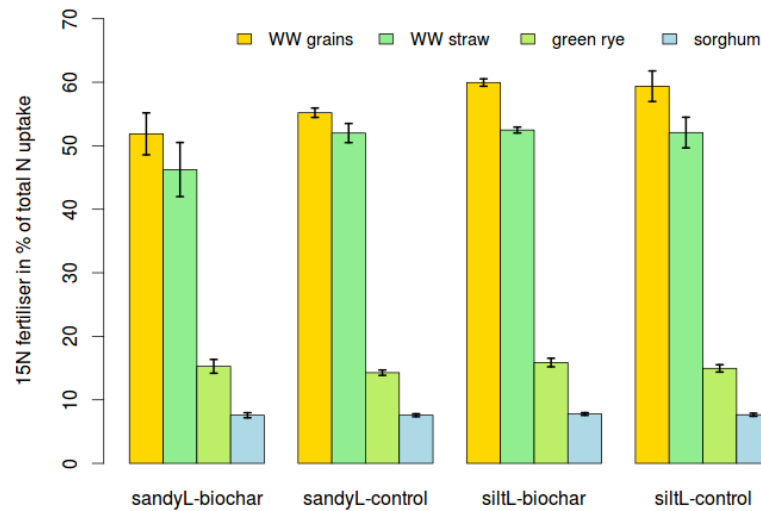
Figure 5.3 – ^{15}N fertiliser uptake as a percentage of total N in plants for winter wheat (WW) grains and straw, the cover crop green rye and sorghum. Error bars indicate the standard error from the 4 replicates.

and warm period in April and June. Average temperatures in Switzerland during this period were 1.5 °C above the 1981-2010 norm. From July onwards, the summer was cold and wet compared with the climatic mean (Meteoswiss, 2015). However, the precipitation sum for the whole year 2014 of 985 mm was lower than in 2013.

Soil VWC appeared to be higher in soils treated with biochar compared with the control, but only for 10 out of 735 days was this difference significant. Hence, there was no evidence that soils treated with biochar held significantly more water than non-treated soils.

5.4 Discussion

5.4.1 N balance

Our results for a temperate winter wheat - cover crop - sorghum rotation showed that biochar application neither led to a higher fertiliser N uptake by the crops nor did it increase or decrease yields. The N use efficiency from the first year's fertiliser application of approximately 40 % throughout the whole rotation was not increased by biochar. Furthermore, N content in 3 ecosystem components, i.e. plant, soil and partly leachate, was not significantly altered in our system by biochar at any time. Reported changes in N transformation with biochar (Prommer et al., 2014; Nelissen et al., 2014) may not immediately change gross N flows in a temperate agricultural system with high N inputs and already high soil fertility. For example, Prommer et al. (2014) have shown that biochar significantly reduces gross rates of soil organic N transformation in the field but not gross mineralisation of organic N. The authors explained their findings by a decoupling of the soil organic and inorganic N cycles and concluded that
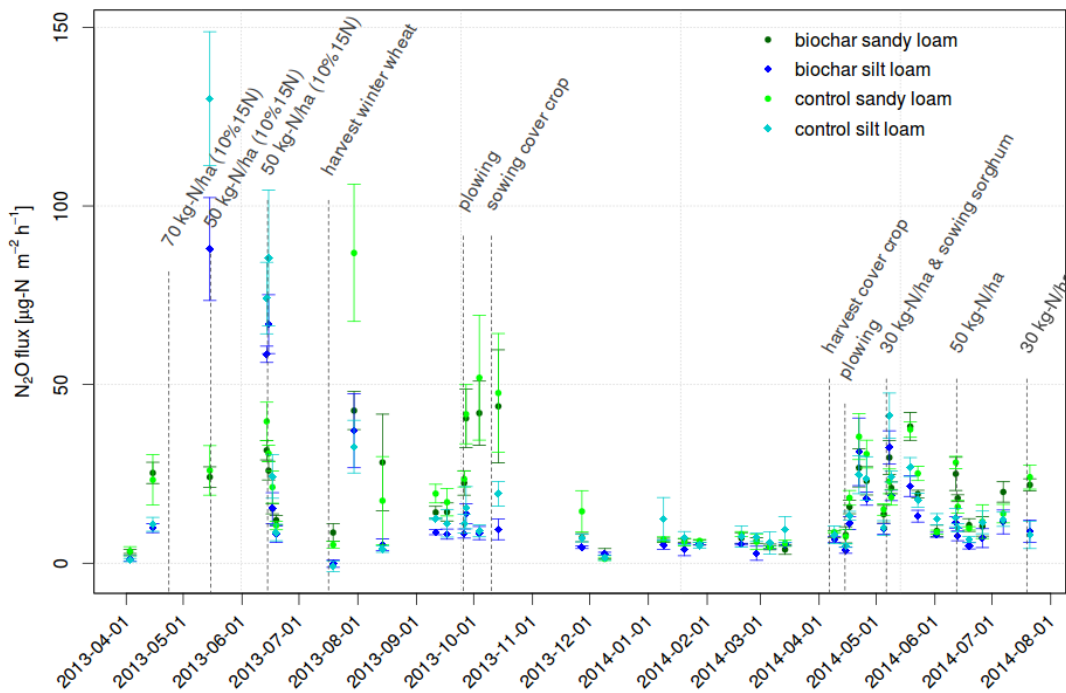
Figure 5.4 – N$_2$O emissions during the 2 years of the experiment with winter wheat in 2013 and sorghum in 2014. Error bars indicate the standard error from the 4 replicates.
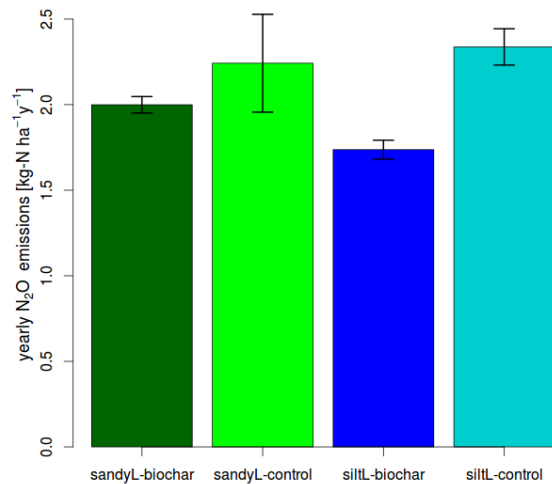
Figure 5.5 – Total annual N$_2$O emissions as calculated from measured events; error bars = 1 SE; annual emissions estimated with fluxes interpolated between days without measurement.
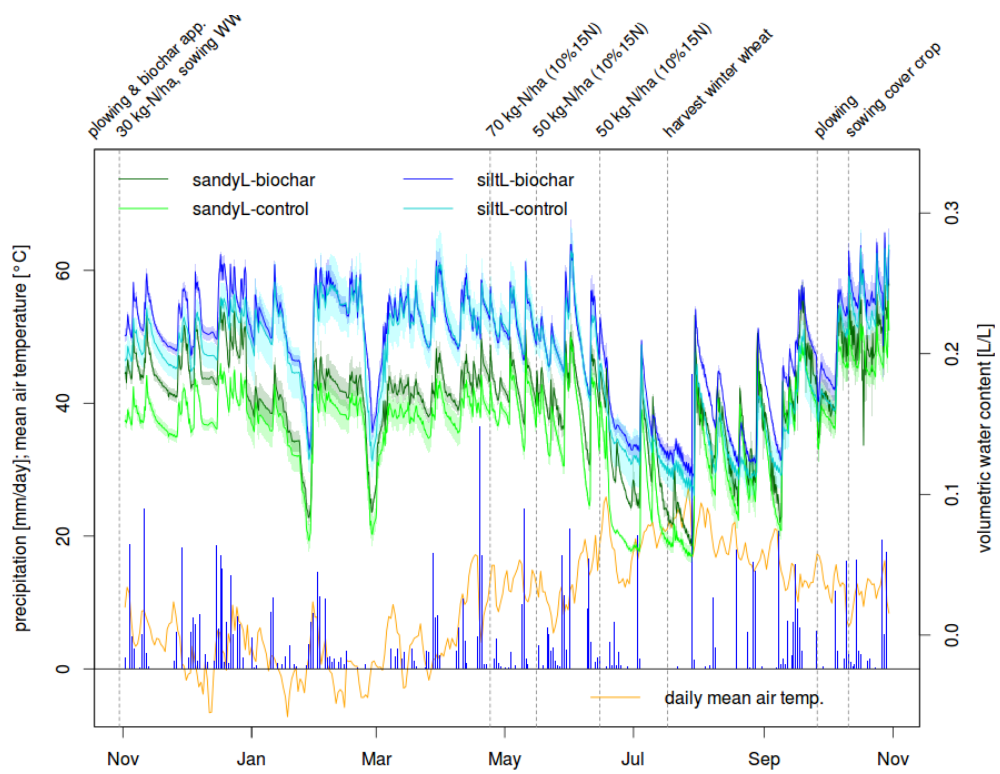
Figure 5.6 – Volumetric water content of the 4 treatment combinations, temperature (orange line) and precipitation (blue bars) during winter wheat cropping in 2013 until November 2013.

