University of Zurich[UZH]

Impact of elevated atmospheric CO2 and nitrogen fertilisation on lipid composition in the Braunschweig FACE experiment

GEO 511 Master's Thesis

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Abstract

Future increase in atmospheric carbon dioxide (CO_2) and different amounts of nitrogen (N) fertilisations have been simulated by a various of experiments. One of them was the free-air carbon dioxide enrichment (FACE) experiment conducted in Braunschweig in an arable crop rotation. Elevated CO_2 concentration have a direct effect on plant waxes and therefore on lipids which are a major component of these waxes. In this thesis the focus lies on the effects of elevated CO2 concentration and reduced N fertilisation on the molecular and isotopic ($\delta^{13}C$) composition of the lipid classes *n*-alkanes and *n*-carboxylic acids in crops and soils. It will be shown the changes of lipid composition under modified CO_2 concentrations and reduced N fertilisation. Had influences on lipid composition. However, the direction of the effect was different for the distinctive crops. In the soils there was no clear trend detectable. For the isotopic values of the bulk C it could be proofed that the labelling of the crops and soils cultivated under elevated CO2 conditions (which were fumigated with air depleted in $\delta^{13}C$) worked. Also, the reduced N fertilisation had a visible influence on the $\delta^{13}C$ -values of the bulk C. Compared to these results, the isotopic values of n-alkanes and n-fatty acids were depleted in $\delta^{13}C$ .The weighted mean average isotopic values of the most abundant lipids revealed that also an effect of N fertilisation on the $\delta^{13}C$ values of the lipids existed. Therefore, future atmospheric concentration might alter the activity of photosynthesis and the biosynthesis of lipids.

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Abbreviations

ACL	average chain length
C	carbon
CO_2	carbondioxid
FACE	free air carbon dioxide enrichement
GC	gas chromatography
GC-irMS	gas chromatography ion ratio mass spectrometry
N	nitrogen
ppm	parts per million
OC	organic carbon
SOC	soil organic carbon
SOM	soil organic matter
CPI	carbon preference index
FID	flame ionisation detector

1. Introduction

1.1. Influence of CO_2-emissions on carbon cycling

Through the anthropogenic CO_2-emissions, humans have perturbated global biogeochemical cycles and the climate system. Since the Industrial Revolution, the CO_2 concentration has increased by almost 40%, and it is very likely that the atmospheric CO_2 concentration reach 550 ppm by the middle of the century and will further rise to about 794 to 1142 ppm till the end of the century (IPCC, 2013). The Global Monitoring Laboratory argue that – even in this year, where a reduction of 8% in CO_2-emissions is expected because of lockdowns due to the virus Sars-Cov-2 – the CO_2 will increase in a similar rate (NOAA, 2020). A part of the surplus of CO_2 gets partly incorporated into land ecosystems and oceans (IPCC, 2013). As soils store at least three times as much carbon in soil organic matter (SOM) as is found in either the atmosphere or in living plants (IPCC, 2013) they play a key role in the storage of organic carbon (OC) in terrestrial ecosystems (Stockmann et al., 2013). However, it is still unclear how the plant-soil system reacts to higher CO_2 concentration as the stabilisation and destabilisation of organic matter in soils is still under debate (Schmidt et al., 2011). Soils, can act as a potential sink for atmospheric CO_2 through the conversion of plant biomass into SOM. Therefore, studies try to figure out, if the soils act as a source or sink for CO_2 and which processes are responsible for an incorporation and stabilisation of plant-derived organic matter (Kögel-Knabner, 2002). That major pool of OC is sensitive not only to the local environment but also to changes in climate. However, the feedbacks between soil organic carbon (SOC) and climate are not fully understood (Schmidt et al., 2011).

An important factor on climate change are agricultural systems, as they account for 13.5% of the greenhouse gas emissions (not only CO_2 but also N_2O and CH_4). However, these systems are not only a factor causing climate change, but they get as well affected by global warming and higher CO_2 concentration (Follett et al., 2011). To be able to determine how plants and soils react to this new CO_2 concentration, many experiments and studies have been conducted in controlled environments or enclosures (Ainsworth & Long, 2005). It could be shown that plants which grow under elevated CO_2 conditions reduce their stomatal conductance and transpiration. Therefore, the water-use efficiency can be enhanced. Plants also exhibit higher rates of photosynthesis, have higher C content, an increased C/N ratio and increased light-use efficiency (Drake et al., 1997) which results in higher yield (Follett et al., 2011). However, the beneficial effect is questionable if the plant stoichiometry gets changed (Loladze, 2002). When soil is poor in N it is possible that the elevated CO_2 has a lower effect on plant growth, as C_3 photosynthesis gets acclimated (Ainsworth and Long, 2005). If CO_2 is elevated increase in C/N ratios in crop residues could be observed (Loladze, 2002). The shoot root ratio decreases under low N concentrations (Gastal & Lemaire, 2002) and the roots might extend to deeper layers of the soil (Svoboda & Haberle, 2006). Also, the N concentration in vegetative plant parts, in seed and grains decreased (Fangmeier et al., 1999). Therefore, the protein concentration in grains is lower (Taub et al., 2008). Which has a negative impact on the nutritional value (Feil, 1997) and functional properties of flour and therefore affects bread baking quality (Williams et al., 1994). It could be shown that through fertilisation of N the decrease in plant N and protein concentrations could only be partially counteracted (Fangmeier et al., 1999). Furthermore, a soil limited in N reduces the possibility of C sequestration (Ainsworth & Long 2005). Fertilisation with N can increase SOC content, but as N fertiliser production is high in CO_2 emissions and fertilisation emits the greenhouse gas N_2O, a possible increase in SOC might be nullified (Follett et al., 2011)

1.2. Free air carbon dioxide enrichment (FACE) experiments

Most of the knowledge about influences of an enriched CO_2 concentration on plants and soils origin from experiments conducted under controlled environment. One of the main drawbacks of these experiments is the limitation in size and the possible creation of a "chamber effect", which results in a greater impact of the chamber than the elevated CO_2 (Ainsworth & Long, 2005). To test if the findings also apply under field conditions free air carbon dioxide enrichment (FACE) techniques were developed in the late 1980's (Hendrey et al., 1993), where plots got artificially enriched in CO_2, while the other conditions were kept natural (Ainsworth & Long, 2005).

1.2.1. FACE experiment in Braunschweig

In 1999 the Thüen-Institute started the FACE experiment in Braunschweig. It is the only one of these experiments, which was conducted on an arable crop rotation system in Europe (Weigel et al., 2006). The local farming practice, which includes annual rotation of cultivated crop species and the turnover of the soil about two times per year due to ploughing, leads to a continuous mixing of "old" soil C and recently translocated C compounds in the soil. To assess the influence of N fertilisation they manured the sample plots with two different amounts of N (Giesemann, 2005).

Many different studies have been conducted in Braunschweig. They showed that a change in decomposer could be detected. There was a higher abundance and diversity of collembolan under elevated CO_2 than under ambient air when nutrients were available (Sticht et al., 2006) also microbial community structures changed which affected fungivorous and bacterivorous nematodes (Sticht et al., 2009). These changes in soil-fauna probably can lead to changes in decomposition processes, soil C-cycle and therefore, have an impact on soil fertility (Sticht et al., 2008). Photosynthesis of crops got stimulated and soil moisture increased. However, yield of crops was lower in the FACE-experiment as when tested in chambers (Weigel et al., 2006). Fine root biomass increased by about 35% in the elevated CO_2 rings (Søe et al., 2004). Pacholski et al. (2015) found positive effects in roots of wheat, barley and sugar beet under elevated CO_2 and this effect was not influenced by N supply. C/N ratios of roots was only slightly influenced when the air in the rings was enriched with CO_2. A higher influence on the C/N ratios was due to changes in N fertilisation and dependent on crop species. C from soil respiration originated manly from recent assimilation (70%) and only 30% originated from older SOM. Soil respiration was not influenced by N fertilisation (Søe et al., 2004). Giesemann & Weigel (2008) detected after the second crop rotation an increase of soil C content in the uppermost 10 cm. Although the results were not yet statistically significant, they determined that the new C input under ambient air conditions measured about 4.9% and 10.7% for the elevated plots. It is very likely, that harvest practices as ploughing twice a year might counteract C sequestration in agricultural systems (Søe et al., 2004).

However, the FACE experiment in Braunschweig only tested the effect of different N fertilisation and elevation in atmospheric CO_2. A future climate change would also influence air and soil temperature as well as rain patterns (Pacholski et al., 2015).

1.3. Lipids as molecular proxies

At the FACE-experiment in Braunschweig no studies about the influence of the different treatment on the lipids in the crops and the soils have been conducted. Therefore, the focus of this thesis lies on lipids which will be used as molecular proxies. They are often referred to as biomarkers (Jansen & Wiesenberg, 2017). These proxies are molecules which are present or absent in a studied specimen and therefore they can give hints to a process in a system, its composition or state (Jansen & Wiesenberg, 2017). Proxies are used as indicators in various study fields: from clinical sciences (Philipp et al., 2016) to soil sciences (Jansen & Wiesenberg, 2017). In soil sciences plant wax lipids and lipids from microorganisms can be used as diagnostic markers (Harwood & Russell, 1984). Because even if lipids are deposited for millions of years in sediments, their molecular distributions stay the same as they are highly resistant to microbial and chemical degradation (Huang et al., 1995). They can be used to apportion the source of the SOC and to determine the turnover rate of C in soil. (Hardwood & Russel, 1984; Kögel-Knabner, 2002; Jansen & Wiesenberg, 2017). During their biosynthesis, plant lipid distribution patterns and isotopic signatures are influenced by several environmental factors and also internal growth factors such as exposition, growth or stage of degradation, as well as in which part of the plant they were integrated (Jansen & Wiesenberg, 2017). It has been discussed that changes of CO_2 in the atmosphere also likely influence the lipid biosynthesis and therefore the lipid composition of plants (Ainsworth & Long, 2005). The group of lipids is very heterogenous and it is defined as a group of organic substances that is hydrophobic but can be extracted with non-polar solvents (Dinel et al., 1990). In this thesis n-carboxylic acids and n-alkanes will be analysed.

1.3.1. Carboxylic acids

Normal carboxylic acids (n-carboxylic acids) can be found in plant biopolyester, plant waxes (Kolattukudy & Walton, 1972) and also in microbial products (Otto et al., 2005). They contribute most to plant leaf waxes and are primary plant biosynthetic products. Therefore they are sensitive to changes in changes in atmospheric CO_2 concentration (Wiesenberg et al., 2008a). Short-chain carboxylic acids ($<C_{20}$) are omnipresent in all living organisms (Hardwood & Russell, 1984). Long-chain carboxylic acids in contrast are mainly produced by plants (Hardwood & Russell, 1984). Therefore long-chain carboxylic acids are frequently used as proxies to investigate the source of SOM (Griepentrog et al., 2015). As carboxylic acids produced by plants are an important food source for soil fauna, it is possible, that changes in the composition of carboxylic acids could have an influence on the microbial community structure and therefore on decomposition processes as well as the cycling of SOM (Feng & Simpson, 2011).

1.3.2. Alkanes

Normal alkanes (n-alkanes) are straight-chain hydrocarbons without functional groups. These chains are stable and can survive in fossil records for millennials (Eglington & Eglinton, 2008). N-Alkanes is one of the most common lipid biomarkers as they are ubiquitous in the environment (Ficken et al., 2000). They get produced by terrestrial and aquatic plants (Ficken et al., 2000) mainly through degradation of carboxylic acids and alcohols (Wiesenberg et al., 2004). In soils, the chain length of the n-alkanes can indicate to different sources (Li et al., 2020). Short-chain n-alkanes ($<C_{20}$) primarily origin from bacteria, plankton and algae and have a strong odd-over-even predominance (Eglinton & Eglinton, 2008). They can also be formed by microbial activity and fossil fuel burning, but then there is no strong odd-to-even C number preference (Bouloubassi et al., 2001). Long chain n-alkanes (C_{21}–C_{37}) are part of the epicuticular leaf wax of terrestrial plants (Eglinton et al., 1962) and contribute to the hydrophobic properties of these waxes. The wax layer serves as the first barrier from the external environment and reduce water loss through

evaporation (Jetter et al., 2006). In vascular plant leaf wax are abundant with C_{27}, C_{29}, and C_{31} (Li et al., 2020).

1.4. Stable Isotope analysis

Stable isotope analysis is gaining in relevance (Giesemann, 2005) as it can be used to study the physiological functioning of plants (Huang et al., 1999), ecosystem dynamics (Giesemann, 2005) and paleoclimatic and paleoenvironmental reconstruction (Huang et al., 1999). Through natural abundance level of $\delta^{13}C$ it is possible to calculate C input and C turnover in systems (Giesemann, 2005).

The use of gas chromatography-isotope ratio mass spectrometry (GC-irMS) enables the use of compound specific isotopic analysis (CSIA) of individual lipids (Hayes, 1993). Therefore, differentiation of compound sources is possible: how fast they are incorporated into soil and how long their residence times are (Wiesenberg et al., 2008b). However, it is under debate if isotopic values of the n-alkanes of the leaf wax have the isotopic signal of the water that the plant used during leaf formation or if it changes through the growing season because of environmental conditions (Bush & McInerney, 2013).

1.5. Research questions

One main aim of this master thesis is to obtain an insight in the lipid composition of different plant and soil samples to draw conclusions about the source of the OC and its turnover rate. With this information, estimations can be drawn of how fertilised agricultural soils with C_3 plants could respond to a future elevation of atmospheric CO_2 concentration.

Therefore, the following research questions shall be answered:

1. How do the lipid distribution patterns change with different CO_2 concentration and different N fertilisation?

2. What are the isotopic compositions of the plant and soil samples? Do the $\delta^{13}C$ values differ between different compounds?

3. How do the results fit in with other findings of other FACE studies?

2. Methods

2.1. Sampling

The plant and soil samples origin from a FACE experiment conducted at the Federal Agricultural Research Centre (FAL). The plots were located in Braunschweig, South East Lower Saxony in Germany [10°26'E 52°18'N, 79 m above sea level] (Figure 1). The mean annual temperature is 8.8 °C and the mean annual precipitation is 618 mm/a. The studied field was cultivated with only C_3-plants for more than 30 years before the FACE experiment started (Giesemann & Weigel 2008). The soil in the rings is cambisol/loamy sand (Parabrown Earth) with a pH of 6.3–6.5. The soil organic C and N amount to 0.99 ± 0.07% respectively 0.09 ± 0.01% (Søe et al., 2004). The FACE experiment in Braunschweig started in 1999 and two crop rotations were conducted. The samples for this thesis were taken from the second crop rotation from the years 2002-2005. This rotation consists of the cultivation of winter wheat (*Triticum aestivum* cv. Batis), winter barley (*Hordeum vulgare* cv. Theresa), rye grass (*Lolium multiflorum* cv. Lippstädter Futtertrio) and sugar beet (*Beta vulgaris* subsp. Rapacea (KOCH) DÖLL cv. Wiebke) which is a common combination for this region (Søe et al., 2004). Before every sowing, the soil got ploughed to a depth of 30 cm. During this process plant residues such as root and stubbles entered the soil (Giesemann & Weigel, 2008).