Figure 5.7 – Volumetric water content of the 4 treatment combinations, temperature (orange line) and precipitation (blue bars) during cover crop and sorghum cropping in 2014 until November 2014.

the combined addition of biochar and fertiliser N would increase soil organic N and enhance soil C sequestration. (Pereira et al., 2015) observed increased N transformation rates with biochar but no change in plant productivity or leaf N content. Vaccari et al. (2011) observed up to 30 % increased biomass production withou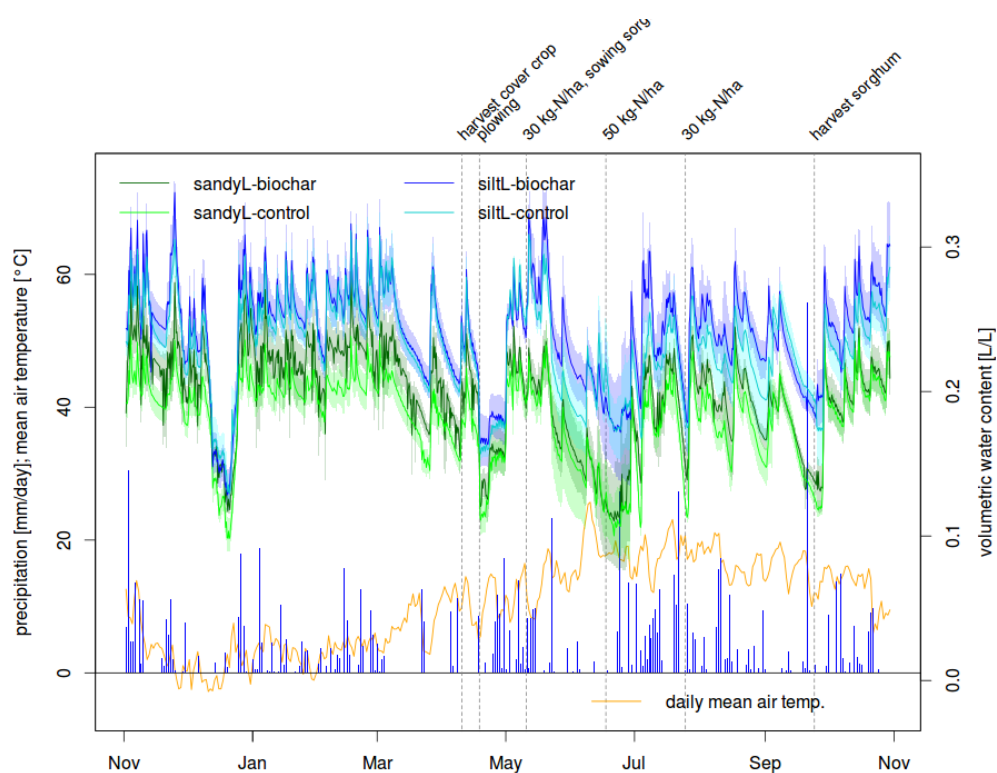t change in grain N content. In agreement with our results, this shows that small changes in N cycling with biochar (i.e. increased N transformation rates or increased biomass production) do not necessarily increase agricultural yields. Our results also showed that plant growth was not limited by factors that were affected by biochar, i.e. pH and base saturation. Biochar-dominated soil properties also did not change soil N content, leaching or plant available N.

In contrast, many studies on biochar and N uptake found an increase in yields (Jeffery et al., 2011; Biederman and Harpole, 2013). For example, van Zwieten et al. (2009) reported a 250 % wheat biomass increased with biochar at 10 t ha^{-1} on a ferralsol and suggested an increase in N use efficiency. Petter et al. (2016) showed an increased N use efficiency with biochar in an upland rice cropping system (soil pH 5.3 and base saturation 41 %). This discrepancy highlights the differential effects biochar application can have on highly weathered tropical soils versus temperate fertile ones. Soils like those in our experiment with naturally high pH and base saturation may not benefit as much as less fertile and highly weathered tropical soils (Crane-Droesch et al., 2013). Jeffery et al. (2011) suggested that the main mechanisms for yield increase may be due to biochar's liming effect, improved water holding capacity and improved crop nutrient availability. In experiments by Karer et al. (2013), positive yield effects were observed during drought situations but no significant effect was discernible in the following years and with other crops. In our case, we could not find increased yields with biochar although winter wheat plants suffered from dry conditions during grain filling after the third fertilisation event.

Fertiliser N use efficiency of the first crop is usually around 30-50 % (Ladha et al., 2005). Our results of fertiliser N uptake by winter wheat grains of roughly 30 % was at the lower end of this estimate. Our yields were depressed due to the application of a growth regulator following constrained chamber height and bird feeding (inhibited grain filling in addition to the estimated 67 % yield loss). Additionally, intensified drought in the sun-exposed concrete block of the lysimeter facility may have impaired plant growth and yield and may have had a negative influence on grain filling. The sorghum yield was also largely consumed by birds, which was not corrected for, but no systematic bias was observed due to such external impacts, hence we assume that bird predation was equally distributed between all lysimeters.

There were 2 significant biochar effects observed for our system, namely reduced bulk N leaching in the second year and reduced N₂O emissions. Despite their environmental relevance, these N fluxes were very small compared with the overall N balance. Furthermore, the high natural variability in a field situation, i.e. soil heterogeneity, field management, weather conditions and extremes (see Figures 5.6 and 5.7), probably reduced the sensitivity of our experiments for small effects that were frequently detected in laboratory incubations (Clough and Condron, 2010).

Because not all N fluxes were measured, there was a quantifiable but unknown gap in the fertiliser N recovery of 13-25 % after 2 years. The extent of this gap may vary due to field variability (larger for the sandy loam than the silt loam) and uncertainties in the balance calculation, but trends between treatments are important. The missing fraction was most likely related to the transformation of fertiliser N into gaseous forms, namely N_2, NH_3 and NO during denitrification, nitrification and ammonium volatilisation that can make up a

significant proportion of the overall N budget Clough et al. (2001); Martinez and Guiraud (1990); Friedl et al. (2015). The imbalance was significantly higher in the silt loam, which is less prone to leaching than the sandy loam. A proportionally higher leaching for both soil types could balance the gap only for the sandy loam, where greater losses due to leaching were measured. Instead, the missing fertiliser ^{15}N can better be explained by the observed N_2O emission patterns. More precisely, although N_2O emissions are often one order of magnitude lower than N_2 emissions (Jambert et al., 1997) and do not contribute significantly to the overall N balance, they may provide semiquantitative information on the overall denitrification rate and hence N_2 loss. A higher denitrification rate (as suggested by higher N_2O accumulation; see below) may explain the larger gap in the ^{15}N balance of the silt loam compared with the sandy loam. Friedl et al. (2015) demonstrated how cumulated N_2 emissions from an intensively managed subtropical pasture can account for up to 40 % of the applied N. Considering this large potential for unmeasured gaseous losses, they may account for the gaps in N recovery. Biochar often reduces N leaching from soil (Güereña et al., 2013; Ventura et al., 2013; Laird et al., 2010). We found no significant effect of biochar on the overall cumulative N leaching during the experiment. However, there was an early tendency for lower N leaching with biochar than without, which turned into a significant difference if only the second winter was considered (see Figure 5.2). This result can be explained by the residence time of N in the soil column after biochar incorporation and the early end of the experiment. Still, we can confirm biochar's potential to reduce N leaching in an open-air field trial. However, the amounts of N leached from the labelled fertiliser (on average 0.4 %) were very low for all treatments. In a long-term ^{15}N study, Sebilo et al. (2013) found that after 3 decades, between 8 and 12 % of the applied fertiliser leaked to the ground water. Martinez and Guiraud (1990) also found much higher N leaching than we did; on bare fallow 18.7 % of the applied N was leached compared with 7.1 % with cover crop. There are several possibilities why in our cover crop system leachate was lower. The calcareous, stony soil used by Martinez and Guiraud (1990) is expected to give more room for leaching N than our silt and sandy loam soils. Furthermore, our fertilisation scheme with 3 doses and overall lower amounts than those applied by Martinez and Guiraud (1990) was optimised to reduce leaching. Our sampling only covered one winter (2013/2014) and ended in autumn 2014 before the next major leaching period. It seems that the ^{15}N fertiliser needs more time than 1.5 years to pass through the lysimeter soil column of 60 cm depth. Lastly, total precipitation in 2014 was lower than in 2013 and possibly hampered the leaching of the ^{15}N signal.

To our knowledge, this is the first study that observed N use efficiencies comparing biochar application on 2 soil types using ^{15}N fertiliser tracing. Previous work in this context relied mostly on short-term laboratory experiments that do not allow investigating the sustainability of biochar effects on the N cycle in soils on larger spatial scales (Scott et al., 2014). For example, Nelissen et al. (2015) showed how significant changes in soil N transformation with fresh biochar completely vanish after 1 year. In order to better understand the underlying mechanisms of biochar-plant-soil-microorganism interactions, more longer-term scale experiments under more natural conditions (with ageing biochar) are needed.

5.4.2 Nitrous oxide emissions

We found an average reduction in soil N_2O emissions of 15 %, which is within the range of a recently published meta-analysis by Cayuela et al. (2015) (mean of 28 ± 16 % in field

experiments). This concordance strengthens the evidence for the effectiveness of biochar to reduce N_2O emissions in a field situation. With the same biochar, (Felber et al., 2013) found a 21.5 % reduction in N_2O emissions during one growing season on a grassland.

With a 0.20 H/C ratio, the biochar we used was in the range of low H/C ratio biochars that Cayuela et al. (2015) identified as being most effective for reducing N_2O emissions from soil. These biochars have a condensed aromatic structure that allows electron transfer across conjugated pi-electron systems (Klüpfel et al., 2014), which might be beneficial for the last step of denitrification (Cayuela et al., 2013). Furthermore, our biochar had a high pH and especially a large liming capacity of 15.4 % $CaCO_3$ equivalents. We observed an increase in soil pH after application of this alkaline biochar (Table 5.2). Several authors have suggested pH effects as a mechanism for reduced N_2O emission from soil (van Zwieten et al., 2010; Zheng et al., 2012). With increasing soil pH, the denitrifying community tends to increase N_2O reduction activity, thereby reducing emissions as N_2O (Čuhel et al., 2010), also shown in biochar-soil slurries by Obia et al. (2015). Although the pH hypothesis is plausible from our observations and data, it is still unclear if reductions in N_2O emissions can solely be assigned to the soil pH increase. Hüppi et al. (2015) , for example, explicitly tested for the pH effect in a field trial and did not observe the same sort of reduction effect with limestone as they found with biochar. Soil pH manipulations and their effects on N_2O emissions are driven by complex interactions (Baggs et al., 2010) and cannot be elucidated by one replicated field plot experiment over medium time scales.

Residual losses in the fertiliser N balance are mostly gaseous fluxes of N_2O, N_2, NOx and NH_3 (Jambert et al., 1997). From our flux measurements, we can roughly estimate the N_2O losses to be at the magnitude of 1 % of applied fertiliser. Butterbach-Bahl et al. (2013) estimated the mean N_2O share of denitrification from agricultural soils to be 15 ± 6 %. If we estimate the N_2 emissions accordingly (i.e. N_2 being 6.7 ± 1.9 times the N_2O emissions), our system lost roughly 7 % of fertiliser as N_2. This percentage accounts for half of the missing N in the sandy loam and about one-fourth in the silt loam. According to Jambert et al. (1997), gaseous N losses from a mineral fertilised maize field can have the following shares: 1 % as NH_3, 40 % as NO, 14 % as N_2O and 46 % as N_2. Hence, NO emissions can be in the same order of magnitude as N_2. Nelissen et al. (2014) tested various fertiliser types and found not only reduced cumulative N_2O (52-84 %) emissions with biochar but also reduction in NO (47-67 %). They explained the reduced emissions by increased NH_3 volatilisation, microbial N immobilisation and non-electrostatic sorption of NH_4^+ and NO_3^- as well as pH effects. However, our data do not suggest that there were large changes in N immobilisation (due to high fertiliser input) or sorption on biochar, because we did not observe changes in soil N content or plant N uptake.

Our observations with regards to fertiliser-derived N_2O suggest that there was no change in the N source for N_2O production in soil when biochar was added. This is the first study to show that the N source for N_2O in an experiment with reduced emissions by biochar in the field was not changed. This finding means that neither fertiliser nor soil-derived N availability for microbial N_2O production was reduced by biochar. The (unknown) processes responsible for N_2O emission reduction may not be fertiliser specific. Further, this finding indicates that reduced N_2O emissions by biochar only depended on increased N_2O reduction (i.e. increased nosZ activity) but did not decrease the amount of N used for denitrification (Obia et al., 2015; Harter et al., 2014).

5.5   Conclusion

Biochar did not change N fertiliser use efficiency or N partitioning among the 3 ecosystem components (soil, plants or leachate) of the agricultural system over the course of our 2 year lysimeter study. Biochar treatment caused a decrease in N_2O emissions but no change in the source of N for N_2O production. Although the observed effects due to biochar application (i.e. reduced N_2O emissions and leaching) apply to fluxes that are small within the overall N balance, they are environmentally significant and important for understanding biochar functioning in agricultural systems. Especially reduced N_2O emissions have a large relevance for climate mitigation and the overall biochar GHG balance. However, a comprehensive life cycle assessment is needed to verify if these improvements can counterbalance possible negative effects from biochar production (e.g. competition for biomass as resource) and other adverse effects (e.g. introduction of organic and inorganic pollutants to soil). We showed that biochar application in temperate agricultural soils has a small but significant potential to reduce environmental impacts of N fertilisation and does not impair crop yields.

5.6   Supplementary material

Yield correction due to bird feeding

The winter wheat grain yield was severely reduced by feeding birds. For ^{15}N balance consideration, we estimated the yield reductions by birds. We used yield data (i.e. N yield and average grain size) from our harvest and compared it with data from a nearby breed examination trial at Agroscope from the same year and with a sister breed of Siala. Besides bird feeding, the yield was also reduced compared with the breed trial because of management inconveniences in our experiment that were associated with reduced plant growth in the lysimeter system (e.g. pronounced drought conditions in the concrete block or the use of growth regulators). Using reported mean grain size from both trials, we estimated a potential N yield reduction of 30 % for our lysimeter system. With reported N contents from the neighbour field, we estimated the expected yield without bird feeding loss and lysimeter management induced yield reductions. The remaining difference between the potential and actual yield after harvest was the average loss from bird feeding (= 67 %). Hence, our obtained grain N content data were multiplied by 3 to represent the actual N uptake by winter wheat grains. For the sorghum yield, loss due to bird feeding was not accounted for in the N balance, because its impact on the ^{15}N fertiliser budget from the previous year was not as pronounced as for winter wheat (roughly 3 % of ^{15}N-labelled fertiliser was recovered in sorghum).

Annual N_2O emission budget

Alternatively to the interpolated annual N_2O emissions, we calculated mean emissions and extrapolated this number over a full year. Comparison of both approaches is useful because these are the 2 most simple estimates that do not require manual manipulation of the data set (such as introducing virtual background emissions) and they cover a wide range of possible estimates. Both estimates showed the same magnitude of emissions and similar treatment effects despite their caveats, suggesting that the true emissions followed the same pattern (see Fig. 5.8).
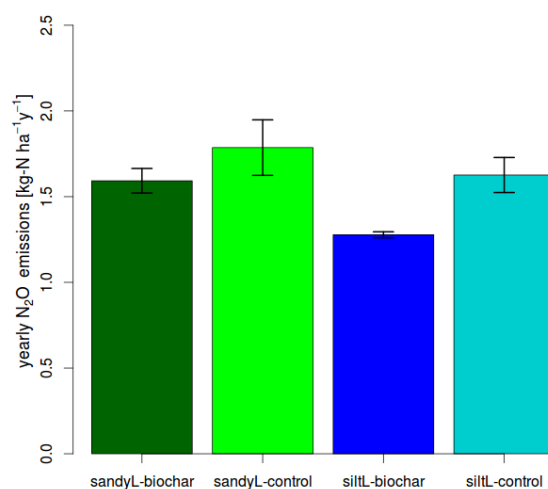
Figure 5.8 – Total annual N_2O emissions as calculated from measured events; error bars = 1 SE; plot represents the mean emission extrapolated to the whole year; error bars indicate the standard error from the 4 replicates. Numbers see Table 3 in the original manuscript.

Table 5.2 – Soil parameters and ^{15}N content in soil (^{15}N at %) at several points in time (mean by treatment ± standard error); sandy loam and silt loam, bc=biochar, ctrl=control, BS=Base Saturation.

Parameter	Unit	Date	sandL-bc	sandL-ctrl	siltL-bc	siltL-ctrl	p-value char	p-value soil
BS (pre bc)	%	2012-10-15	78.0 ± 0.9	79.0 ± 2.3	56.3 ± 1.4	58.0 ± 1.9	0.43	<0.001
BS	%	2012-10-24	90.2 ± 1.1	80.0 ± 1.9	76.2 ± 1.7	59.5 ± 1.7	<0.001	<0.001
CEC (pre bc)	cmol+/kg	2012-10-15	13.6 ± 0.2	13.6 ± 0.2	11.9 ± 0.2	11.9 ± 0.2	0.95	<0.001
CEC	cmol+/kg	2012-10-24	13.6 ± 0.3	13.6 ± 0.3	11.8 ± 0.1	12.2 ± 0.1	0.31	<0.001
N(min), (pre bc)	$mg\,N\,kg^{-1}$ soil	2012-10-15	3.1 ± 1.1	3.3 ± 1.5	0.5 ± 0.2	0.2 ± 0.1	0.97	0.01
N(min)	$mg\,N\,kg^{-1}$ soil	2012-10-24	1.8 ± 0.3	1.5 ± 0.4	0.8 ± 0.3	0.8 ± 0.2	0.74	0.01
N(min)	$mg\,N\,kg^{-1}$ soil	2013-04-08	5.3 ± 0.4	6.3 ± 0.4	2.7 ± 0.5	3.0 ± 0.8	0.26	<0.001
N(min)	$mg\,N\,kg^{-1}$ soil	2013-05-08	6.4 ± 0.8	7.2 ± 0.5	2.4 ± 0.4	3.0 ± 0.8	0.33	<0.001
N(min)	$mg\,N\,kg^{-1}$ soil	2013-06-13	3.8 ± 0.2	3.8 ± 0.6	1.1 ± 0.3	1.3 ± 0.4	0.78	<0.001
N(min)	$mg\,N\,kg^{-1}$ soil	2014-07-04	1.3 ± 0.1	1.3 ± 0.1	0.5 ± 0.1	0.7 ± 0.1	0.29	<0.001
N(min)	$mg\,N\,kg^{-1}$ soil	2014-04-14	1.1 ± 0.2	1.3 ± 0.1	0.8 ± 0.2	0.7 ± 0.2	0.83	0.02
pH (pre bc)		2012-10-15	6.95 ± 0.03	6.93 ± 0.09	5.88 ± 0.07	5.96 ± 0.11	0.74	<0.001
pH		2013-04-16	7.95 ± 0.12	7.31 ± 0.09	7.54 ± 0.16	6.35 ± 0.07	<0.001	<0.001
pH		2013-07-19	7.24 ± 0.15	6.77 ± 0.05	6.55 ± 0.06	5.94 ± 0.06	<0.001	<0.001
soil ^{15}N	^{15}N at%	2013-07-16	0.68 ± 0.05	0.71 ± 0.03	0.80 ± 0.05	0.71 ± 0.02	0.40	0.15
soil ^{15}N	^{15}N at%	2014-09-16	0.60 ± 0.03	0.57 ± 0.02	0.61 ± 0.03	0.59 ± 0.02	0.30	0.53

Table 5.3 – Yearly mean N_2O emissions and ^{15}N content of N_2O emissions at 2 sampling dates - treatments: SandL = sandy loam, siltL = silt loam, bc=biochar, ctrl=control, cum N_2O = cumulative N_2O by daily linear interpolation

Parameter	Unit	Date	sandL-bc	sandL-ctrl	siltL-bc	siltL-ctrl	p-value char	p-value soil
cum. N_2O	$kg\,N\,ha^{-1}y^{-1}$	2013-2014	2.00 ± 0.05	2.24 ± 0.29	1.74 ± 0.06	2.34 ± 0.11	0.02	0.60
yearly mean N_2O	$kg\,N\,ha^{-1}y^{-1}$	2013-2014	1.60 ± 0.08	1.79 ± 0.16	1.28 ± 0.02	1.63 ± 0.10	0.03	0.04
soil N_2O	^{15}N at%	2013-05-16	4.94 ± 0.55	5.91 ± 0.33	8.52 ± 0.17	8.65 ± 0.10	0.13	<0.001
soil N_2O	^{15}N at %	2014-05-08	1.16 ± 0.14	1.40 ± 0.13	1.11 ± 0.06	1.11 ± 0.06	0.27	0.14

Safe application of nonlinear flux calculation schemes for static chamber N$_2$O measurements - a tool

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Abstract

The static chamber approach is often used for greenhouse gas (GHG) flux measurements, whereby the flux is deduced from the increase of species concentration after closing the chamber. Since this increase changes diffusion gradients between chamber air and soil air, a nonlinear increase is expected. Lateral gas flow and leakages also contribute to nonlinearity. Several models have been suggested to account for this nonlinearity, the most recent being the HMR (Hutchinson-Mosier R-script) model. However, the practical use of these models are challenging because the researcher needs to decide for each flux whether a nonlinear fit is appropriate or exaggerates flux estimates due to measurement artifacts. In the latter case a flux estimate from the linear model is a more pragmatic solution and introduces less arbitrary uncertainty to the data.