Figure 1. Study area Braunschweig in Germany (google maps, http:// maps.google.com).

For the experiment they installed six rings with a diameter of 20 m on the field which measures 22ha. Four of them have an artificial fumigation system (engineered by Brookhaven National Laboratory [New York, USA] (Giesemann & Weigel, 2008)). Two of them have a computer-controlled system which enriches the air with CO_2 (*Figure 2*). This concentration amounted to 550 ppm during the growing seasons at daylight hours and had a $\delta^{13}C$ value of atmospheric CO_2 of -20.5 to -22‰ As the $\delta^{13}C$ values of tank stock solution ranged between −48‰ and −45‰. Therefore, it can be used as a tracer (Giesemann, 2005). No enrichment criteria were night time, wind speeds above 6.0-6.5 m/s and air temperature lower than 5° C. Two plots had only blowers, which fumigate with ambient air (with a CO_2 concentration of 370 ppm and a $\delta^{13}C$ value of -7.5‰ to -9.8‰) and two rings had only vertical vent pipes without blowers (Figure 3). To prevent a fumigation of the ambient rings, they were located 100 m away from each other (Weigel und Dämmgen, 2000 in Søe et al., 2004).

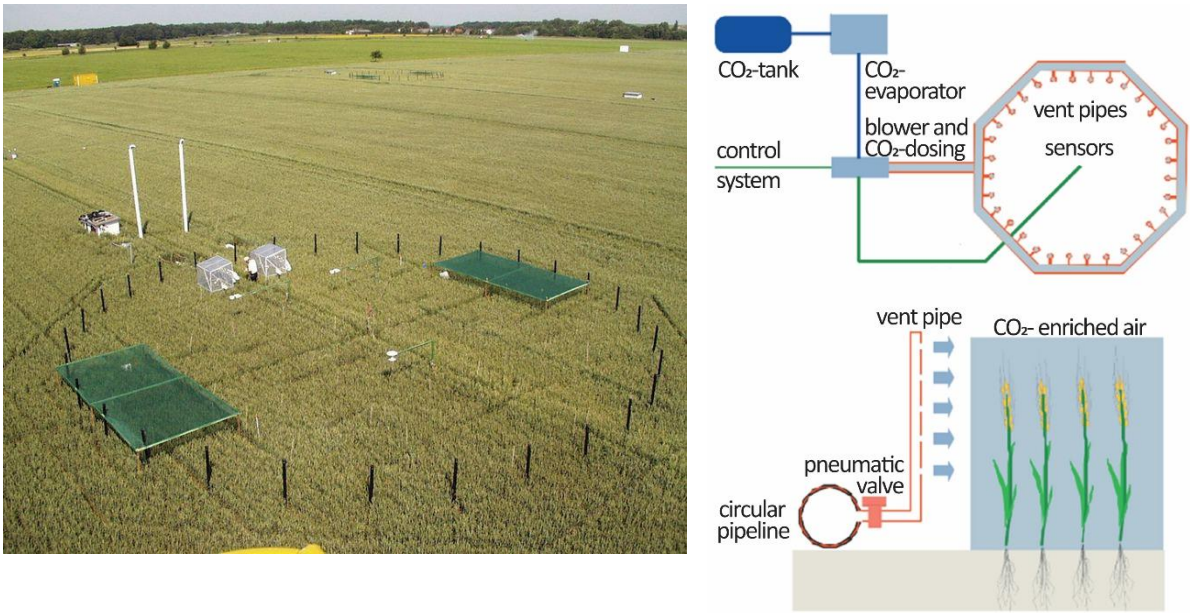

Figure 2. On the left is the top view of a ring installation in the FACE experiment in Braunschweig with winter wheat. (© Thünen-Institut/BD) and on the right is the schematic view of the installation modified according to Thünen-Institut (2016).

For the N fertilisation UAN (urea ammonium nitrate) and CAN (calcareous ammonium nitrate) were applied to an amount of 150kg N/ha. To test the influence of N fertiliser, only one-half of each ring received the full amount of N (N100). The other halves of the plots received only half of this amount (N50) (Søe et al., 2004)). Also, other management measures were applied as typical for local farming practice (Giesemann & Weigel, 2008).

These half rings were divided into quarters. From these quadrants eight positions were determined, where soil cores have to be taken. (Figure 3). From these positions, three (3, 6 and 8) were too close to the vent pipes and differed significantly to the other samples (Giesemann & Weigel, 2008). Therefore, they got excluded. The samples were taken with a hand auger to 10cm depth at the end of each vegetation period (Giesemann & Weigel, 2008). From the five taken cores a mixed sample got created. In the N50 plot halves of the ambient and elevated rings there existed no samples for rye grass from the end of the period. Because of that, the samples were taken during the start of the vegetation period. For the plant samples there existed above-ground biomass specimen of wheat, barley and sugar beet and for the root samples there were only wheat and barley samples available.

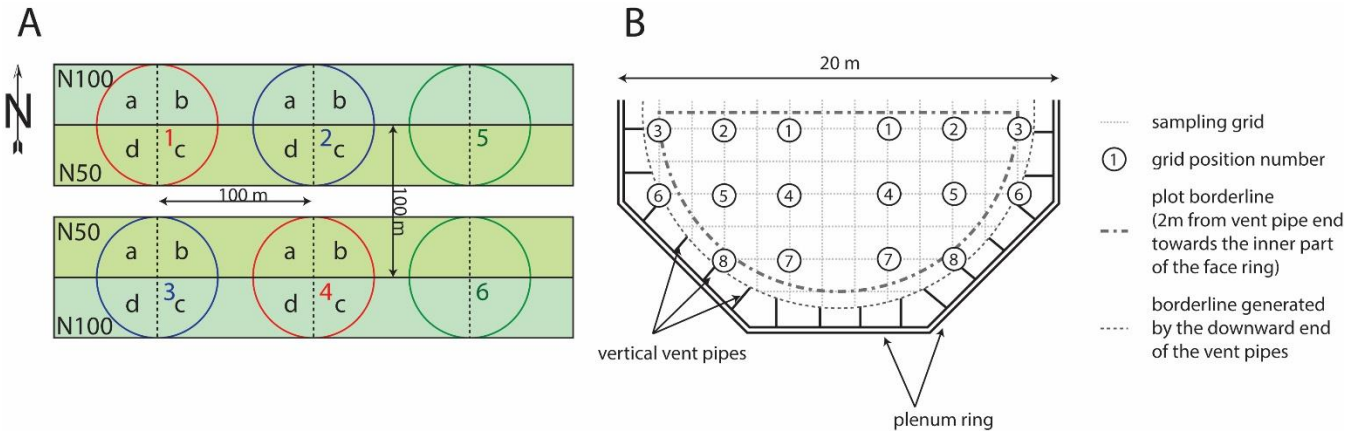

Figure 3. A) Sample setting: the red rings (1 & 4) were enriched with CO_2. The blue rings (2 & 3) had blowers which fumigated with ambient CO_2. The green rings (5 & 6) served as control rings and had only vertical vent pipes without blowers. The rings were divided into two plots which were fertilised with different amounts of N fertiliser. (N100 fertilisation typical for North Germany and N50 was half of the normal amount). To prevent elevated CO_2 concentration in the ambient rings they were placed 100m away from each other (modiefied according to Annette Giesemann, unpublished data). B) The rings with diameter of 20m were sampled as fallows: The rings were divided into quadrants. In each quadrant they took 8 samples at the grid line intersects (Giesemann & Weigel 2008).

2.2. Laboratory

2.2.1. Elemental Analysis

To specify the C and N content, the $\delta^{13}C$ and $\delta^{15}N$ values an elemental analysis was conducted. A part of the samples had to be put in tin capsules and got analysed in a Thermo Fisher Scientific Elemental Analyser coupled to a Delta V isotope mass spectrometer. Each sample got measured in duplicates. As the soil in the experiment in Braunschweig did not contain carbonate, a pre-treatment with acid was not necessary (Giesemann, 2005). For some samples there was not enough material to do a lipid extraction and elemental analysis. For these samples the extractable lipid free material, which was left after extraction, was used. Because of that C and $\delta^{13}C$ values were corrected with an approximation, to obtain values which are close to the untreated samples.

2.2.2. Lipid Analysis

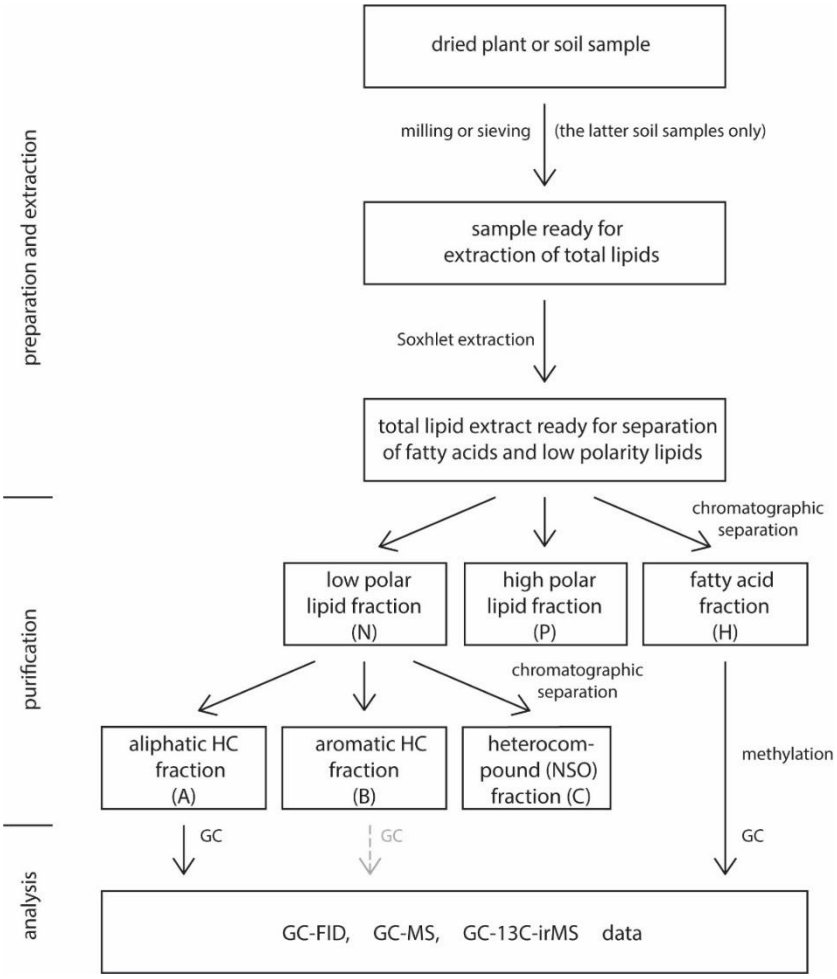

Figure 4 Schematic overview of sample preparation, lipid extraction, purification and analysation. Of all the separated fractions only fraction A and H were analysed. Fraction B was only used to ensure that the separation into the A fraction was successful. Modified according to Wiesenberg & Gocke (2017). HC= hydrocarbon, NSO = heterofunctionalised organic compounds containing nitrogen, oxygen, and/ or sulfur.

To analyse specific lipid classes the samples had to be prepared, extracted with a Soxhlet extractor, purified through chromatography and analysed with gas chromatography (GC) (Figure 4). The following procedure was performed as described in Wiesenberg & Gocke (2017), who provide a detailed guideline for lipid analysis.

Preparation and extraction

Before the fractions could be separated the samples had to be prepared and extracted. As the samples from Braunschweig were already milled or sieved when the thesis started, they only had to be weighed in. This was conducted with a Mettler Toledo XS205 Dual Range scale (d= 0.01mg/ 0.1mg). For the specimens which stem from above-ground biomass 1 to 1.5 grams were taken for extraction. For soils the mass of sample which was taken amounted from 11.2 to 44.3 g. The root samples measured from about 0.1 to 0.5 g as there was a limitation in existent material. Afterwards, each sample was put into a thimble within a Soxhlet extractor. The round bottom flask on the bottom of the Soxhlet extractor was filled with a mixture of dichloromethane (GC grade) : methanol (GC grade) (97:3, v:v), and was put into a water bath (54 ± 1°C). Through the heating

the solvent condensed, evaporated again, flushed the samples and thereby freed the lipids. After at least 20 cycles the total lipid extract in the solvent of the round bottom flask was disconnected from the Soxhlet extractor. Subsequently, the condensed total lipid extracts got purified into different fractions.

Purification

Chromatography was used to separate the total lipid extract into five different classes. This partitioning into fractions of different polarity enhances the qualitative and quantitative evaluation of lipid data obtained during GC analysis (Wiesenberg & Gocke, 2017). For the first separation 1.5-2.0 g KOH-coated silica gel 60 was put into a 6 ml glass column. To elute the low polar lipid fraction (N) 30 ml dichloromethane (GC grade) was used, fatty acids (H) were eluted with a mixture of 20 ml dichloromethane (GC grade) : formic acid (high purity) (99:1, v:v) and the high polar lipid fraction (P) was eluted with 4 ml of a mixture dichloromethane (GC grade) : methanol (GC grade) (1:1, v:v). With the second chromatography fraction N got further separated. To do so a glass pasteur pipette was filled with about 1 g activated silica gel (100 Å). Then the aliphatic hydrocarbon (HC) fraction (A) was eluted with 5 ml n-hexane (GC grade), the aromatic HC fraction (B) was eluted with a mixture of n-hexane (GC grade) : dichloromethane (GC grade) (1:1, v:v) and heterocompound (NSO) fraction (C) was eluted with 4 ml of the mixture dichloromethane (GC grade) : methanol (GC grade) (97:3, v:v). After all fractions got separated fraction H needed to be methylated that the GC is able to detect the fatty acids. For this 300 µl of dichloromethane and 500 µl of boron trifluoride/methanol was added to this fraction (BF3-CH3OH). As a methyl group was introduced, measurements had to be corrected.

2.3. Analysis

After the purification of the different fractions and the methylation of the fatty acids, an internal deuteriated standard (50 µl of d_{50} *n*-C_{24} alkane, respectively 50 µl d_{39} *n*-C_{20} carboxylic acid) was added to each sample of fractions A respectively to H. Therefore, the peaks obtained in the Agilent Technologies 7890B GC Systems equipped with a flame ionisation detector (FID) could be quantified and analysed. For this thesis only the peaks of different n-alkanes (within fraction A) and the fatty acids got analysed. they should be dissolved in n-hexane (GC grade). Measurement of n-alkanes was performed at a concentration of 10–20 µg/µL with 1 µL injected at 70°C in splitless mode. The temperature of injector and detector were set to 320°C (held 2 min). The oven gets further heated by 10°C/min until 120°C are reached and afterwards heated by 5°C/min until 320°C are reached (held 20 min) (according to Wiesenberg & Gocke, 2017). For methylated fatty acids, samples were injected via splitless mode at 50°C. (held 2 min). Injector and detector temperature had to be set to 320°C (2 min). Then oven temperature was increased by 5°C/min until 120°C were reached (held 2 min) and further heated at a rate of 2°C/min until 210°C are reached (held 3 min), then it is heated by 3°C/min until 320°C (held 20 min9.