We present a tool to simulate, visualise and optimise the flux calculation scheme for a specific static nitrous oxide (N$_2$O) chamber measurement system. We offer an automatic, reproducible and dynamic selection criterion for a reduced tradeoff between bias and uncertainty (i.e. accuracy and precision). The decision procedure and visualisation tools will be implemented in a package for the R software. Finally, we demonstrate with this approach the performance of the applied flux calculation scheme for a specific flux dataset to estimate the actual bias and uncertainty of the dataset's cumulated flux.

6.1 Introduction

Since more than 30 years, trace gas emissions from soil are frequently measured with static (non-steady state) chambers, especially for greenhouse gases (GHG) like N_2O. There are several guidelines available for the practical ahndling, chamber layout, experimental design and determination of the concentration within the chamber during deployment (de Klein and Harvey, 2013; Collier et al., 2014). To calculate a flux from the concentration measurements in the chamber headspace researchers very often simply use a least squares linear regression (LR) (Rochette and Eriksen-Hamel, 2008; Conen and Smith, 2000). Such LR based flux estimates are least sensitive to measurement uncertainty (good precision) due to analytical limitations or variations due to gas sampling in the field (Venterea et al., 2009). However, the gas concentration in a static chamber theoretically follows a nonlinear shape during chamber closure because of underlying processes from diffusion theory and leakages (Anthony et al., 1995). Estimating parameters for the non-linear behaviour of a flux curve introduces large uncertainties. Depending on chamber height and deployment time, the estimated flux may increase with the assumption of a non-linear behaviour (Venterea and Baker, 2008) relative to the more conservative LR. Given a certain system, the uncertainty related to the flux calculation scheme is the largest single error source for the estimated flux (Levy et al., 2011). The magnitude of this error is hardly understood within the possible parameter space as it depends on the chamber system, flux calculation procedure and flux size. Venterea (2013) present a comprehensive summary of the available nonlinear flux calculation schemes (FCS) within the methodology guidelines by de Klein and Harvey (2013). They list pro- and contra-arguments for the commonly used procedures (conventional FCS: Linear regression (LR), Hutchinson and Mosier (HM), Quadratic regression (QR); as well as advanced FCS: Non-steady state diffusive flux estimator (NDFE), HMR (R script by Pedersen et al. (2010), based on the Hutchinson-Mosier equation) and chamber bias correction method (CBC by Venterea (2010)). From their analysis it is clear, that none of the methods can directly be applied to a measured flux dataset. Whereas LR produces a considerable bias (Pedersen et al., 2010) all non-linear estimates have large uncertainties for small fluxes (Parkin et al., 2012) and deviation from the theoretical curvature. Venterea (2013) modelled this deviation by switching on and off different processes in soil diffusion models and compared the results to the available FCS's. They showed that under varying conditions most commonly used FCSs tend to substantially underestimate the theoretically modeled flux. If one aims at reducing bias of flux estimates, the user has to combine a linear with nonlinear method depending of the properties of each single flux within the dataset. The HMR tool (R packages available on CRAN) from Pedersen et al. (2010) offers a manual screening of each flux and strongly recommends expert knowledge to chose between HMR, LR or zero flux estimates. As this is very subjective and not practical for large datasets, many users introduce some thresholds of certain indicators like $F_{nonliear}/F_{linear}$ (g-factor) or statistical goodness of fit outputs like R^2, p-values, standard errors (SE) or the Akaike information criterion (AIC). The choice of these thresholds is arbitrary, poorly justified and rarely documented. There is a need for a better understanding of the type of criterion that is chosen and the values of the thresholds needs to be related to the uncertainty of the concentration estimate by a specific measurement system. Most critical for nonlinear estimates are those measurement systems that use only 4 time points with concentration measurements on a GHG gas chromatography system by offline manual vial sampling. A commonly used calculation tool within the gas flux community of the German Soil Science Society (Deutsche Bodenkundliche Gesellschaft DBG) is implemented and maintained by R. Fuss and available

on bitbucket (Fuss, 2015). This tool offers different FCSs as well as an additional decision mechanism (see "RF2011") described in Leiber-Sauheitl et al. (2014). In addition to LR and HMR, a robust linear regression according Huber and Ronchetti (1981) is implemented to reduce the sensitivity of least square regression to outliers. RF2011 choses between HMR, LR and robust linear depending on lower p-value and AIC (Burnham and Anderson, 2004) of the fits and does not allow the absolute value of the nonlinear estimate to deviate by more than the g-factor (default is 4) from the absolute value of the linear estimate. Although this mixed FCS has been used in some studies (Walter et al., 2015; Deppe et al., 2016) and is easy to implement, its performance for different systems has not been analysed systematically. There is no general rule or understanding for the thresholds and statistical decision criteria. It is very difficult for users of FCSs to estimate the impact of a certain FCS for their specific measurement system and how to choose the appropriate parameters. For this reason, we present a tool that i) allows for a better understanding of the behaviour of the FCS used and ii) provides a decision rule for the best trade-off between uncertainty and bias a FCS introduces to the dataset. We look for a relationship between measurement error of the concentration (standard deviation by the GC) and a dynamic threshold that allows to separate the LR and HMR regime in order to minimise bias and uncertainty together. The tool provides an automatic procedure for a safe use of nonlinear FCS also for unexperienced users. Commonly used decision trees can be pruned and simplified.

6.2 Methods

6.2.1 Model framework for FCS visualisation and testing

In a first step, a simulation framework is setup that scans through a common range of curved chamber concentrations and flux strength. The HMR parameterization (Pedersen et al., 2010) of the Hutchinson-Mosier equation (Hutchinson & Mosier, 1981) for nonlinear flux estimates is used as approximation for any nonlinear flux curvatures (varying κ in Eq. 6.1).

$$C(t) = \varphi + f_0 \frac{exp(-\kappa \cdot t)}{-\kappa \cdot h} \tag{6.1}$$

h = chamber height
t = time (after chamber closure)
$C(t)$ = gas concentration at time t
$\varphi > 0$ model parameters
$f0$ = flux at time zero, when the chamber is closed
Parameter κ (for $\kappa > 0$ and for $-\infty < f_0 < \infty$) is estimated by the ordinary least squares method.

The HMR model was used to simulate synthetic concentrations for a common range of fluxes and for the non-linear shape parameter κ. κ could be tentatively related to soil texture and moisture classes (dry/wet) that influence diffusion coefficients if an assumption is made regarding the depth of the gas source (Venterea, 2013). However, in this exercise we just scan through a commonly observed range of κ (see Fig. 6.1) without relating it to soil or chamber properties and the underlying diffusion theories that themselves are also prone to bias and uncertainty (Venterea, 2013). It is assumed that within the chosen range of κ, the resulting concentrations-time curves capture any diffusion characteristics of a typical soil-air system at
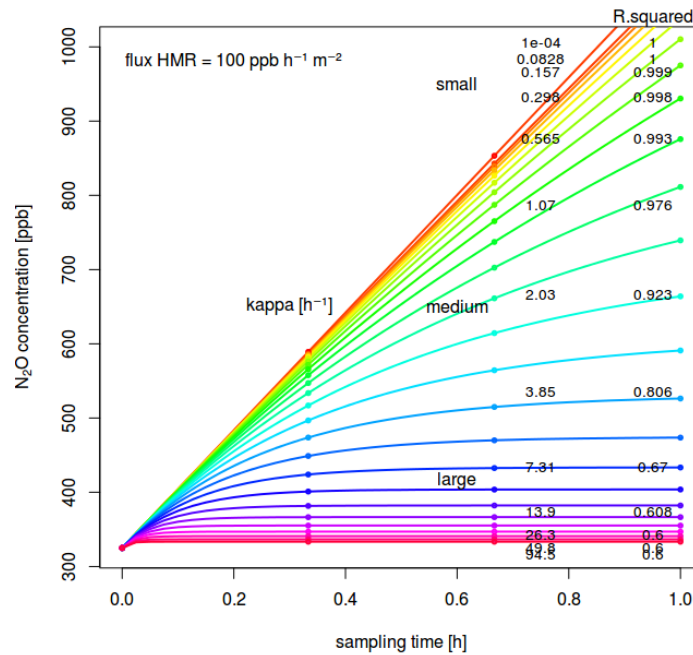
Figure 6.1 – Example of simulated concentration-time curves depending on κ and a specific flux size (chamber height: 0.13 m), R^2 of a linear fit are given for 4 sampling time points.

different water levels.

For the system specific input, a chamber area (i.e. 0.071 m^2) and height (i.e. 0.13 m) were defined without loss of generality. A measurement device (i.e. gas chromatography GC) precision was then assumed as a constant standard deviation over the calibrated concentration range. Using this input data, a series (i.e. $n = 100$) of synthetic chamber concentrations are calculated for different flux sizes (i.e. $f0; 0 - 100 ppb m^{-2} h^{-1}$) and nonlinear shapes ($\kappa$ from 0.0001 to 100 h^{-1}). Each of these replications follow the perfect HMR derived flux curve but in addition a random noise according the GCs precision is added. The simulated chamber concentrations are then fed back to the flux estimation script (i.e. gasfluxes on bitbucket, ref URL) which uses the HMR routine (Pedersen et al., 2010) again, applied with additional linear vs. nonlinear decision rules (see section "decision rules").