To detect the stable carbon isotope values ($\delta^{13}C$) of the different compounds a Trace 1310 GC with split/splitless injector and FID coupled to a ConFlo IV and Delta V Plus isotope ratio mass spectrometer (irMS),was used. Measurements for n-alkanes were performed at 70°C (held 4 min) to 320°C (held 35 min) at 5°C/min. For the methylated fatty acids the programme was set to 70°C (held 4 min) to 150°C at 20°C/min and afterwards to 320°C (held 40 min) at 5°C/min. A constant flow of He was used as a carrier gas (according to Hirave et al., 2020)

To detect the CSI values ($\delta^{13}C$) of individual aliphatic hydrocarbons and carboxylic acids GC^{13}C-irMS was used. Each sample was measured in triplicates.

Carbon isotopic values were expressed in ‰ relative to the Vienna Pee Dee Belemnite (V-PDB) standard:

$$\delta^{13}C = \left[\left(\frac{^{13}C_{Sample}/^{12}C_{std}}{^{13}C/^{12}C_{std}}\right) - 1\right] \times 10^3 \quad (1)$$

where $^{13}C/^{12}C_{std} = 0.0112372$.

To be able to illustrate the changes in lipid distribution patterns, selected molecular ratios were determined:

Compounds with relatively longer chain length ($\geq C_{25}$ for alkanes and $\geq C_{20}$ for FAs) are more abundant in higher plant biomass. Shorter chain lengths can typically be found in the organic matter produced by microorganisms (Hardwood & Russell, 1984). Therefore, as chemotaxonomic ratio the average chain length (ACL) will be used to asses if the lipids in the soil stem more from plants or microorganisms.

$$ACL = \sum(z_n * n) / \sum z_n; \quad (2)$$

with n= number of carbons within compound, z_n= quantity of the respective compound (Wiesenberg & Gocke, 2017)

To assess the degradation of organic material, the carbon preference index (CPI) for the different fractions can be calculated. If the chain length is dominated by odd-over-even for alkanes and even-over-odd for fatty acid and alcohols, then the biomass originates from higher plants or is fresher (Eglington et al., 1962). Therefore, the CPI is higher for fresh plant material.

$$CPI_{alkanes} = [\sum C_{25-33\ odd} / \sum C_{24-32\ even}) + (\sum C_{25-33\ odd} / \sum C_{26-34\ even})] / 2 \quad (3)$$

$$CPI_{acids} = [\sum C_{20-32\ even} / \sum C_{19-31\ odd}) + (\sum C_{20-32\ even} / \sum C_{21-33\ even})] / 2 \quad (4)$$

(Wiesenberg & Gocke, 2017)
CPI (odd over even or even over odd carbon preference index) (Huang et al., 1999)

To obtain averages of the CSIA results the mean of the most abundant compounds ($\delta_{Malkanes}$ and δ_{Macids}) were used (four for alkanes and six for carboxylic acids).

$$\delta_{Malkanes} = (A \times \delta_A) + (B \times \delta_B) + (C \times \delta_C) + (D \times \delta_D) \quad (5)$$

$$\delta_{Malkanes} = (A \times \delta_A) + (B \times \delta_B) + (C \times \delta_C) + (D \times \delta_D) + (E \times \delta_E) + (F \times \delta_F) \quad (6)$$

A, B, D, E and F are the relative proportion of the most abundant compounds and the corresponding δ are their $\delta^{13}C$ isotopic values (Wiesenberg et al., 2008b).

3. Results

The values used for the graphics are listed in the tables at the end of this section (Table 1 & Table 2)

3.1. C and N content

The total OC content of the above-ground biomass of wheat, barley and sugar beet ranged from about 39 to about 47 % (Figure 5). The roots of wheat and barley had OC content which ranged from 44 to 48%. For the soils under the different crops, the OC content ranged from 0.7 to about 1.4% (Figure 6). For the wheat straw the OC concentration increased by 2.6% when CO_2 concentration was higher for the lower and normally fertilised plots. The combined effect increased C concentration by 1%. C content for barley stayed almost the same for all treatments. For sugar beet the C content decreased under elevated CO_2 by 3.5%. Less N-fertiliser under ambient air and enriched CO_2 had no visible effect on the C content of the leaves compared to the C content of standard conditions. However, the standard error of the leaves under decreased N fertilisation and increased CO_2 concentration is rather high. It is possible that a lower N fertilisation dose lead to similar C content as the unfertilised plants have. The root samples of barley had about similar results for elevated as for ambient CO_2 conditions. When the plants got less N fertiliser, the samples under elevated CO_2 obtained 4.5% higher C concentrations. In the ambient plots, the concentration stayed more or less the same. For the roots of wheat, the fertilisation effect was reversed. The normally fertilised root samples had 3.8% higher C content under elevated than under ambient CO_2. When the plants received a lower fertilisation dose, the wheat roots had similar C concentrations in both CO_2 treatments as when full amount of fertiliser was applied. For the soils it can be seen that the soils with reduced N fertilisation had 0.2 to 0.3% higher C-contents under elevated CO_2 than under ambient CO_2. Only the soils under sugar beet had also higher C concentrations under enriched air than ambient air when the fertilisation dose was typical for that region. When looking at the C concentration of the soils of the different treatments during time (Figure 6), then it can be seen, that the C concentration of the soils decreased for ambient air by 0.5% and increased for CO_2 enrichment by 0.4-0.6% when fertilisation was applied as usual and when the fertilisation dose was halved. The lower fertilised plot under ambient air also had from the start to the end of the crop rotation period an increase in C content by 0.6%.

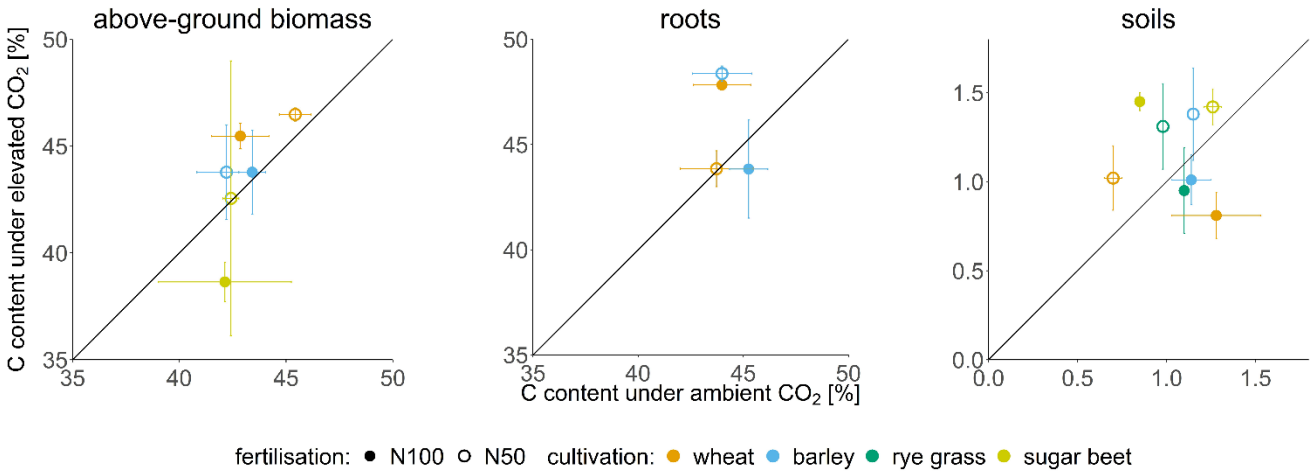

Figure 5. The Carbon content [%] under elevated and under ambient CO_2 concentration from the different plant species and different fertilisation treatments. The error bars depict the standard error of two measurements. For the soils a different scale got used as for the plants, as the C concentration of the soils were much lower than from the plant specimens.

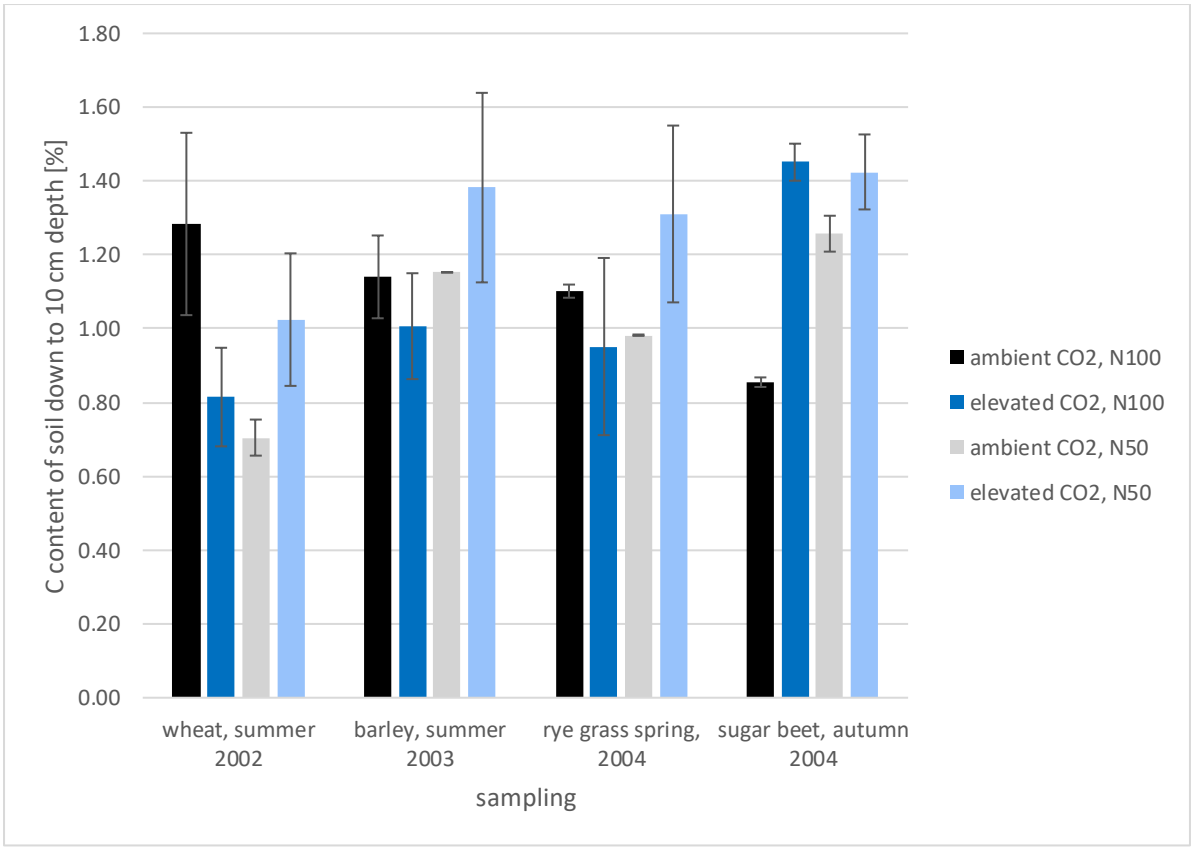

Figure 6. Mean soil C content [%] under different CO_2 and fertilisation treatments. The standard error is the error of two measurements.

The C/N ratios were typical for the sample types. The straw samples of wheat and barley measured about 60 to about 180. Their roots had C/N ratios which ranged from 40 to 100. The

C/N ratios of sugar beet leave samples were in the same range as those of the soil samples which was about 10 to 13.

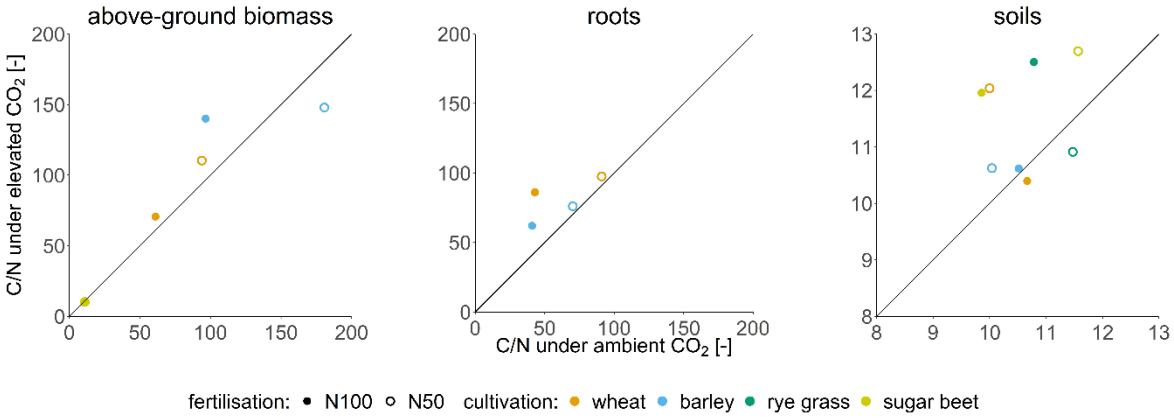

Figure 7. The C/N ratio of the plants and soils cultivated under ambient CO_2 in respect to the samples influenced by elevated CO_2. For the normal and reduced fertilisation.

The C/N ratios of the plant samples wheat and barley were similarly affected by an elevation of CO_2 (Figure 7). All samples had a 7 to 99% higher C/N ratio under elevated than ambient CO_2-condition independent of the N fertilisation. Only the barley sample with lower fertilisation had a 18% higher C/N ratio under ambient CO_2 than under elevated CO_2. A lower fertilisation with no changes in air CO_2 concentration resulted in a 54% for wheat, and 87% for barley higher C/N ratios. The combined treatment (elevated CO_2 and low fertilisation) increased the C/N ratio by 81% for wheat and 53% for barley compared to the control treatment. The sugar beet leaves had similar C/N ratios for all treatments and the C/N ratio of the different soil samples were pretty similar. Most soil samples had a slightly higher C/N ratio under elevated CO_2 than under ambient conditions. A fertilisation effect is not visible. When we look at the changes during the crop rotation period (Figure 8). It can be seen that at the start of the second crop rotation period the C/N ratio under enriched CO_2 was 6% lower than in the control plot. However, at the end of the crop rotation period the C/N ratio of the elevated plot had 21% higher C/N ratio as the ambient plot. With a lower fertilisation dose under ambient air, the C/N ratio was again lower by 6% compared to control plots at the start of the second crop rotation period and in the end, it was 17% higher than under ambient air. For soil samples under enriched CO_2 and lower N fertilisation the C/N ratio was about 13% higher than under ambient air. It was about the same when cultivated with barley and rye grass and increased in the end to about 29% more under combined effects than under control conditions.

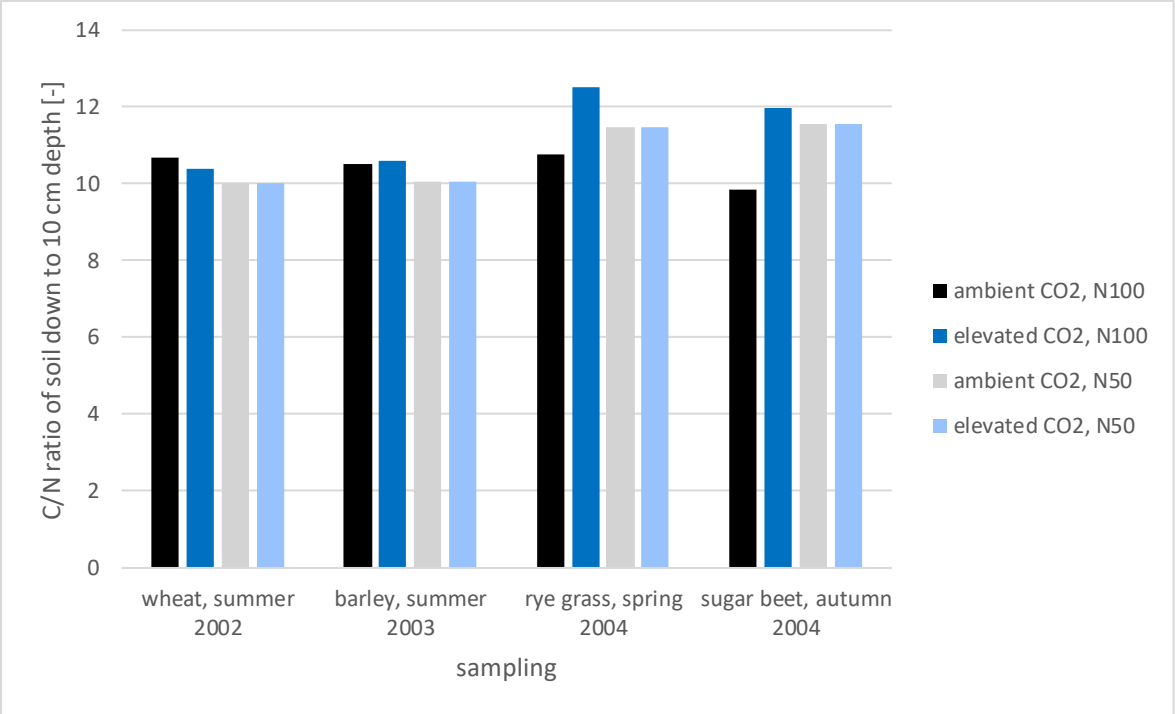

Figure 8. C/N ratios of the soil samples under different treatments and distinct crops. The second crop rotation started with wheat and ended with sugar beet.

3.2. Lipid extracts

The free lipid extracts got weighted and the free extractable lipid content of the samples was calculated. The extractable lipid content was about four to five times higher for sugar beet than for wheat and barley because for sugar beet the leaves were used and for the other two crops the straw got extracted. The sugar beet leaves concentration of extractable lipids was around 80 to 100 mg/g. The extracts of wheat and barley were similar for the above-ground biomass and the roots and ranged between 13 and 23 mg/g (Figure 9). The extract concentrations from the soil samples were lowest between 0.3 and 0.6 mg/g. The sugar beet leaves, the straw and roots of wheat and barley had similar lipid concentrations under ambient and elevated CO_2 conditions. A lower dose of fertiliser resulted in slightly higher lipid concentrations for barley straw then for wheat straw under ambient and elevated CO_2-conditions. Where the concentrations for wheat straw was slightly higher for samples with the full fertiliser dose. The sugar beet samples had lower lipid contents in the plots where the fertilisation rate was halved. For the soils there could be seen that the samples of wheat, barley and sugar beet soils had slightly lower lipid concentrations under elevated CO_2. Only for the rye grass plots increased the concentrations under elevated CO_2. Less fertiliser resulted for wheat in higher lipid contents and for sugar beet and barley for lower contents under ambient and elevated CO_2 compared to the samples of the corresponding CO_2-treatment.

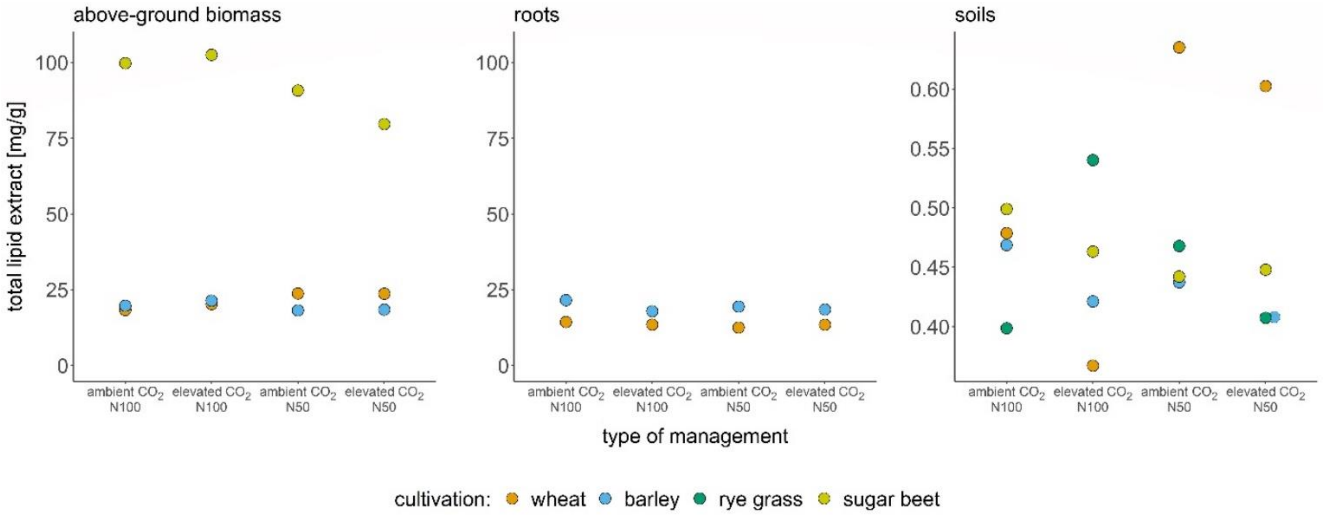

Figure 9. The concentrations of the free extractable lipids of the samples in mg/g from different cultivations in respect to the different treatments. The y-axis of the soil samples has a different scale than the plant samples have as the lipid concentrations were much lower.

3.3. Carboxylic acids

The carboxylic acids in the plant samples showed a predominance of saturated C_{16} and unsaturated C_{18}. In all samples even number chain homologues predominated over the odd numbered homologues. More short chain (<20 Cs) compared to long chain (≥20 Cs) acids were found for soils versus plant biomass. All plant samples but sugar beet leaves had a depletion of unsaturated C_{18} acids under elevated CO_2. For sugar beet the abundance of unsaturated C_{18} increased under higher CO_2 concentration.

The ratios of n-$C_{16:0}/C_{18:0}$ were highest for leave and straw samples which ranged from 8 to 11 (Figure 10). For the root samples ratios of n-$C_{16:0}/C_{18:0}$ were a bit lower and ranged from 2 to 5. All plant samples (normally and lower fertilised samples) had 13 to 32 % higher ratios of n-$C_{16:0}/C_{18:0}$ under elevated CO_2 concentration than under ambient CO_2. So, these samples had a relative higher amount of $C_{16:0}$ in relation to $C_{18:0}$ compared to samples grown under ambient CO_2 condition. The only exception are the lower fertilised barley straw samples which had the same ratios under ambient and elevated CO_2. When fertilisation amounts were lower the ratios of n-$C_{16:0}/C_{18:0}$ were also 5-31% lower under ambient respectively elevated CO_2. The soil samples had similar ratios of n-$C_{16:0}/C_{18:0}$ as root samples and ranged from 2 to 4. All normally fertilised soil samples except the sample cultivated with sugar beet had 7-37% higher ratios of n-$C_{16:0}/C_{18:0}$ under enriched CO_2 than under ambient air. The ratio of sugar beet sample decreased by 12% under elevated CO_2. All samples with lower fertilisation had ratios which were about 5-37% lower for enriched CO_2 than for ambient air. A lower fertilisation under ambient air resulted with 6-86% higher ratios compared to normally fertilised soils under ambient air.

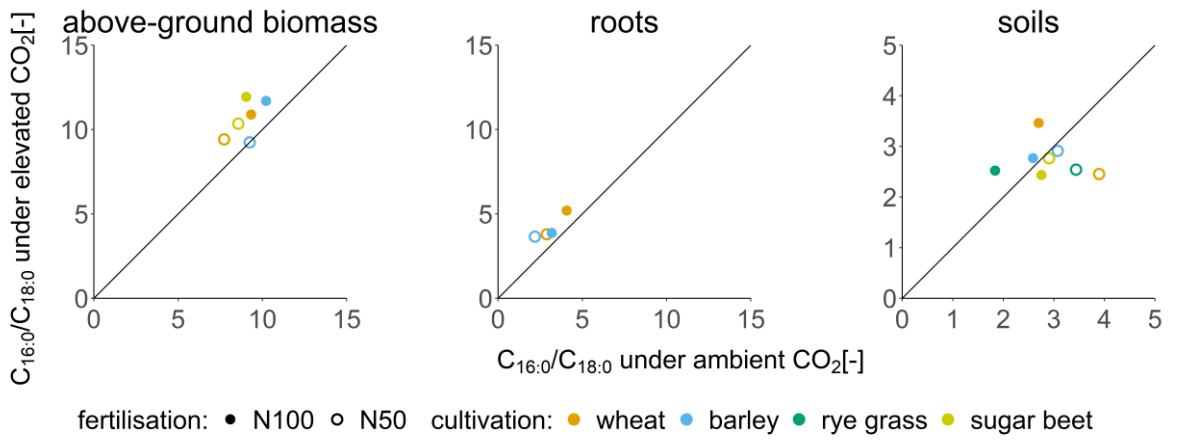

Figure 10. Ratios of saturated carboxylic acids $C_{16:0}$/ $C_{18:0}$ [-] of the samples cultivated under elevated CO_2 versus ratios of $C_{16:0}$/ $C_{18:0}$ [-] under ambient CO_2 with different fertilisation and cultivated with different crops. The soil samples have a different scale as plant samples have, to enhance visibility of datapoints.

When looking at the ratio of $C_{18:2}/C_{18:1}$ it can be seen that for plant samples (normal amount and reduced fertiliser) $C_{18:2}/C_{18:1}$ ratios were 14-58% higher under elevated CO_2 treatment than under ambient air (Figure 11). Only wheat samples which were normally fertilised had a 11% higher $C_{18:2}/C_{18:1}$ ratio under ambient air than enriched CO_2. For normally fertilised soils it can be seen that the $C_{18:2}/C_{18:1}$ ratios decreased by 18-56% when the air was enriched with CO_2. For the less fertilised plots the opposite effect could be observed. The $C_{18:2}/C_{18:1}$ ratio increased by 17 to 89% for barley, rye grass and sugar beet under elevated CO_2. For wheat the concentration of unsaturated C_{18} in the samples was too low to calculate a ratio.

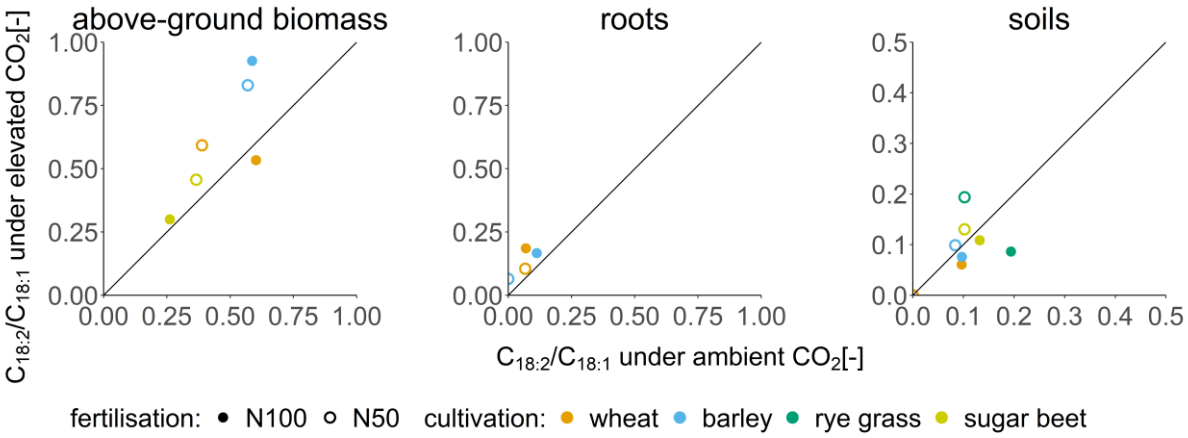

Figure 11. Ratio of unsaturated carboxylic acids $C_{18:2}$/ $C_{18:1}$ [-] of the samples cultivated under elevated CO_2 versus ratios of $C_{18:2}$/ $C_{18:1}$ [-] under ambient CO_2 with different fertilisation and cultivated with different crops. The soil samples have a different scale as plant samples have to enhance distinction of datapoints.

The ratio of $C_{18:1+18:2}/C_{18:0}$ which ranged from 1 to 7 for plant samples increased also under enriched CO_2(

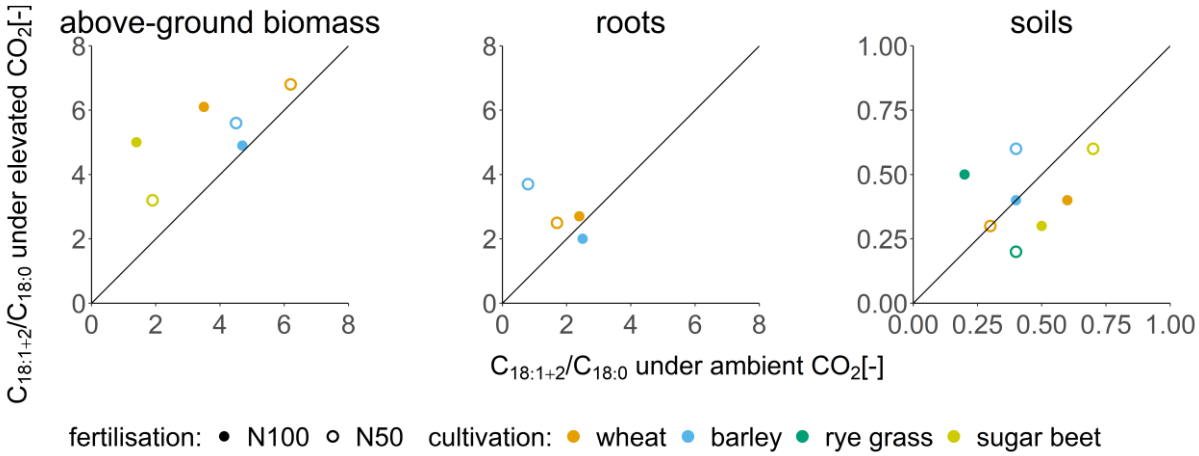

Figure 12). Again, only one plant type – here it was the normally fertilised barley root samples - was a bit higher under ambient air than elevated CO_2. A clear influence of fertilisation is not visible. The proportion of unsaturated C_{18} to saturated C_{18} acids of the soil samples is lower than that of the plant and ranges from 0.2-0.7. A clear pattern that gives indication on the influence of fertilisation and CO_2 concentration is not detectable.