Figure 6.2 exemplifies how the simulation output is visualised. On the x-axis (front to back) the predefined HMR flux ($f0$; 0-100 $ppb m^{-2} h^{-1}$) is plotted against the y-axis (left to right) showing given values for κ (0.0001-100 h^{-1}). Onto this 2d surface of given HMR derived chamber concentrations the flux calculation script estimates the fluxes again, shown on the z-axis as coloured surface. In this example only a small noise of 0.1 ppb standard deviation is introduced to all the 4 concentration measurements. The results from the simulations are shown as the median of 100 replications in the red surface and a purely linear estimate is the blue surface. The contour plot on the bottom of the 3d plot is also the resulting linear flux estimate. Whereas the linear model provides a smooth transition from pre-given HMR concentration shapes with large κ (very high nonlinearity) to small κ (perfectly linear behaviour), the HMR decision suggestion suddenly jumps from nonlinear to linear estimates. At this point,
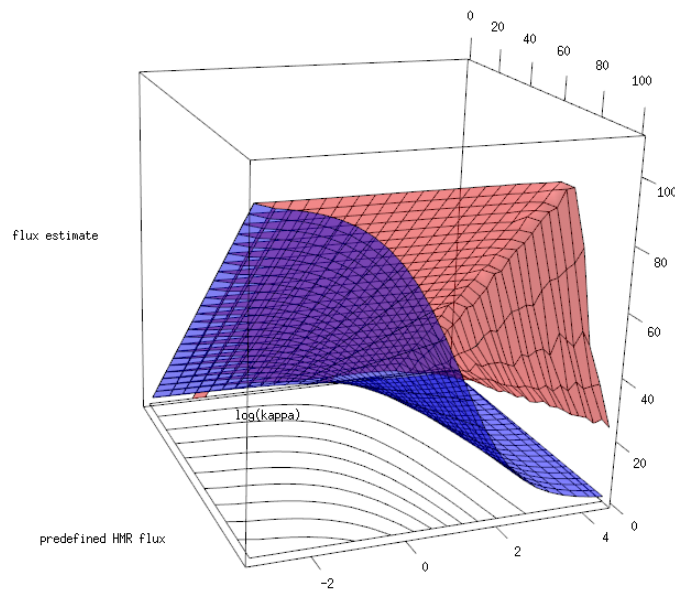
Figure 6.2 – Basic behaviour of the estimated/calculated flux (median of 100 varied input data) from the two different FCS (LR in blue and HMR in red). Added noise to the synthetic concentrations is very small (0.1 ppb SD).

the HMR procedure itself does not accept the estimated κ. In addition to the HMR nonlinear fit, also a quadratic regression is calculated for method comparison (Wagner et al., 1997). It basically fits the equation $C(t) = at^2 + bt + c$ to the data and solves for the flux rate b. This representation of chamber nonlinearity has no basis in diffusion or any other physical theory, but it empirically fits the chamber behaviour and creates less uncertainty than HMR (Venterea, 2013).

6.2.2   FCS decision rules

Eight different FCSs were selected that each apply different decision rules between linear and nonlinear in the interpretation of chamber flux data. The synthetically generated concentrations by the HMR equation (Eq.1) are then fed to these calculation procedures. Note that HMR itself is a decision rule on its own, because it also uses linear regression for fluxes where no nonlinearity parameter (κ) could be fitted. Especially with the added noise from measurement uncertainty, the HMR decision rule often cannot retrieve a flux which concentrations are originally calculated by the HMR equation (see Fig. 1 at $\log(\kappa) > 4$). All the other methods presented here are either commonly used as calculation procedure in publications with static chamber flux datasets or they were suggested to offer robust solutions to decide between nonlinear and linear flux estimates:

- **LR:** This method always applies linear regression (LR) by the least squares estimate.
- **HMR:** The nonlinear flux estimate by Pedersen et al. (2010), κ is estimated by minimising the mean squared error (MSE). If no local optimum is found for any κ, then the tool decides for linear regression too.
- **AIC:** AIC of the HMR fit needs to be smaller than the linear AIC. Note that AIC is biased

towards the more complex model if the number of datapoints is small. This bias is reduced (but not removed) by applying a correction for finite sample size (AICc, see Cavanaugh (1997)). However, AICc is not defined for $n < 5$.

- **f.detect:** The linear estimated flux needs to be at least twice as high than the minimal detectable flux (f.detect) in order to allow for the nonlinear HMR procedure. The detection limit is calculated that is calculated according to Parkin et al. (2012) approximation for HMR fluxes:

  $f.detect = 13.20 \cdot t[4]^{-0.9973} \cdot GC.sd \cdot A$

  where $t[4]$ is the time of the last concentration sample, $GC.sd$ is the precision of the GC as standard deviation at atmospheric concentrations and A is the area of the chamber. This approximation is only valid for 4 time points.

  The f.detect is also calculated for all the methods within the simulation run at a predefined flux of zero. The 95 % confidence interval is chosen as f.detect (according Parkin et al. (2012)). In case of more than 4 time points of chamber concentration, the f.detect is used from the 95 % confidence interval of the HMR decision rule.

- **G-factor = $F_{nonliear}/F_{linear}$:** The nonlinear flux calculation is not allowed to increase the linear flux estimate by more than the g-factor. The default for a maximum g-factor of 4 is taken from the flux script by R. Fuss.

- **Dynamic R^2:** Nonlinear flux calculation is allowed if the linear R^2 exceeds a dynamic threshold, depending on the minimal detectable flux (f.detect), the linear flux estimate (f.lin) and the minimal R^2 (R.lim) for large κ's as follows:

$$\textbf{dyn.rsq: } R^2 \leq 1 - \frac{1 - R.lim) \cdot f.lin^2}{(3 \cdot f.detect)^2 + f.lin^2} = \frac{(3 \cdot f.detect)^2 + R.lim \cdot f.lin^2}{(3 \cdot f.detect)^2 + f.lin^2} \quad (6.2)$$

$R.lim$ is the lowest R^2 value possible for data without measurement uncertainties following the HMR model. This R^2 value is observed for large κ's and depends on the number of measurements in time. It can be shown that with 4 and 5 concentrations measured at equidistant time points the $R.lim$ is 0.6 and 0.5, respectively. To illustrate this: $cor(t = 0, 1, 2, 3, C = 320, 1000, 1000, 1000)^2 = 0.6$. In analogy to the definition of the coefficient of determination (R^2) we use squared ratios between total error and a combination of systematic error and uncertainty. The dyn.rsq formula requires the R^2 for small fluxes to be close to 1 in order to use nonlinear HMR estimates. Large fluxes can have a R^2 as low as $R.lim$ (i.e 0.6 for 4 time points) that the nonlinear estimated can be trusted. The shape between these two extremes is governed by the factor f.detect is multiplied with. Because at the minimum detectable flux the nonlinear estimate should not significantly increase the more pragmatic and stable linear estimate, we only allow for a roughly 5 % increase, hence this is achieved by multiplying f.detect with factor 3. R^2 as decision criteria has a behaviour similar to other parameters and could be translated into a certain κ or a g-factor that is accepted for a specific system. By choosing higher and more pragmatic detection limits, one can decrease uncertainty for a minimal cost in bias.

- **RF2011 (new):** This FCS is proposed by R. Fuss and implemented in the actual gasfluxes function within the package on bitbucket (Fuss, 2015; Leiber-Sauheitl et al., 2014). As explained in the introduction, there are known issues with the actual setup. The p-value is a misleading criteria for small κ's, whereas the AIC is too relaxed for large κ's.

Furthermore, in this kind of simulations (with few large outliers) there is only a very small effect of the robust LR compared to least square regression.

- **Quadratic regression (QR):** The nonlinear estimate is calculated from the following quadratic regression (QR); $C(t) = at^2 + bt + c$, where b represents the flux at time of chamber deployment (Wagner et al., 1997). The quadratic function itself has no basis in physical processes like diffusion theory but is often used for nonlinear chamber estimates (Venterea, 2010). However, although the bias in our simulation framework is larger for QR than HMR, the uncertainty is much lower.

- **QR.plus:** In this simulation framework a specific flux selection scheme is used which follows a decision tree to select a linear or nonlinear flux. The FSC was developed and used by Verhoeven and Six (2014) and Angst et al. (2014). Linear or nonlinear fluxes are selected based on a set of criteria that incorporates concentration changes, shape of curve and equipment precision. In brief;

  a) When the total concentration change over the time course was less than 1 SD of the GC, the flux was

  determined to be below the detection limit and set to a value of zero.

  b) When the R^2 of the LR was greater than 0.95, a linear flux calculation was chosen.

  c) When the R^2 of the LR was between 0.8 and 0.95 the QR was chosen.

  d) All fluxes with an R^2 of the LR less than 0.8 were considered too noisy and were tossed.

  e) Concentrations that were below the initial sample were tossed.

6.2.3 Quality measures

Bias/Accuracy: The bias from the perfect HMR pre-given fluxes is calculated for each modeled datapoint, for the different decision rules and for a range of measurement precision.

$$\textbf{FCS Bias Index} = \sum_{f_0 vs.\kappa}^{sims} (1 - \frac{median(flux_{simulated})}{flux_{predefined}}) \cdot \#sims^{-1} \qquad (6.3)$$

Equation 6.3 describes how the bias is calculated as the deviation from the predefined nonlinear flux to the actual value of the FCS according its decision rule. To compare the different schemes, the mean of all simulated grid points is calculated. A zero bias factor in this simulation environment is neither reachable nor desired, because for large κ's the nonlinear flux estimate is uncertain, unstable and the difference to linear flux estimation is large (Venterea et al., 2009).

Uncertainty/Precision: The measure for uncertainty of the setup is the average interquartile range (IQR) between 5 and 95 % of all the modeled grid points. It is given as absolute flux number in $ppb\,m^{-2}\,h^{-1}$ to show a quantitative effect on a potential flux dataset:

$$\textbf{Uncertainty Index} = \sum_{f_0 vs.\kappa}^{sims} mean(90\%IQR) \cdot \#sims^{-1} \qquad (6.4)$$

The uncertainty measure can be applied to a certain flux dataset with respect to the simulated standard deviation (SD). The fluxes from the dataset are categorized into to the simulated

framework. Fluxes larger than the maximum modelled flux are appointed to the largest flux category of the simulation framework (i.e. 100 $ppb\,m^{-2}\,h^{-1}$). From the combination of both datasets the relative standard deviation (RSD) is calculated for a specific FCS as follows:

$$\text{FCS RSD [\%]} = \frac{\sqrt{\sum_{f_0 vs.\kappa}^{sims}(fluxes \cdot SD^2_{simulated})}}{\sqrt{n_{fluxes}} \cdot mean(flux_{dataset})} \tag{6.5}$$

The performance of different FCS can be screened with respect to bias and uncertainty (see case studies).