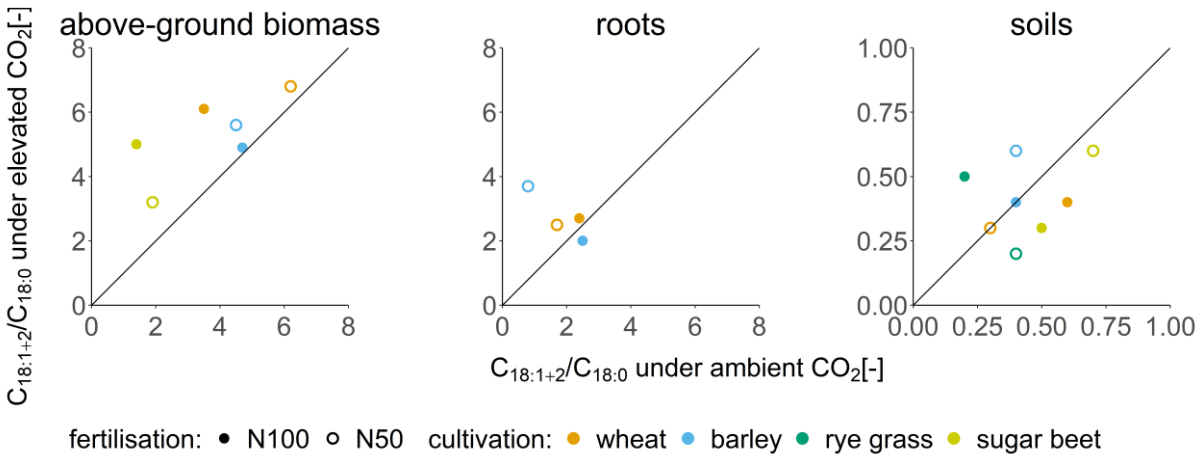

Figure 12. Ratio of $C_{18:1+2}/C_{18:0}$ under ambient versus elevated CO_2 with different fertilisation and cultivated with different crops. For soil samples a different scale as for the plant samples was used, to enhance distinction of datapoints.

For plants no clear influence of treatment was visible for plant samples. The chain lengths varied according to the plant species. The soil samples showed under elevated CO_2 45-89% longer carboxylic acids under normally fertilised plots compared to less fertilised plots. Under ambient air a lower fertilisation had no clear trend under all crops.

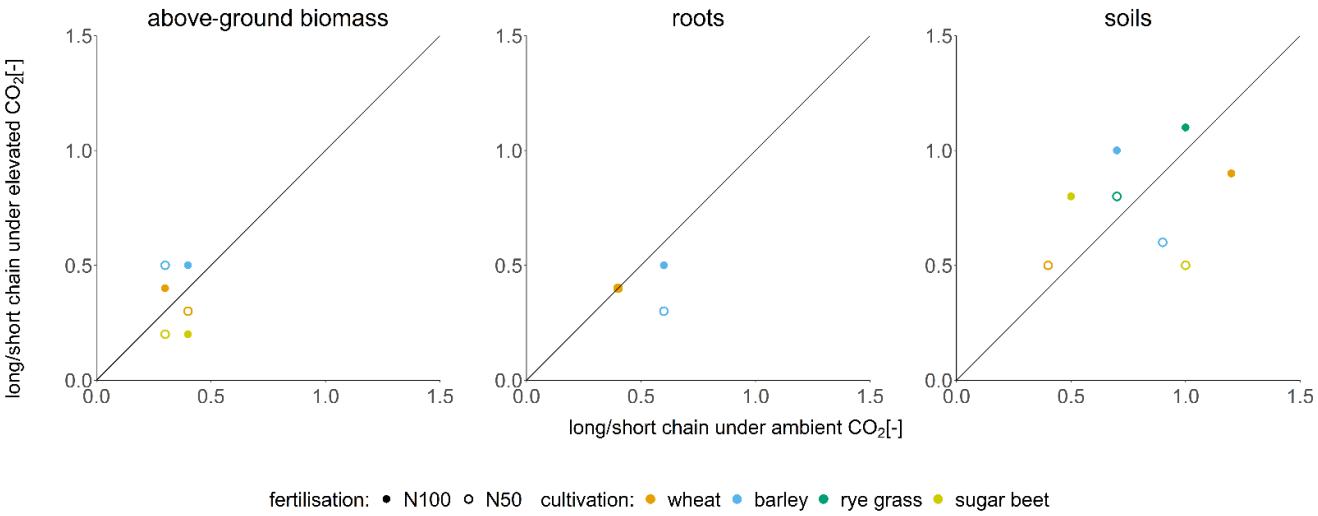

Figure 13. Ratio of long (≥C_{20}) over short chains (<C_{20}) under ambient vs elevated CO_2 with different fertilisation and cultivated with different crops. For the roots C_{20} was not included in the calculation as the samples were contaminated by mistake with an addition of C_{20}.

The ACL were similar for all samples and ranged from about 17 to 21 µg/g (Figure 14). The samples had similar results for all treatments. Under elevated CO_2 a lower fertilisation decreased the ratio of the average chain length in most cases. Only for the above-ground biomass of sugar beet and wheat ACL values from elevated CO_2 plots were a bit higher when the amount of fertiliser was reduced. The plant samples had a similar distribution pattern as the corresponding soil samples had.

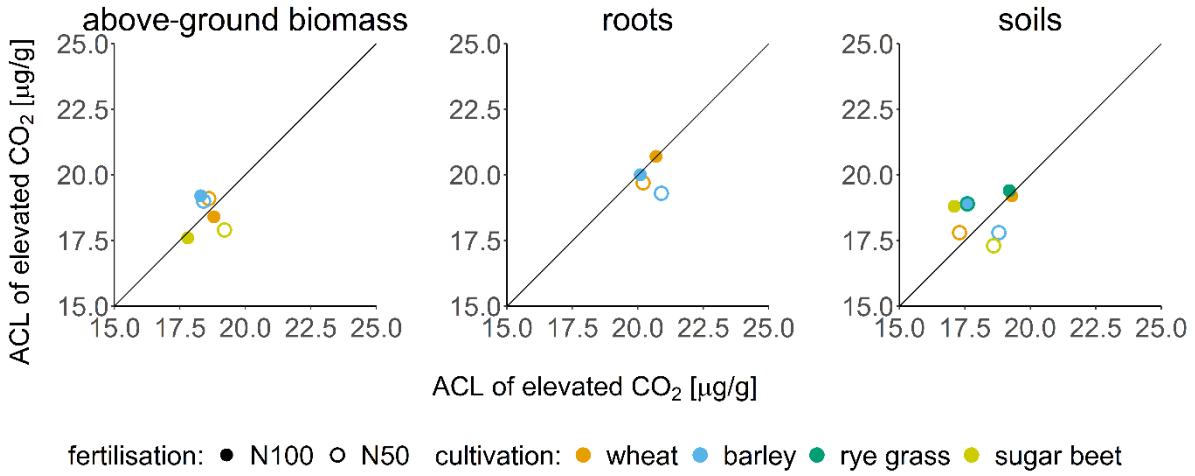

Figure 14. ACL values [µg/g] of the samples cultivated under elevated CO_2 versus ACL under ambient CO_2 with different fertilisation rates and different crops. The scale of the soils was different as the scale of above-ground biomass and the roots.

The CPI values of the *n*-carboxylic acids of the plant samples ranged from about 2-9 (Figure 15). A clear influence on above-ground biomass samples according to treatment is not clear. For roots a normal fertilisation lead to higher CPI values under enriched CO_2 concentration versus ambient air. For the less fertilised samples, the CO_2 effect was the other way round and CPI values were higher under ambient air than enriched CO_2 concentration. CPI values of soils were lower than

most values of plants and ranged from 3-4. Samples which were normally fertilised had higher CPI values than most samples that were manured with less N fertiliser.

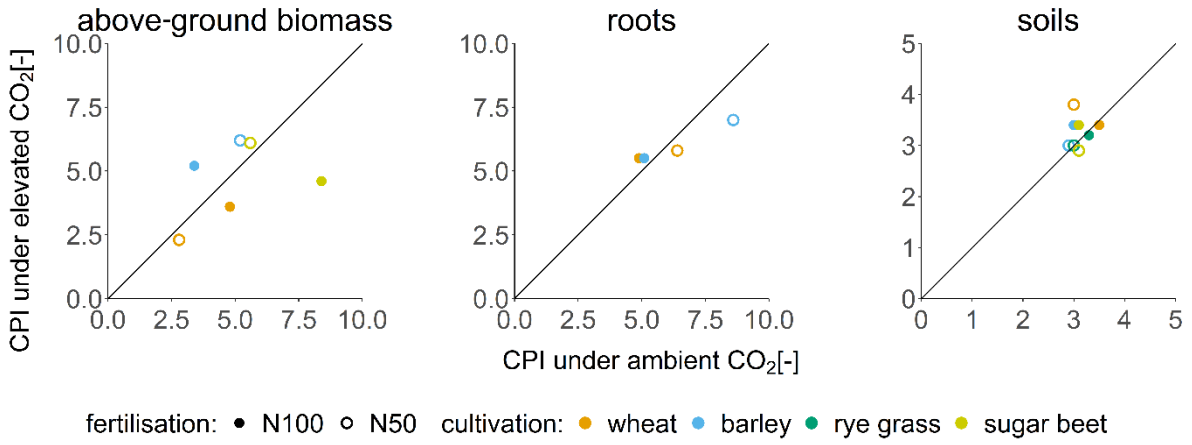

Figure 15. CPI values [-] of the samples cultivated under elevated CO_2 versus CPI under ambient CO_2 with different fertilisation and cultivated with different crops. The above-ground biomass has a different scale as roots and soils have.

3.4. Alkanes

The samples had highest amount in n-C_{29} and n-C_{31} alkanes for all samples only some wheat root samples had no n-C_{31} values. That is probably due to the small amounts of samples which was used for extraction. As comparison of the different lipid distribution pattern is difficult, different ratios were calculated.

When looking at the C_{29}/ (C_{29} +C_{33}) ratio of plant samples a typical range for different plants is visible. The values of the ratios of these samples range from about 0.7 to 0.9 (Figure 16). Values for sugar beet leaves were higher than for wheat and barley. The ratios were similar for all treatments. For sugar beet and barley lower fertilised samples had slightly lower (8-10%) ratios for plots fumigated with ambient air but the samples grown under enriched CO_2 had similar results for both fertilisation treatments. For wheat, fertilisation had no impact on the ratio under ambient air, but the sample from the plot which was enriched with CO_2 had a 6% higher ratio under elevated air than the less fertilised sample. The ratios of the barley root samples were pretty similar under both CO_2 treatments and were a bit higher when a lower amount of fertiliser was applied to the plots. The wheat samples obtained lower ratios for ambient air plots but the fertilisation type had nearly no influence. The soil samples are rather close to each other differences between treatments were very low. However, a lower fertilisation resulted in slightly higher ratios under elevated CO_2 and also for all, but the barley soil samples, under ambient air. These samples also showed slightly lower ratios under ambient than elevated plots. Only for barley the ratios were a bit higher under elevated CO_2.

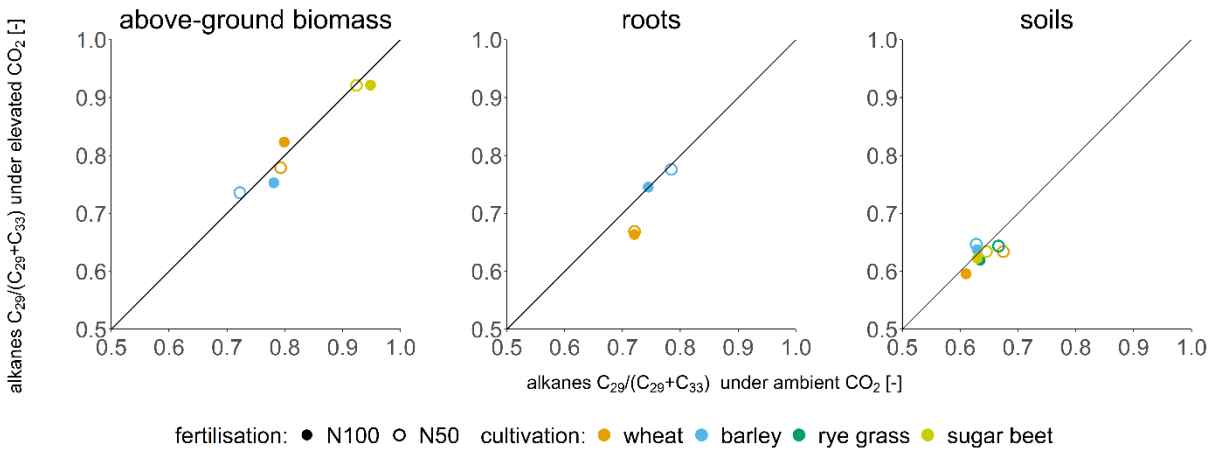

Figure 16. Ratio of C_{29}/ $C_{29}+C_{33}$ [-] under ambient CO_2 versus elevated CO_2 with different fertilisation rates and different crops.

ACL values of plant samples also showed specific ranges for different species. The ACL values of the n-alkanes in the plant samples ranged from about 28 to 43 µg/g and was highest for sugar beet leaves. The ACL values of the different plants were very similar under every treatment. Only the sample of sugar beet, which was grown under enriched CO_2 and was less fertilised had a 19-20 % higher ACL value compared to the other three treatments.

When a lower dose of fertiliser got applied the roots had a bit higher ACI values under elevated than ambient CO_2. For soils, samples the ACL values ranging from 27 to 28. So, the ACL values of the different samples were very similar. The samples cultivated with barley had the highest values and rye grass had the lowest values, while wheat and sugar beet ranged in the middle. For all samples (aside from the soil samples cultivated with wheat, where ACL stayed the same) a smaller amount of fertiliser increased the ACL values under elevated CO_2 even a bit (Figure 17).

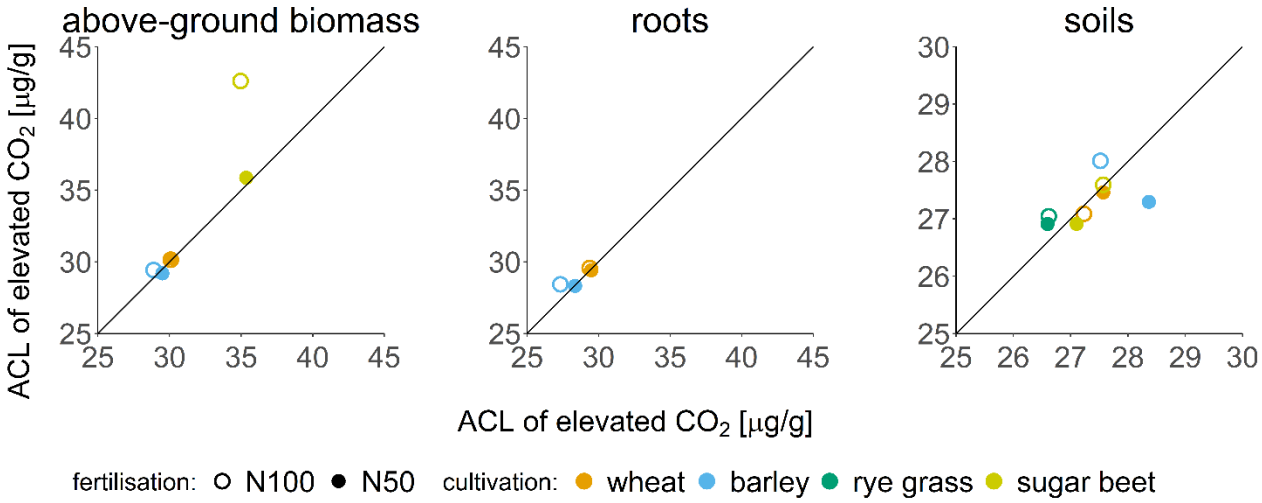

Figure 17. ACL values [µg/g] of the samples cultivated under elevated CO2 versus CPI under ambient CO_2 with different fertilisation rates and different crops. The scale of the soils was different as the scale of above-ground biomass and the roots.

The CPI values of the *n*-alkanes of the plant samples had similar ranges for the different samples belonging to one species. Samples of sugar beet leaves ranged from 6 to 7, for wheat straw around 40 to 45 and barley straw from 19 to 23 (Figure 18). For sugar beet the CPI values are very similar

and no differences between different treatments are visible. For wheat and barley straw the normally fertilised samples had only slightly higher CPI values under elevated than ambient CO_2. The samples which received half the nitrogen had 10-12% higher CPI values under ambient than elevated CO_2. The CPI value of the roots from the different plant species were closer together as those of above-ground biomass of the same species and ranged for wheat from about 7 to 9 and for barley from 5 to 6. Here the amount of fertiliser had a different effect compared to the above-ground biomass. The roots normally fertilised had a slightly higher CPI value under enriched CO_2 concentration plots compared to the plots fumigated with ambient air. The roots which received only half of the fertiliser amount had 11-26% higher CPI values under elevated CO_2 compared to the roots under ambient CO_2. For the soils which range from about 5 to 8, no real trend for the CPI values depending on the treatment is visible. The soil samples cultivated with wheat did not have higher CPI values compared to those of barley or sugar beet as was visible in the above-ground biomass and roots).

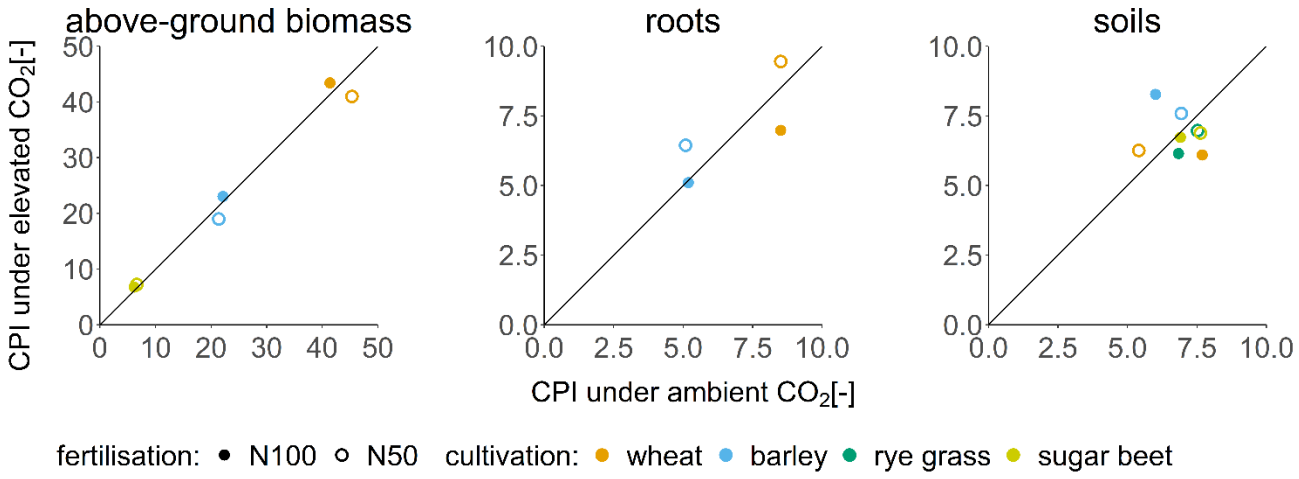

Figure 18. CPI values [-] of the samples cultivated under elevated CO_2 versus CPI under ambient CO_2 with different fertilisation and cultivated with different crops. The above-ground biomass has a different scale as roots and soils have.

3.5. Isotopic ($\delta^{13}C$) values

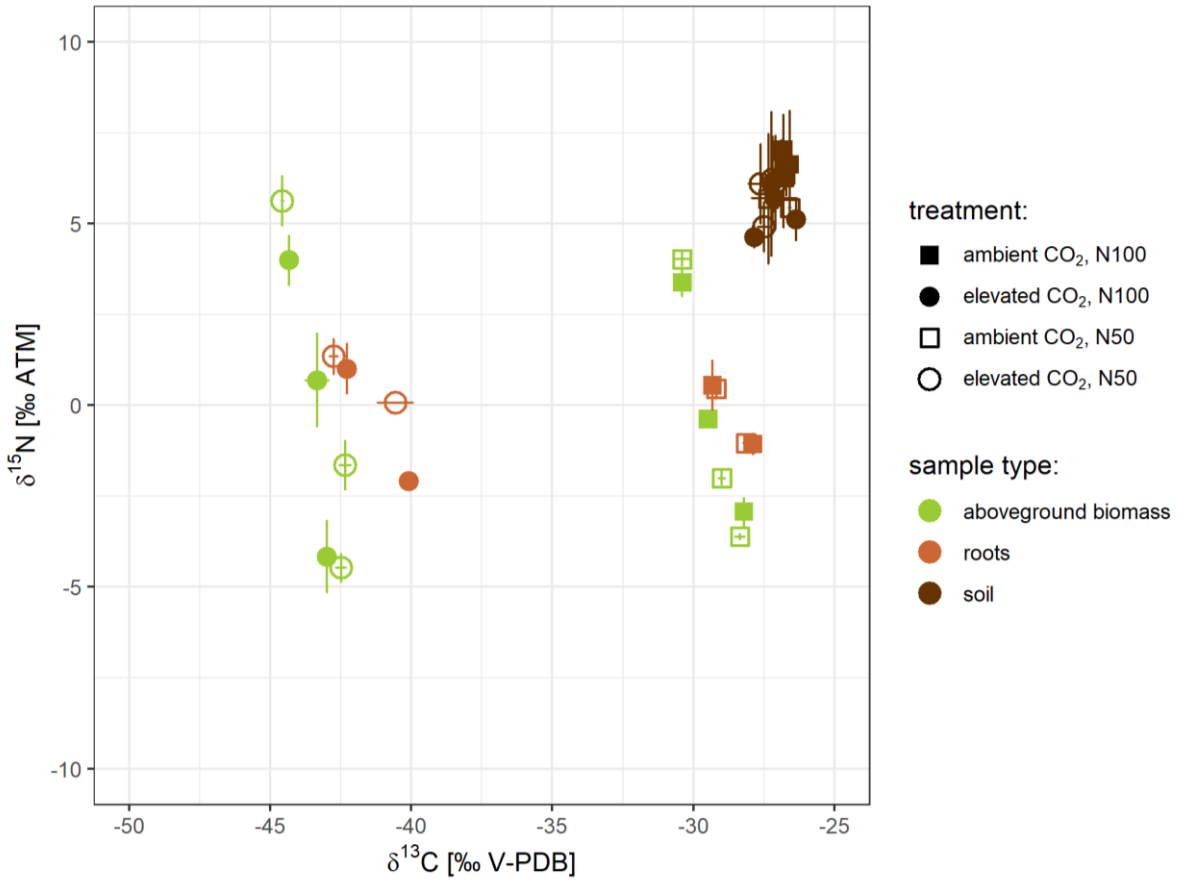

Figure 19. $\delta^{13}C$ and $\delta^{15}N$ bulk values of the different samples. The error bars indicate the standard deviation of the two measurements.

The $\delta^{13}C$ values of plant samples under ambient CO_2 concentration ranged from -28 to -30 ‰. V-PDB. Plant samples grown on the plots with elevated CO_2 condition were more negative and ranged from -40 to -45 ‰ VPDB (Figure 20). Which accounts for a depletion of about 12-15‰ V-PDB compared to the plants grown under ambeint CO_2 conditions. For the soils under elevated CO_2 conditions the $\delta^{13}C$ values are only slightly depleted (0.1-1.3‰ V-PDB) than for the plots fumigated with ambient air (Figure 20). For wheat with normal fertilisation and rye grass cultivated with less fertiliser had even slightly higher $\delta^{13}C$ values under ambient air than under same fertilisation under elevated air (0.4‰). When looking at the changes in the soil during time (Figure 21), it can be seen that under each crop the $\delta^{13}C$ values were more negative for soils under elevated CO_2 concentration then the soils under ambient air with the same fertilisation. Only the wheat sample that was normally fertilised had a lower $\delta^{13}C$ value when fumigated with enriched CO_2 contrations than under ambient air. The $\delta^{13}C$ values also got more negative during time but not constant, as under rye grass the change was more visible than under sugar beet. The fertilisation had no visible effect on the $\delta^{13}C$ values of the plant and soil samples when conditions stayed the same. For the $\delta^{15}N$-values a specific range depending on their species can be observed. A lower fertilisation resulted in more negative $\delta^{15}N$ values for the straw of wheat and barley. The leaves of sugar beet and the two root samples are oppositely affected by a lower N-fertilisation. These samples with halve the N dose, have similar $\delta^{15}N$ values as the normally fertilised samples under ambient CO_2-conditions but obtain higher $\delta^{15}N$ values in the elevated CO_2 plots. However,

as the $\delta^{15}N$ values are not really relevant for this thesis they will not be further discussed and only serve for a better overview in the figures.

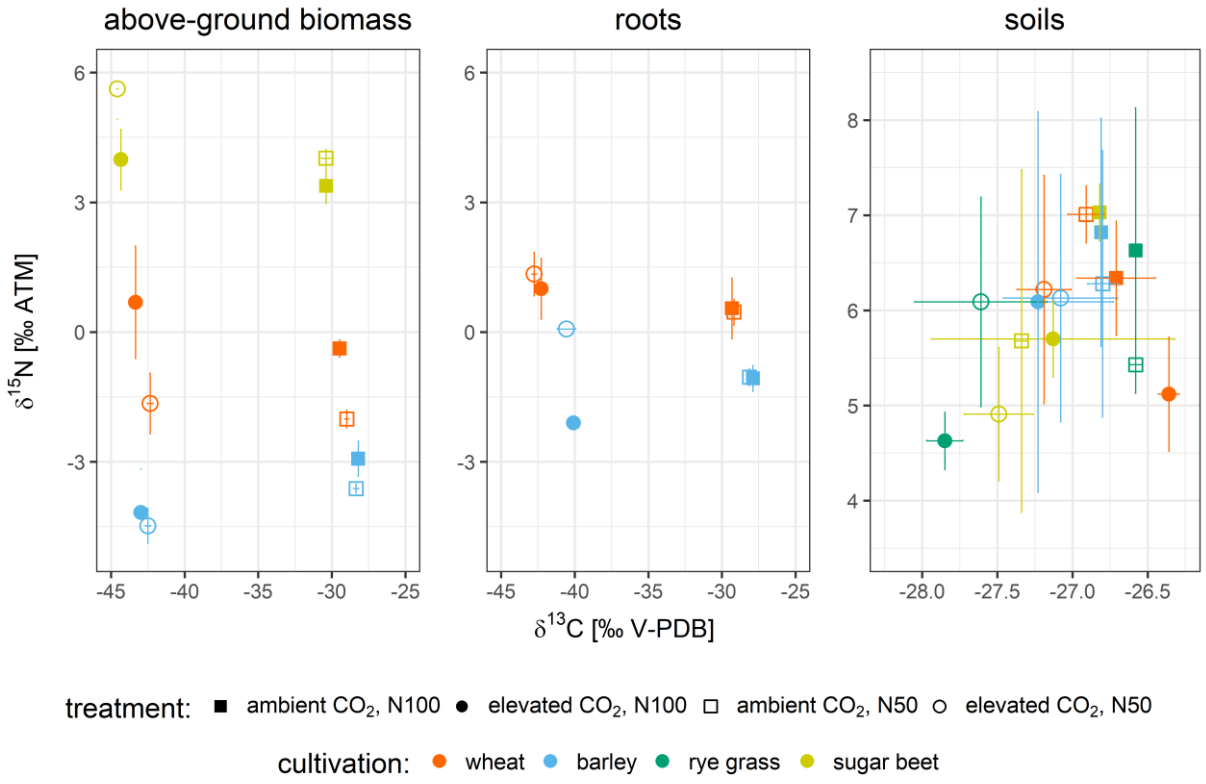

Figure 20. $\delta^{13}C$ values of bulk C in respect to their $\delta^{15}N$ of the plant and soil samples under different CO_2 concentration and fertilisation treatments. As the individual values of the soil samples are very close to each other they have different scales as the above-ground biomass and roots. Error bars indicate the standard deviation of two different measurements.

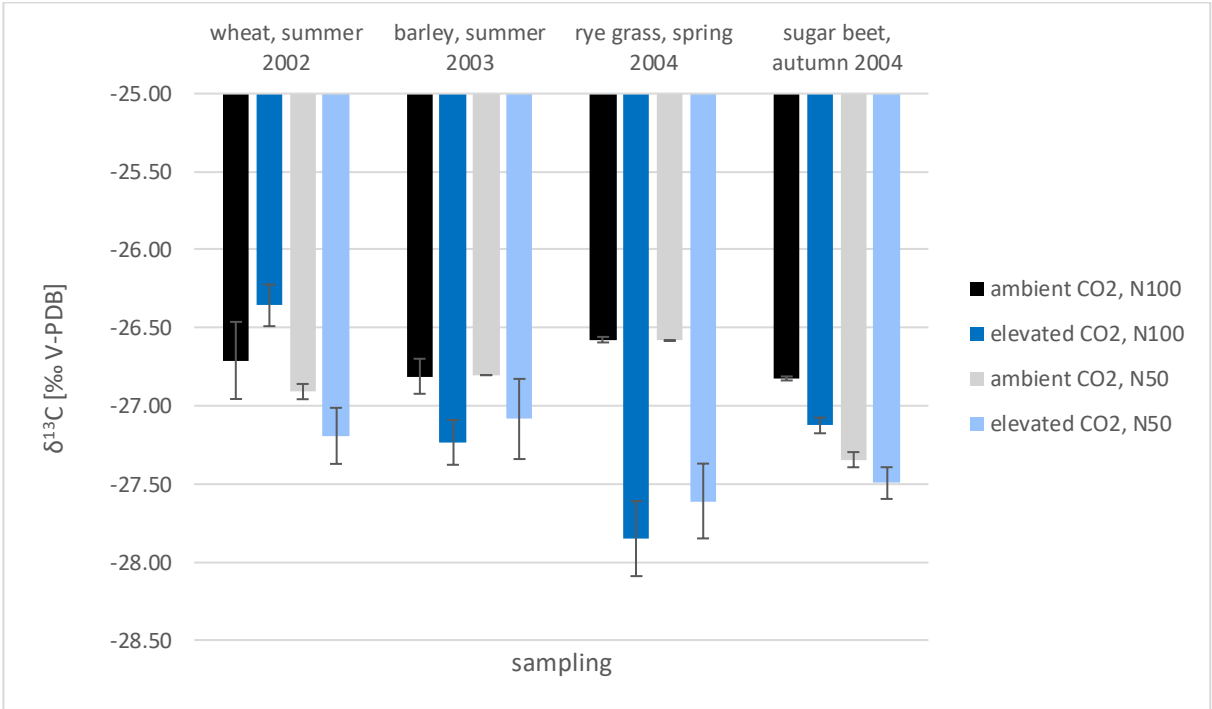

Figure 21. δ¹³C values of soil bulk C under different treatments. On the plots the plant wheat, barley, rye grass and sugar beet were cultivated in this order. Error bars are the standard deviation of two measurements.