6.2.4   Goals for optimized FCS decision rules

From visualising the behaviour of a certain FCS decision rule for a specific chamber measurement system and its uncertainties, the appropriate procedure be identified. An optimized scheme should fulfil the following goals:

1. Small uncertainty considering the 90 % confidence interval (CI) and standard error (if compared to a FCS that toss fluxes). In the first step the uncertainty from monte carlo simulations is considered within the model framework and in a second step these uncertainties can be applied to the frequency of a real flux dataset on the model frame.
2. The bias from the theoretically given nonlinear HMR shaped fluxes should be minimized but balanced to the uncertainty estimate. (more details about goal 1 and 2 see Venterea et al. (2009)).
3. An optimal decision scheme should be as simple as possible, i.e. use as less parameters for decision making as possible (pruning decision trees).
4. Arbitrary thresholds should be avoided (i.e. g-factor < 4, $R^2 > 0.8$, $\kappa < 1$).
5. The desired FCS should provide a smooth transition from large κ (poorly defined non-linearity => LR is prefered) to medium κ (large increase in HMR to linear flux estimate, large uncertainty of nonlinear estimate) up to small κ where a safe use of HMR can be assured. The threshold between the different regimes should be smooth as there is an uncertainty in any threshold value (κ, R^2, detection limit, g-factor etc.) especially when there are real measured fluxes close to the threshold.
6. The threshold parameters should be based on statistical principles or physical theories. If possible the parameter should have a meaningful unit and value (i.e. minimal detectable flux or CV of a GC).
7. The detection limit should be low (can be retrieved from the model simulation).
8. Pragmatic and simple to use (no additional measurements of temperature, bulk density or water content of the soil needed, no expert knowledge about how to set the thresholds and which method to chose necessary).

6.2.5   Model application on a real flux dataset

We present one case study in the results section as example for the application of our tool. Two other case studies are shown in the supplementary material. The aim is to choose the appropriate FCS decision rules depending on the properties of the measurement system (i.e. chamber size, sampling interval, measurement precision etc.) After visualising the performance of the flux calculation method for the system, the user can also check in which

region of the f0-κ space the data is gathered. If there are many measurements with fluxes of large uncertainty (high uncertainty index i.e. medium κ-values and large flux size) one might choose more restrictive decision criterion for HMR fluxes (i.e. higher detection limit for the dynamic R2 method) or change to more robust estimates, like QR or always LR.

With the application of the modelled properties of a specific FCS to a real flux dataset, we calculate the uncertainty and bias of the dataset. Both bias and confidence intervals of the mean flux will be presented as example of the systems performance. However, for practical application (i.e. with publishing the dataset) these quality indices should refer to the mean cumulative flux per treatment that are discussed in the publication. The errors of the mean values have the same statistical properties and can be used to compare different methods.

6.3 Results - Case study 1

6.3.1 System specifications of a manual chamber measurement system

Chamber size, measurement intervals, user input:
Chamber volume (V): 0.0085 m^3
Chamber area (A): 0.0707 m^2
Sampling time (min): 0, 12, 24, 36 min
Precision of the measurement device (GC.sd): 3 ppb SD (taking into account the handling of the vials, assumed to be constant for the usual concentration range)

Model input and assumptions
Atmospheric N_2O concentration (c0): 325 ppb
Maximum simulated flux (fmax): 100 $ppb\,m^{-2}\,h^{-1}$
Increments of simulated fluxes (f0): 4 $ppb\,m^{-2}\,h^{-1}$
Number of simulated fluxes (length(f0)): 26
Sequence of simulated κ (kappa): 0.0001 to 95 h^{-1} logarithmic scale with length 25
Number of monte carlo simulations (carlo): 100

The chambers design of case study 1 is described in Flessa et al. (1995) in detail. They consist of a base ring that is permanently installed in the field and an opaque chamber with 30 cm diameter and 12 cm height. A stainless steel vent is installed for pressure equilibration. Additional rings can be installed between base ring and chamber to account for the actual plant height. In this case, a fan was installed to assure gas mixing in the larger chamber volume. Further details about the field experiment and sampling method can be found in Krauss et al. (2016).

6.3.2 Simulated model space

Figure 6.3 shows the decision behaviour of the most recommended method "dyn.rsq" with blue surface as median, green 5 % and red 95 % confidence interval. Up to rather large κ (~20 h^{-1}) nonlinear fluxes are accepted and estimated with small uncertainty. The jump from linear to nonlinear estimates can cause a large increase in flux estimates. This can create large uncertainty to the flux dataset as the estimated κ is uncertain in itself. Note, that f0=0 indicates the detection limit.
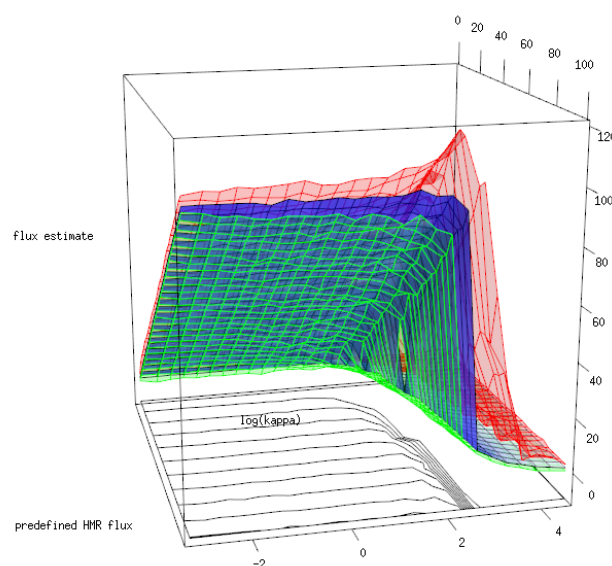
Figure 6.3 – Visualisation of dyn.rsq decision rule results, blue surface is the median, red the 95%confidence interval (CI) and green the 5%CI from the simulations, GC precision 3 ppb SD, $N = 100$

In figure 6.4 the median flux estimate of different decision rules is compared. Here we see how RF2011 drops back to linear for small κ's and creates a larger bias. For small κ's, toward the perfectly linear fit, a nonlinear estimate can be used without adding to uncertainty.

6.3.3 Decision rule performance in the model space

Figure 6.5 visualizes the trade off between uncertainty and bias. The decision rules have a different emphasis on bias or uncertainty. The sensitivity to measurement precision is shown as a line with increasing SD of the GC. The choice of method might not only depend on which methods performs best, but also on the system's specific precision. Furthermore, depending on the experiment (treatment effects or absolute emissions) the user has different requirements in terms of bias and uncertainty. However, the dynamic R^2 (dyn.rsq) method is well balanced between the different methods and it uses most available data about the measurement system and flux strength. If the dyn.rsq method is used with a more restrictive minimal detection limit, the uncertainty can further be reduced with a minimal increase of bias.

6.3.4 Flux detection limits

The simulation provides an estimate for the minimal detectable flux. From the first given flux size of zero to the HMR equation, the measurement uncertainty is introduced in the monte carlo simulation, hence a 95 % confidence interval for all κ is a robust measure for the minimal detectable flux (Tab. CS1). Several decision rules are close to the always linear decision, because they successfully identify the small fluxes populated around zero to be calculated linearly only. Problematic with respect to high uncertainty for small fluxes are the
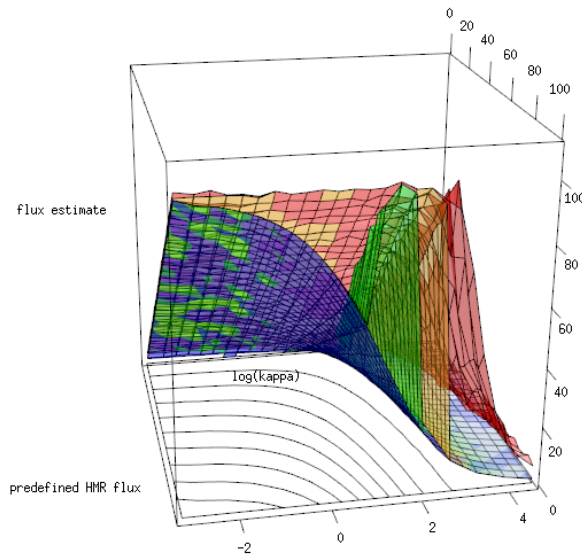
Figure 6.4 – Visualisation of different methods using HMR simulated concentrations ($t = 4$), for different κ (0-95 h^{-1}) and flux size (0-100 $ppb\,m^{-2}\,h^{-1}$); linear = blue, red = HMR, green = RF2011, orange = dyn.rsq, noise on simulated measurements = 3 ppb SD, $N = 100$

Table 6.1 – Detection limit: Calculated from the 95 % confidence interval in $ppb\,m^{-2}\,h^{-1}$ from n=2500.

linear.f0	HMR.f0	f.detect	AIC.dec	g-factor	QR plus	dyn.rsq	RF2011
1.29	18.99	1.29	1.39	1.62	15.32	1.46	1.28

methods HMR.f0 and QR.plus. The additional rules introduced to the QR scheme, like tossing out concentrations, are responsible for larger minimal detection limits.

6.3.5 Model application to the actual flux dataset

After exploring the model space with a possible range of κ's and flux sizes, we apply the scheme to an actual dataset of a two years field measurement campaign (Krauss et al., 2016).

Figure 6.6 visualises the fluxes from the dataset as histogram in the model space (shown in fig. 6.3 and 6.4). Bar height indicates the number of fluxes that were measured within the range of flux sizes and κ's determined by the model space. The truncated bar goes up to roughly 870 counts and represent small fluxes with undefined κ in the first row with the smallest κ. Counts that exceeded the model space where set to the maximum value available on the simulated framework. If κ could not be fitted, the linear regression is used hence these cases are shown with the smallest kappa value (around zero). The numerous fluxes where κ could not be fitted are found on the far left row in the back of fig. 6.6. With the colour code the uncertainty of the decision model "dyn.rsq" is applied to the frequency bars of the dataset. The uncertainty associated with these fluxes is small, because they are calculated with linear regression and are from small flux size anyways. Most fluxes are found in areas with rather small uncertainty
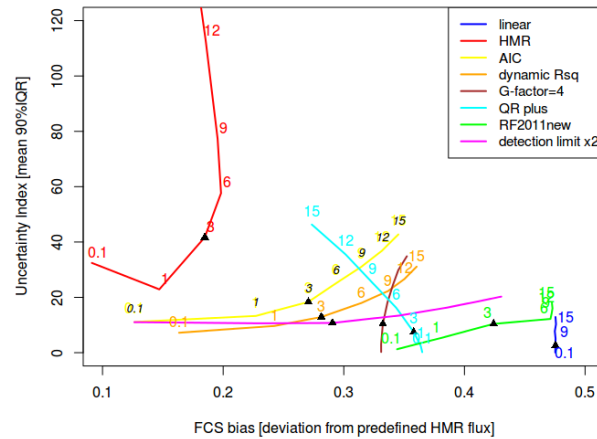
Figure 6.5 – Method performance in terms of bias (x-axis, the larger the value the larger the bias) and uncertainty (y-axis, index calculated from the mean 90 % interquartile range), depending on measurement precision (SD of the GC indicated by the coloured numbers). The triangles show the given GC precision (3 ppb SD) of the CS1 system.