The mean average isotopic composition [‰ V-PDB] of long chain n-carboxylic acids of the six most abundant acids and the four most abundant n-alkanes were compared to each other (Figure 22.). For the samples of plants, the isotopic values were similar for n-alkanes and n-carboxylic acids. The δ¹³C values of normally and lower fertilised samples are 8-35% respectively 17-32% more negative under elevated CO_2 as under ambient air. Root samples had slightly more negative δ¹³C values for long chain n-alkanes compared to long chain n-carboxylic acids.

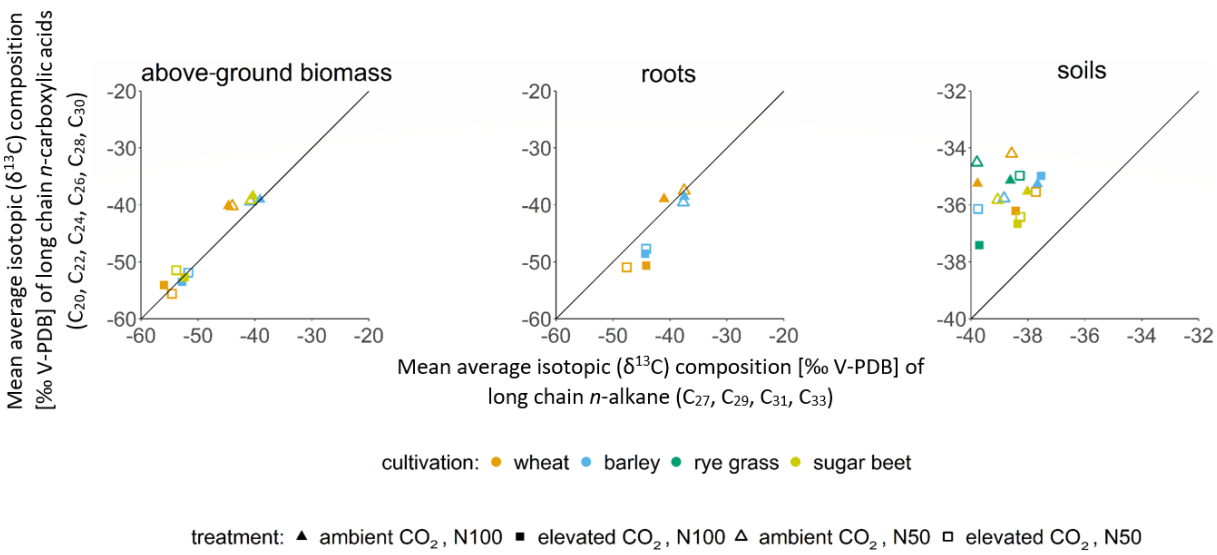

Figure 22. Mean average isotopic composition [‰ V-PDB] of most abundant long chain n-carboxylic acids versus n-alkanes. The carboxylic acids of root samples were contaminated with an addition of C_{20} and because of that were excluded from calculation in these samples.

The δ¹³C values are in most cases less negative as for plant samples. In the soils a δ¹³C shift under elevated CO_2 was not visible. For some samples the δ¹³C values were more negative under ambient CO_2 than under elevated CO_2. However, there can be seen that for soil samples the long chain n-carboxylic acids had less negative δ¹³C values than the long chain n-alkanes. The δ¹³C values of n-alkanes were on average 35% and the n-carboxylic acids were 30% more negative than bulk δ¹³C values. When examine the δ¹³C values of most abundant n-carboxylic acids and alkanes, it can be observed that in the time span of the experiment δ¹³C got more negative under elevated CO_2 treatment compared to the values under ambient air (Figure 23). When soil samples under elevated CO_2 were lower fertilised δ¹³C were depleted compared to normally fertilised soils under most cultivations. For n-alkanes a clear effect of treatment over time is not clearly visible (Figure 24).

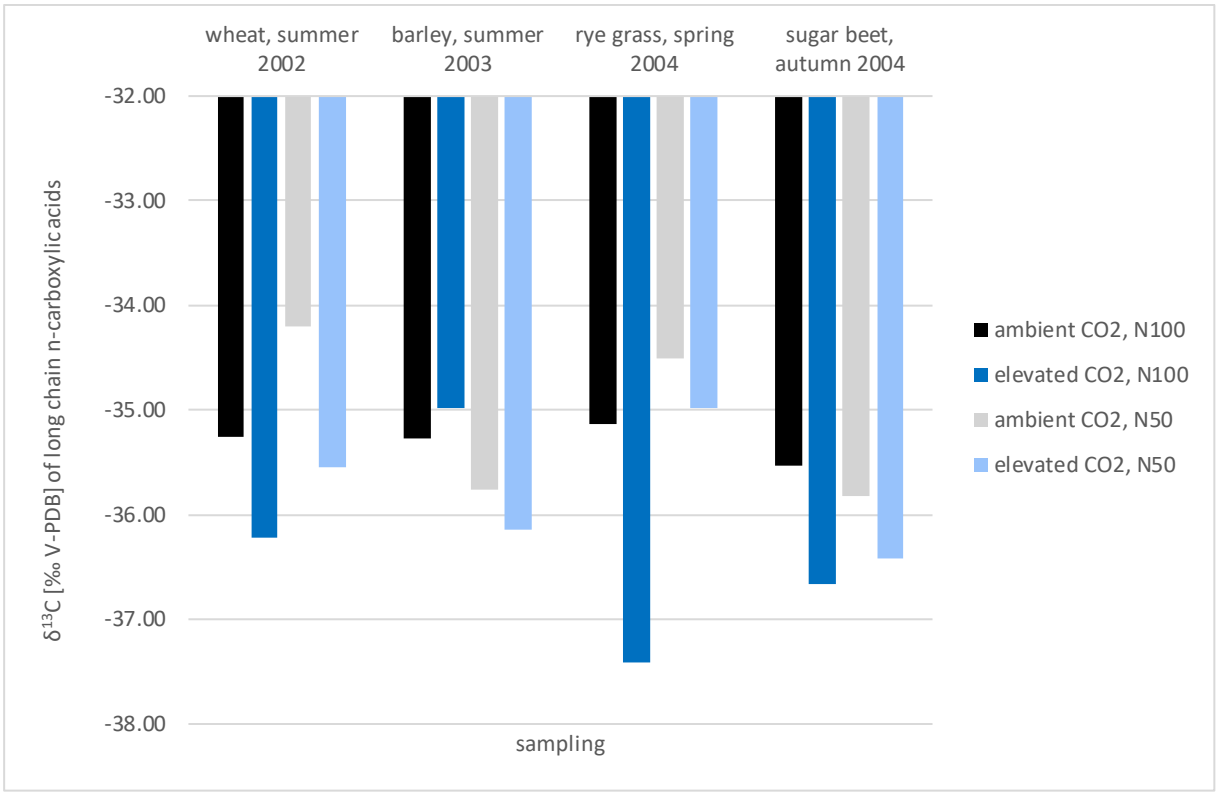

Figure 23. Mean average isotopic composition [‰ V-PDB] of most abundant long-chain n-carboxylic acids under different treatments. On the plots the plant wheat, barley, rye grass and sugar beet were cultivated in this order.

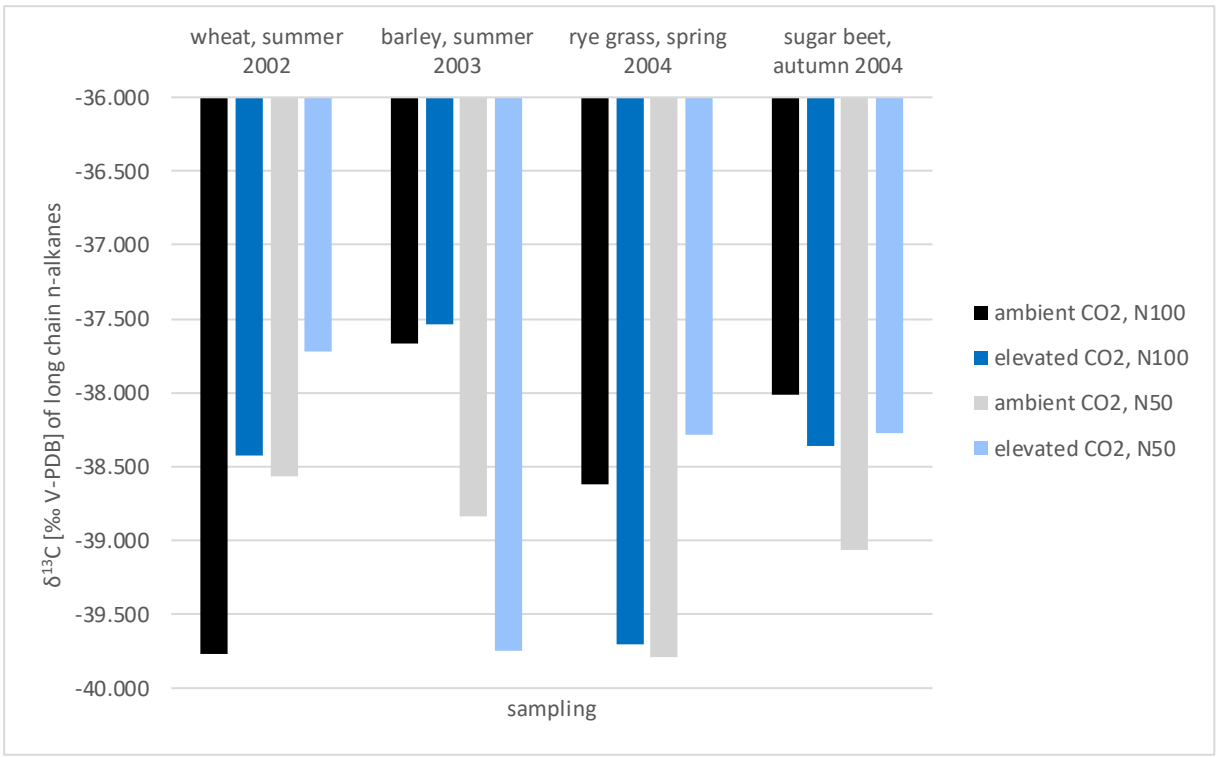

Figure 24. Mean average isotopic composition [‰ V-PDB] of most abundant long-chain n-alkanes under different treatments. On the plots the plant wheat, barley, rye grass and sugar beet were cultivated in this order.

*Table 1. The values of the elemental analysis (total OC, isotopic composition and the corresponding standard deviations as well as C/N ratio) of the individual samples. *These results were obtained from samples which were already extracted and therefore had a lower C content as unextracted samples. These values were corrected using an approximation.*

plant species	sample type	treatment	mean $\delta^{13}C$ [‰ V-PDB]	std $\delta^{13}C$ [‰ V-PDB]	mean $\delta^{15}N$ [‰ V-ATM]	std $\delta^{15}N$ [‰ V-ATM]	total OC[%]	std OC [%]	C/N [-]	normalised extract weight [mg/g]
wheat	above-ground biomass	ambient CO2, N100	-29.47	0.25	-0.38	0.2	42.86	1.35	61.1	20.28
wheat	above-ground biomass	elevated CO2, N100	-43.33	0.43	0.69	1.3	45.47	0.60	70.7	23.72
wheat	above-ground biomass	ambient CO2, N50	-28.99	0.13	-2.01	0.2	45.43	0.75	93.9	18.37
wheat	above-ground biomass	elevated CO2, N50	-42.34	0.21	-1.65	0.7	46.48	0.31	110.3	23.83
barley	above-ground biomass	ambient CO2, N100	-28.21	0.07	-2.93	0.4	43.42	0.63	96.6	21.41
barley	above-ground biomass	elevated CO2, N100	-42.98	0.35	-4.17	1.0	43.78	1.97	140.0	18.46
barley	above-ground biomass	ambient CO2, N50	-28.35	0.18	-3.62	0.1	42.21	1.38	180.7	19.78
barley	above-ground biomass	elevated CO2, N50	-42.49	0.19	-4.48	0.4	43.78	2.22	147.9	18.21
sugar beet	above-ground biomass	ambient CO2, N100	-30.39	0.18	3.38	0.4	42.14	3.11	10.5	102.55
sugar beet	above-ground biomass	elevated CO2, N100	-44.33	0.18	3.99	0.7	38.64	0.93	10.4	79.71
sugar beet	above-ground biomass	ambient CO2, N50	-30.40	0.31	4.02	0.2	42.42	0.37	11.0	99.82
sugar beet	above-ground biomass	elevated CO2, N50	-44.57	0.05	5.62	0.7	42.55	6.44	10.1	90.79
wheat	soil	ambient CO2, N100	-26.71	0.26	6.34	0.6	1.28	0.25	10.7	0.37
wheat	soil	elevated CO2, N100	-26.36	0.07	5.12	0.6	0.81	0.13	10.4	0.60
wheat	soil	ambient CO2, N50	-26.91	0.12	7.01	0.3	0.70	0.05	10.0	0.48
wheat	soil	elevated CO2, N50	-27.19	0.18	6.22	1.2	1.02	0.18	12.0	0.64
barley	soil	ambient CO2, N100	-26.81	0.02	6.82	1.2	1.14	0.11	10.5	0.42
barley	soil	elevated CO2, N100	-27.23	0.50	6.09	2.0	1.01	0.14	10.6	0.41
barley	soil	ambient CO2, N50	-26.80	0.10	6.28	1.4	1.15	0.00	10.0	0.47
barley	soil	elevated CO2, N50	-27.08	0.38	6.13	1.3	1.38	0.26	10.6	0.44
rye grass	soil	ambient CO2, N100	-26.58	0.02	6.63	1.5	1.10	0.02	10.8	0.54
rye grass	soil	elevated CO2, N100	-27.85	0.12	4.63	0.3	0.95	0.24	12.5	0.41
rye grass	soil	ambient CO2, N50	-26.58	0.04	5.43	0.3	0.98	0.00	11.5	0.40
rye grass	soil	elevated CO2, N50	-27.61	0.44	6.09	1.1	1.31	0.24	10.9	0.47
sugar beet	soil	ambient CO2, N100	-26.82	0.01	7.03	0.3	0.85	0.01	9.9	0.46
sugar beet	soil	elevated CO2, N100	-27.13	0.81	5.70	0.4	1.45	0.05	12.0	0.45
sugar beet	soil	ambient CO2, N50	-27.34	0.04	5.68	1.8	1.26	0.05	11.6	0.50
sugar beet	soil	elevated CO2, N50	-27.49	0.23	4.91	0.7	1.42	0.10	12.7	0.44
wheat	roots	ambient CO2, N100	-29.32	0.00	0.55	0.7	43.99	1.36	43.1	13.54
wheat	roots	elevated CO2, N100	-42.27	0.20	1.00	0.7	47.83	0.21	86.0	13.48
wheat	roots	ambient CO2, N50	-29.19	0.09	0.46	0.3	43.73	1.74	91.0	14.39
wheat	roots	elevated CO2, N50	-42.75	0.16	1.34	0.5	43.85	0.85	97.5	12.57
barley	roots	ambient CO2, N100	-27.89	0.17	-1.07	0.3	45.26	0.91	41.0	17.93
barley	roots	elevated CO2, N100	-40.08	0.11	-2.10	0.1	43.83	2.33	61.9	18.52
barley	roots	ambient CO2, N50	-28.13	0.11	-1.04	0.2	43.99	1.41	70.3	21.59
barley	roots	elevated CO2, N50	-40.56	0.64	0.07	0.0	48.37	0.36	76.0	19.50