Table 6.2 – FCS decision rule performance applied to the total dataset. Deviation from perfect HMR estimate (FSC Bias Index) and relative standard deviation (RSD) in percent the mean flux for all methods applied to the CS1.

	linear	HMR.f0	f.det	AIC.dec	g-factor	qr.plus	dyn.rsq	RF2011
Bias Index	0.1532	-0.0393	0.0407	0.0053	0.0151	0.0716	0.0058	0.1364
RSD [%]	1.69	236.96	14.51	50.49	19.69	8.18	6.93	7.61

(note the logarithmic scale of the colour code, indicating the blueish tones are small). However there is a number of fluxes that are in a slightly critical region (i.e. greenish colours, especially the large fluxes). These fluxes need special attention and maybe screened manually using a filter the fig. 6.6 may suggests.

Finally, we can apply the bias and uncertainty indicators to the dataset (also see Tab CS1.2 for the results of the other methods):

- FCS Bias Index: $1 - \frac{sum(predefined\ fluxes)}{sum(FSC\ estimated\ fluxes)} = 1 - 0.9948 = 0.0052$ By following the dyn.rsq decision rule, the total dataset reaches 99.48 % of the initial prescribed/modelled HMR model flux. In other words, with the recommended decision rule (dyn.rsq), 99.42 % of the total flux of the dataset is in agreement with the ideal HMR flux model calculation.
- The deviation factor from always linear estimate is: 1.174. Hence the datasets flux size increases in total by 17.4 % with the recommended method (dyn.rsq) from accounting for nonlinearity in the chamber gas concentration.
- FSCs RSD is 6.93 % of the datasets mean flux of 47.4 $ppb\,m^{-2}\,h^{-1}$. This indicates an error of roughly 7 % due to the uncertainty from the nonlinear flux calculation. If treatment means are similar than the overall mean of the dataset, this error can also be assumed for each cumulative treatment flux.

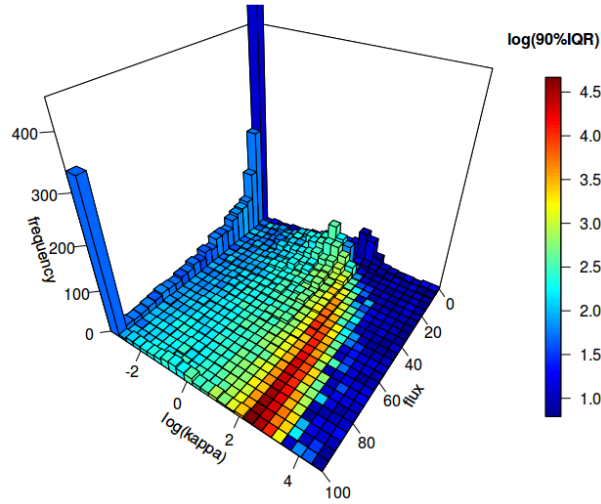For further case studies, see supplemental material.

Figure 6.6 – Histogram of the CS1 flux dataset for the modeled $\kappa/f0$ space with shading of the log(90%Inter Quantile Range) at each dyn.rsq model datapoint. Truncated bar goes up to 872 counts. Fluxes larger than the simulation (>100 $ppb\,m^{-2}\,h^{-1}$) are summed up in the largest flux ($fmax = 100$) columns on the left-front row.

6.4 Discussion

6.4.1 Recommendations for CS1

The system has a high performance in terms of its small bias and low uncertainty. Firstly the uncertainty is low because of the high precision of the GC that corresponds well to the sampling interval during chamber closure (the GC actually was down to 1 ppb SD at atmospheric concentrations). Secondly the flux estimation scheme (linear/HMR and decision criteria dyn.rsq) does efficiently balance bias and uncertainty (see Tab CS1.2). Thirdly, most fluxes are located on less problematic areas within the model space of both low impact on bias and uncertainty. Further improvements can be achieved by further restricting nonlinear estimates for large κ's and consequently applying the dyn.rsq decision criterion or similar methods.

From our simulations and the case study, the goals for an optimal FCS introduced in the method section can be discussed:

Uncertainty

The smallest uncertainty is always achieved by the purely linear flux estimate. The use of HMR needs strict restrictions to prevent unstable estimates that introduce a large uncertainty to the dataset. Using a dynamic R^2 threshold always has a better performance than AIC for any precision (fig. CS1.3). AIC seems to be a too relaxed criteria for the decision between linear and HMR and it allows for too high an uncertainty with high κ's being accepted as nonlinear estimate. The g-factor (<4) has a very small uncertainty for high precisions but is getting worse than dyn.rsq with lower precisions (>9 ppb). The reduction in uncertainty is provided at a comparable low cost of bias with the proposed dynamic R^2 scheme. Uncertainty-wise, the RF2011 scheme performs also well, but for small κ's it switches to LR although HMR would work for these conditions. Especially the criteria that p-values need to be smaller favours the

decision for 'linear' for small κ's, which is not desired. This effect is similar to the QR.plus method that does only allow nonlinear estimates for $R^2 < 0.95$. QR in general had an apparently good performance in terms of uncertainty in systems with high precision, but it gets weaker toward higher variability in the input data.

Bias

The HMR only method creates the smallest bias, however at the cost of a dramatically increasing uncertainty. In contrast, the purely linear scheme has a large bias that is stable over the tested range of precision. In our simulation framework a zero bias cannot be achieved and is also not recommended, as the HMR collapses anyway at a certain point, depending on the precision. According to how the FSC bias is estimated here, the decision rules perform in the following order: HMR>>AIC>dyn.rsq>f.detect>g-factor>QR.plus>RF2011>linear. For f.detect and QR.plus this also depends on the precision, whereas f.detect performs better with high precisions and QR.plus is strangely worse with higher precisions. The latter is probably an artefact of the additionally applied rules (i.e. nonlinear only for $R^2>0.95$).

Simplicity of the scheme

In terms of simplicity the ranking of the screened methods can be evaluated as follows: LR > HMR > AIC > f.detect > dyn.rsq > RF2011 > QR.plus (> CBC, see Venterea (2010)). The less additional information needed to use the FCS the more generally it can be applied. However it makes sense to use at least the measurement precision as additional input and balance with this information the performance in terms of bias and uncertainty automatically (like dyn.rsq). A system with an unknown measurement precision cannot handle the optimal choice between linear and nonlinear schemes. In contrast the CBC (Venterea, 2010) would additionally need measurements of the soil water content and bulk density from the field.

Arbitrary thresholds

Few schemes with arbitrary thresholds were chosen in this study (i.e. g-factor = 4). But many users come up with certain thresholds for R^2 or other parameters from their experience (i.e. R^2 = 0.8). The detection limit itself is not very straightforward to calculate and gets also arbitrary when a multiple of it is used. Also statistical performance indicators like AIC, p-value or SE are rather arbitrary as it is not understood why their values should decide between linear and nonlinear calculation. RF2011 uses several arbitrary thresholds like, p-value, g-factor = 4 and AIC together.

Smooth transition between nonlinear and linear

Linear and QR performs well with respect to a smooth transition from nonlinear to linear estimates. Most other scheme suddenly drop from HMR to linear. If a given dataset tends to realise fluxes within the range of this sudden drop, the detection limit should be increased or QR or LR scheme should be applied. The uncertainty in the estimate of κ introduces otherwise a variations of several 100 percents of the flux estimate.

Low detection limit

The detection limit is related to the uncertainty for low fluxes. The most stable approach is just using LR. The schemes (i.e. f.detect, AIC, dyn.rsq, RF2011) that recognise such low fluxes and calculates them with LR perform well. In spite of its simplicity QR deals well with small fluxes but the additional rules (QR.plus) introduced undermine this reliability.

Basis on statistical principles and physical theories

Applying LR to the chamber flux is appropriate to know the average flux over the deployment time. However LR reflects the effect of the chamber itself (i.e. reduced emissions due to reduced concentration gradient) and hence cannot be interpreted as the actual emission. Rather it is a very robust approach that gives reliable results to compare treatments. But if the actual emission should be measured without the artifact introduced by the chamber itself, nonlinear flux estimates are to be preferred. This is the purpose of the HMR scheme, being derived by diffusion equations with certain assumption to solve numerically. In contrast, the QR is also used to describe emissions without the effect of the chamber itselfs but it is not based on physical principles and just an empirical nonlinear solution. Although based on diffusion theory, HMR is not a perfect model. Venterea (2013) points out the errors of nonlinear FCSs towards the theoretical curvature. Compared to the other schemes tested, HMR has an average performance and generally underestimates the theoretically modeled flux. However, in practical situations we do not think that the deviation from theoretical diffusion models introduces the most significant uncertainty and bias to the flux estimates. Rather the largest problems for the flux estimates are measurement precision, vial handling and most important probably sudden random (i.e. non steady state) fluctuation in the chamber concentration from wind gusts. Such features will dominate the concentration value much stronger than theoretical deviations of the HMR model. The theoretical diffusion models themselves are just approximations and cannot describe the dynamics below the chambers in detail. Further, the soil below individual chambers differ in its diffusion properties that cannot be estimated by water content or structural information (i.e. cracks and bioturbation). Using the HMR procedure allows the numerics to fit the κ parameter to match all these unknown effects best to the measurements. The challenge is to find the limits to the underlying assumptions and consequently use LR for those cases. This is exactly what the dynamic R^2 wants to provide in an automated routine.

At the moment there is no single best FCS decision rule. However through appropriate visualisation it becomes clear, which decision tools is the most appropriate for a given dataset. The best balance between uncertainty and bias is achieved by combining the detection limit with the actual flux estimate, hence the dynamic R^2 (dyn.rsq) method. By tuning the detection limit (choose a more or less stringent method) one can also influence the balance between uncertainty and bias. Choosing a higher and more cautious detection limit, one can reduce uncertainties in certain areas of the model frame, and thereby increase bias.

6.5 Conclusion

- Large uncertainties for static chamber N_2O measurements can be introduced by the FCS. The situation of each specific measurement system should be analysed to choose

the best combination of LR (enhancing bias/reduce uncertainty) and HMR (enhancing uncertainty/reduce bias).

- We recommend the dynamic R^2 threshold to optimally balance bias and uncertainty with respect to measurement precision and chamber setup. We show the practical performance of this FSC decision rule with example case studies. It can be applied without expert tuning or additional data input.
- For treatment comparisons, one could increase the estimated flux detection limit, choose the QR for nonlinear fits or always treat the fluxes with LR.
- Visualizing the parameter space of the specific measurement system helps to choose the appropriate decision rule to balance bias and uncertainty. The understanding of the FCS helps to interpret data with respect to bias and uncertainty.
- Quality indicators of bias and uncertainty from the FCS applied to a certain dataset should be delivered with the publication of a dataset.

6.6 Supplementary material

Case study 2: Manual chamber measurement system (low precision, GC, t=4)

System specifications (user input)
Chamber volume (V): 0.004 m^3
Chamber area (A): 0.0317 m^2
Sampling time (min): 0, 12, 24, 36 min
Precision of the measurement device (GC.sd): 14 ppb SD (taking into account the handling of the vials, assumed to be constant for the usual concentration range)

Model input and assumptions
Atmospheric N_2O concentration (c0): 325 ppb
Maximum simulated flux (fmax): 100 $ppb\,m^{-2}\,h^{-1}$
Increments of simulated fluxes (f0): 4 $ppb\,m^{-2}\,h^{-1}$
Number of simulated fluxes (length(f0)): 26
Sequence of simulated κ (kappa): 0.0001 to 95 h^{-1} logarithmic scale with length 25
Number of monte carlo simulations (carlo): 100
For the description of the decision rules used, see method section above.

Results and recommendations for the CS2 system

- Do not toss fluxes automatically
- Do not use zero flux if the concentration range is small (apparent detection limit)
- Do not omit $t(1)$ vial concentrations when it is higher than $t(2)$
- Use nonlinear estimate for high R^2 up to the dynamic threshold depending on the flux detection limit and actual linear flux estimate.
- Use HMR for nonlinear estimates instead of quadratic because it is more based on diffusion theory and cannot have the wrong curvature (negatively bent). For the rather low measurement precision still QR might be appropriate.
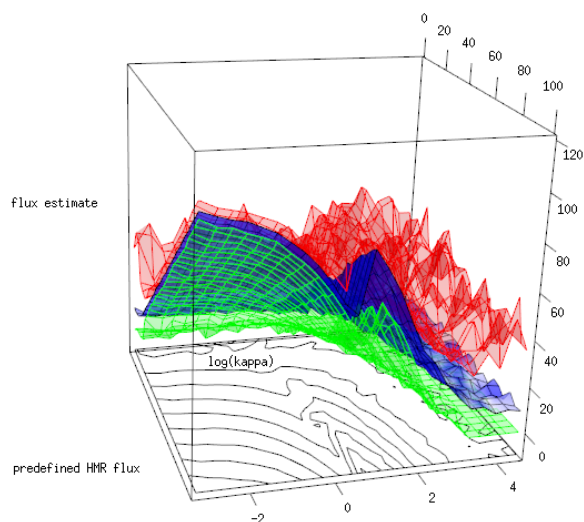
Figure 6.7 – Visualisation of QR.plus decision rule results, blue surface is the median, red the 95%confidence interval (CI) and green the 5 % CI from the simulations, GC precision 14 ppb SD, N=100, the confidence intervals are huge for small fluxes and medium range kappas. Uncertainty for large κ is comparable low, but the bias is large bias. Standard error of the simulation is very high because of flux tossing and sample outtakes.
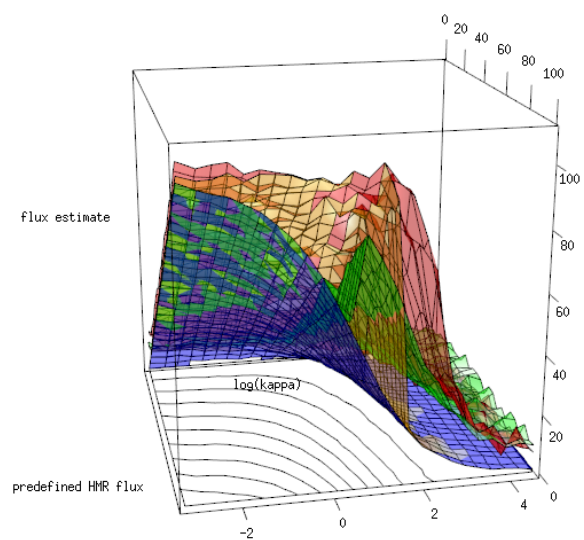

Figure 6.8 – Visualisation of different methods of CS2; linear = blue, red = HMR, green = QR.plus, orange = dyn.rsq, noise on simulated measurements = 14 ppb SD, N=100
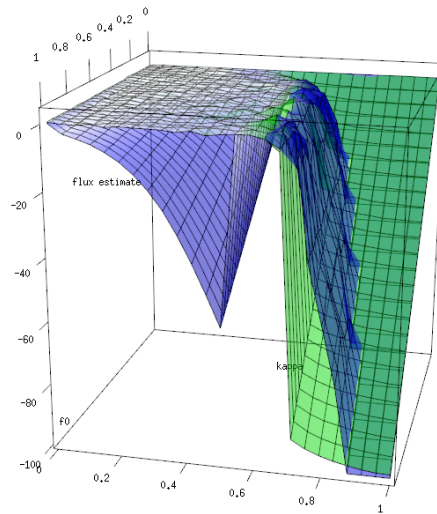
Figure 6.9 – The bias with respect to HMR fluxes comparing QR.plus (blue) with dyn.rsq (green) is shown. There is a large bias for small κ range because of linear use for $R^2 > 0.9$. The bias is smaller in medium κ range compared to dyn.rsq but the resulting uncertainty is large. There is a slightly larger bias for large κ because of zero flux decision instead of convervative linear estimates.

Case study 3: Automatic chamber system (t=12)

System specifications (user input)
Chamber volume (V): 0.0216 m^3
Chamber area (A): 0.09 m^2
Sampling time (min): 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 min
Precision of the measurement device (infrared filter detector): 2.5 ppb SD

Model input and assumptions
Atmospheric N$_2$O concentration (c0): 325 ppb
Maximum simulated flux (fmax): 100 $ppb\,m^{-2}\,h^{-1}$
Increments of simulated fluxes (f0): 4 $ppb\,m^{-2}\,h^{-1}$
Number of simulated fluxes (length(f0)): 26
Sequence of simulated κ (kappa): 0.0001 to 95 h^{-1} logarithmic scale with length 25
Number of monte carlo simulations (carlo): 100

This system uses automatic chambers with a continuous measurement device (infrared filter

Table 6.3 – Detection limit: Calculated from the 95 % confidence interval in $ppb\,m^{-2}\,h^{-1}$ from n=2500.

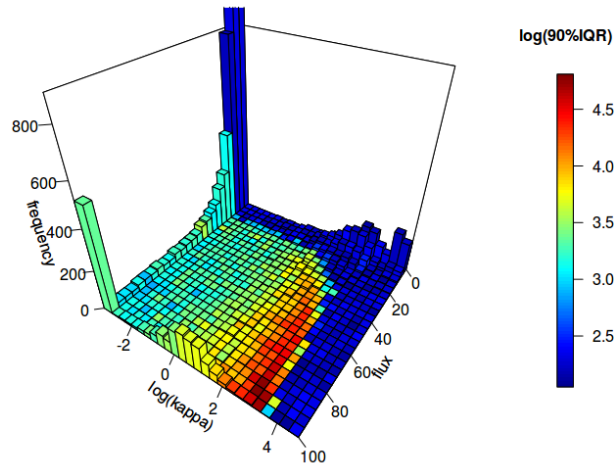| linear.f0	HMR.f0	f.detect	AIC.dec	g-factor	QR plus	dyn.rsq	RF2011
3.83	55.23	3.83	5.68	5.00	45.76	4.62	3.88

Figure 6.10 – Histogram of the CS3 flux dataset for the modeled $\kappa/f0$ space with shading of the log(90 % Inter Quantile Range) at each dyn.rsq model datapoint. Truncated bars in the back go up to 1935 and 3384 counts. Fluxes larger than the simulation (>100 $ppb\,m^{-2}\,h^{-1}$) are summed up in the largest flux ($fmax = 100$) columns on the left-front row.

Table 6.4 – FCS decision rule performance applied to the total dataset. Deviation from perfect HMR estimate (FSC Bias Index) and relative standard deviation (RSD) in percent the mean flux for all methods applied to the CS1.

	linear	HMR.f0	f.det	AIC.dec	g-factor	qr.plus	dyn.rsq	RF2011
Bias Index	0.138	-0.295	0.138	0.069	0.0151	0.034	0.015	0.135
RSD [%]	8.32	11298.56	8.32	3562.82	414.94	303.88	8.60	

technique). The dataset is from a field experiment with biochar, control a lime treatment (Hüppi et al., 2015). The published data was evaluated with the RF2011 method. For the description of the decision rules used in this analysis, see the method section above.

The systems from CS3 achieves the following performance when dyn.rsq is applied:

- FCS Bias Index: $1 - 0.9665 = 0.0335$ By following the dyn.rsq decision rule, the total dataset reaches 96.65 % of the initial prescribed/modelled HMR model flux.
- The deviation factor from always linear estimate is: 1.121. Hence the datasets flux size increases in total by 12.1 % with the recommended method (dyn.rsq) from accounting for nonlinearity.
- FSCs RSD is 303 % of the datasets mean flux of 37.6 $ppb\,m^{-2}\,h^{-1}$. This number suffers from a rather low resolution of the simulation framework towards very small κ. The lowest values should coincide with a linear estimate, hence the uncertainty of the left most row in the back should be very low.

Recommendations for the CS3 chamber system

- The system has an apparently high cost of additional uncertainty for reducing the bias

towards the HMR initialisation.
- Although the number of concentration measurements per flux is large (11) the FCSs are rather unstable due to the high uncertainty to time ratio of the measurement.
- Critical fluxes should be screened manually, where the uncertainty is large and flux size is high (fig. CS3.2 in front)
- The measurement precision needs to be determined more exactly.
- Detection limit could be interpreted more restrictive.

The presented tool can be used for concentration data points per chamber of any number.

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