Table 2. The values of the GC-FID and GC-IRMS (the ratios of n-*alkanes,* n-*carboxylic acids and mean average isotopic composition of most abundant alkanes respectively carboxylic acids) of the individual samples. Root samples were contaminated with additional C_{20}. Therefor this acids were excluded for calculations.*

plant species	sample type	treatment	n-alkanes			n-carboxylic acids						CSIA	
			C29/(C29+C31)	ACL [µg/g]	CPI	16:0/18:0	18:2/18:1	(18:1+18:2)/18:0	long/short	ACL [µg/g]	CPI	$\delta M_{alkanes}$ [‰ V-PDB]	δM_{acids} [‰ V-PDB]
wheat	above-ground biomass	ambient CO2, N100	0.40	30.1	41.4	9.33	0.60	3.49	0.44	18.76	4.81	-39.1	-39.1
wheat	above-ground biomass	elevated CO2, N100	0.38	30.2	43.4	10.88	0.53	6.13	0.30	18.36	3.57	-52.8	-53.5
wheat	above-ground biomass	ambient CO2, N50	0.42	30.1	45.3	7.73	0.39	6.20	0.27	18.57	2.82	-40.8	-39.4
wheat	above-ground biomass	elevated CO2, N50	0.40	30.1	41.0	9.41	0.59	6.79	0.42	19.06	2.32	-51.7	-51.9
barley	above-ground biomass	ambient CO2, N100	0.49	29.5	22.2	10.22	0.59	4.73	0.28	18.31	3.36	-40.4	-38.4
barley	above-ground biomass	elevated CO2, N100	0.52	29.2	23.0	11.69	0.93	4.94	0.48	19.17	5.22	-52.3	-52.7
barley	above-ground biomass	ambient CO2, N50	0.55	28.9	21.4	9.24	0.57	4.50	0.36	18.43	5.17	-40.7	-39.2
barley	above-ground biomass	elevated CO2, N50	0.52	29.4	19.0	9.24	0.83	5.57	0.46	18.96	6.16	-53.8	-51.5
sugar beet	above-ground biomass	ambient CO2, N100	0.55	35.4	6.2	9.04	0.26	1.42	0.25	17.83	8.41	-44.7	-40.2
sugar beet	above-ground biomass	elevated CO2, N100	0.57	35.9	6.7	11.93	0.30	5.03	0.15	17.63	4.64	-56.0	-54.1
sugar beet	above-ground biomass	ambient CO2, N50	0.60	35.0	6.7	8.57	0.37	1.90	0.42	19.16	5.64	-44.0	-40.2
sugar beet	above-ground biomass	elevated CO2, N50	0.55	42.6	7.2	10.35	0.46	3.21	0.22	17.88	6.07	-54.6	-55.6
wheat	soil	ambient CO2, N100	0.36	27.2	7.7	2.70	0.10	0.63	1.23	19.33	3.47	-39.8	-35.3
wheat	soil	elevated CO2, N100	0.39	27.1	6.1	3.46	0.06	0.44	0.90	19.17	3.40	-38.4	-36.2
wheat	soil	ambient CO2, N50	0.40	27.6	5.4	3.89	0.00	0.28	0.42	17.33	3.05	-38.6	-34.2
wheat	soil	elevated CO2, N50	0.40	27.5	6.3	2.45	0.00	0.30	0.48	17.85	3.85	-37.7	-35.5
barley	soil	ambient CO2, N100	0.41	27.5	6.0	2.59	0.10	0.40	0.69	17.59	2.97	-37.7	-35.3
barley	soil	elevated CO2, N100	0.38	28.0	8.3	2.77	0.08	0.38	1.02	18.90	3.42	-37.5	-35.0
barley	soil	ambient CO2, N50	0.38	28.4	6.9	3.07	0.08	0.41	0.88	18.77	2.92	-38.8	-35.8
barley	soil	elevated CO2, N50	0.40	27.3	7.6	2.91	0.10	0.64	0.57	17.77	2.96	-39.8	-36.1
rye grass	soil	ambient CO2, N100	0.39	26.6	6.8	1.84	0.19	0.17	0.99	19.24	3.31	-38.6	-35.1
rye grass	soil	elevated CO2, N100	0.39	27.0	6.1	2.52	0.09	0.53	1.10	19.43	3.17	-39.7	-37.4
rye grass	soil	ambient CO2, N50	0.40	26.6	7.5	3.44	0.10	0.42	0.70	17.58	3.02	-39.8	-34.5
rye grass	soil	elevated CO2, N50	0.39	26.9	7.0	2.54	0.19	0.23	0.76	18.87	2.99	-38.3	-35.0
sugar beet	soil	ambient CO2, N100	0.40	27.6	6.9	2.75	0.13	0.55	0.47	17.13	3.10	-38.0	-35.5
sugar beet	soil	elevated CO2, N100	0.40	27.6	6.7	2.43	0.11	0.34	0.81	18.77	3.44	-38.4	-36.7
sugar beet	soil	ambient CO2, N50	0.40	27.1	7.6	2.91	0.10	0.67	1.03	18.59	3.12	-39.1	-35.8
sugar beet	soil	elevated CO2, N50	0.38	26.9	6.9	2.76	0.13	0.62	0.55	17.30	2.85	-38.3	-36.4
wheat	roots	ambient CO2, N100	0.44	29.5	8.5	4.08	0.07	2.38	0.45	18.93	4.86	-41.0	-38.9
wheat	roots	elevated CO2, N100	0.45	29.4	7.0	5.19	0.19	2.66	0.42	18.84	5.51	-44.2	-50.6
wheat	roots	ambient CO2, N50	0.44	29.4	8.5	2.89	0.07	1.73	0.42	18.58	6.39	-37.5	-37.5
wheat	roots	elevated CO2, N50	0.43	29.6	9.5	3.79	0.10	2.54	0.42	16.92	5.77	-47.6	-51.0
barley	roots	ambient CO2, N100	0.63	28.4	5.2	3.19	0.11	2.50	0.56	18.47	5.14	-37.5	-38.6
barley	roots	elevated CO2, N100	0.64	28.3	5.1	3.87	0.17	1.98	0.55	18.16	5.47	-44.3	-48.6
barley	roots	ambient CO2, N50	0.62	27.3	5.1	2.19	0.00	0.82	0.62	18.77	8.60	-37.6	-39.5
barley	roots	elevated CO2, N50	0.59	28.4	6.4	3.65	0.06	3.73	0.30	18.13	6.97	-44.2	-47.7

4. Discussion

4.1. Elevated CO_2-concentration

The C content of the plant samples of 39-48%, was about the same as Wiesenberg et al. (2008a) found in their study for plant samples. It is a typical content for many plants. In this study the C-content increased for the straw and root samples of wheat and barley under elevated CO_2.

Wiesenberg et al. (2008a) observed an increase in 0.3% C content under elevated CO_2 conditions after ten years in a grassland FACE experiment (Eschikon, CH). The increase from the start to the end of the second crop rotation in this thesis are in the same range. However, the content varied strongly depending on the crops which were used for cultivation. Dietzen et al. (2019) who analysed soil samples of a temperate heath could detect a significant accumulation of C under elevated CO_2 even under dry conditions and higher temperatures. Giesemann & Weigel (2008) could see in the two crop rotations in Braunschweig also a slight increase of C content under elevated conditions, which also variated depending on the crop. They detected a higher C accumulation under elevated CO_2 after the cultivation of sugar beet which could also be detected in this thesis. However, the changes of C content were not statistically significant. These smaller changes compared to the results of Dietzen et al. (2019) are likely due to the cultivation of the agricultural plots where the upper layer was regularly ploughed and the C pools got mixed.

The C/N ratio of the plant biomass increased for most of the plants under elevated CO_2 slightly, which leads to changes in the quality of the biomass (Weigel et al., 2005). Also, the C/N ratio of the soil samples increased during time. Griepentrog et al. (2015) who have investigated influence of elevated CO_2 and N deposition on trees in a FACE experiment, found lower N concentration in spruce samples under elevated CO_2 condition versus ambient air. Which resulted in slightly higher C/N ratios.

The content of the extractable lipid was lower for straw and root samples than for specimens with leaves. The lipid contents of the plants were similar for samples under elevated CO_2 conditions and ambient air. Also, Wiesenberg et al. (2008a) found similar lipid values for rye grass and clover under different CO_2 treatments which was consistent with literature, but can also change with plant. The lipid extract of the soils analysed by Wiesenberg et al. (2008a) had higher concentrations as the extracts of the soils in this thesis. Under enriched CO_2 conditions the extracts increased for both soils compared to the ambient air. However, in this thesis some samples also had lower contents under higher CO_2 concentration.

It could be seen that for the grass samples had a decrease in unsaturated C_{18} acids under elevated CO_2 conditions. However, sugar beet samples had higher abundance of unsaturated C_{18} acid under elevated CO_2 concentration compared to the samples of the control plot. In the FACE experiment in Eschikon also a depletion of unsaturated C_{18} acids under enriched CO_2 concentration could be observed for rye grass and clover (Wiesenberg et al., 2008a). This depletion was explained due to stress and resulted in higher abundance of C_{16} acids. In this thesis the proportion of C_{16} to C_{18} in plant samples increased under elevated CO_2 concentration by 13 to 32%. An increment of about 7-37% could also be observed in most soil samples under elevated CO_2. The ratio of $C_{18:2}/C_{18:1}$ also increased by most plant samples by 14-58% and for soil samples by 18-56% under elevated CO_2 concentration in comparison to control samples. The proportion of unsaturated to saturated C_{18} acids in soils and plants was higher under elevated CO_2 conditions than under ambient air, which also was observed in Wiesenberg et al. (2008a), although they also considered the saturated and unsaturated C_{16} acids. The ratio of long chain to shot chain acids was lower for plants as for soils. That was consistent with Wiesenberg et al. (2008a) they also found a higher ratio for soils as

plants. As plants are abundant with short chain homologues (Walton, 1990 in Wiesenberg et al., 2008a). For soils a higher long/short chain ratio could be due to faster degradation and lower input of short chain acids and a selective preservation and stabilisation of longer chain acids (Wiesenberg et al., 2008a). ACL and CPI values were not really influenced by elevated CO_2 concentration.

Because of the changes in proportions of different carboxylic acids, it could be shown, that the carboxylic acid composition changed due to an elevation in CO_2.

Most samples had slightly higher $C_{29}/(C_{29}+C_{33})$ ratios under ambient air than under enriched CO_2 concentrations. This is in contrast to the results of Wiesenberg et al. (2008a) who had higher values under elevated CO_2 conditions. ACL values could be seen to have specific ranges also in soil according to the plant species. A higher CO_2 concentration did not impact ACL values visibly. CPI values were higher for above-ground biomass and lower for roots and soil samples when CO_2 concentration was elevated versus control plots. As the $\delta^{13}C$ values in plant samples were depleted by 12‰-15‰ V-PDB under elevated CO_2 concentration, the labelling of plants with isotopically-depleted C was detectable. This depletion was slightly higher as was detected by Wiesenberg et al. (2008b), who observed a depletion by 9.2 ‰ and 9.5‰. Even for the soils, slightly more negative values could be detected under elevated CO_2 in bulk $\delta^{13}C$. Also, Giesemann & Weigel (2008) could detect a decrease in bulk $\delta^{13}C$ in the Braunschweig FACE experiment. The soils in the grassland FACE experiment in Eschikon measured a depletion in bulk $\delta^{13}C$ values by 3‰-4.4‰. Whereas, the soil samples in this experiment had much smaller changes from 0.1‰-1.3‰. Therefore, it can be expected that the labelled CO_2 entered the soil. A depletion could also be due to a an enhanced isotopic fractionation in plants during biosynthesis (Zhao et al., 2001). However, the signal in soils was so small because through ploughing old and new C was mixed that the signal of $\delta^{13}C$ values was depleted. The bulk isotopic values were higher compared to the isotopic values of carboxylic

4.2. Lower N-fertilisation

When the fertilisation rate of samples was halved it could not be seen a consistent influence on plant and soil samples. However, over time it could be seen that there is an increase of about 6% in C content in soil over crop rotation period when soils were less fertilised. For normally fertilised soils the C content even decreased by about 0.2 %. C/N ratio of most plant samples were higher under reduced fertilisation and the soil C/N ratio decreased overtime by 17% when the plot was fertilised with a reduced amount of N. These findings could also be observed by Griepentrog et al. (2015). They saw that higher N deposition increased N content of spruce needle and lead to higher C/N ratios under lower fertilised samples

Lipid content of different plants were not equally affected by a lower N fertilisation. No clear trend could be observed. Griepentrog et al. (2015) neither found different lipid extract content under lower N deposition compared to higher N deposition.

A lower fertilisation of ambient air plots decreased $C_{16:0}/C_{18:0}$ values for plants but increased it for soils. $C_{16:0}$ was less abundant in plant samples but showed a higher abundance in soil samples. The proportion of $C_{18:2}/C_{18:1}$ in plants were not consistently influenced by lower fertilisation but soils had a slight decrease in $C_{18:1}$ under lower fertilisation. There could not be seen an influence of fertilisation on the proportion of unsaturated versus saturated C_{18} acids and long/ short chain fatty acids. CPI values increased in roots under lower fertilisation and decreased in soils compared to normally fertilised soils.

For n-alkanes the content of C_{29} compared to C_{33} increased slightly in soils when the plots recived less fertiliser. ACL and CPI values of n-alkanes did not change when fertilisation changed under ambient plots.

For the $\delta^{13}C$ a slight depletion under lower N deposition could be observed. That is consistent with Gripeentrog et al. (2016). In their experiment also $\delta^{13}C$ values of carboxylic acids decreased with lower N deposition (Griepentrog et al., 2015) which could not be seen in this thesis but in the thesis of Huang et al. (1999).

4.3. Combined effect (elevated CO_2-concentration & lower N-fertilisation)

When combining the effects of lower fertilisation and higher CO_2 a change of C could not be seen compared to control plots. However, when looking at the C content in soils during time it can be seen that plots with combined effects had highest C contents, but at the last measurement C content of lower fertilised and higher fertilised plots under elevated CO_2 were similar. The plant samples had higher C/N ratios under combined effects than under ambient CO_2 also in Griepentrog et al. (2015) spruce needles were significantly affected. When looking at C/N ratios a lower fertilisation only had similar ratios as combined effects.

For $C_{16:0}/C_{18:0}$ ratios were similar under combined effects and control plots. Influences which could be seen under the other treatments, disappeared under combined effects. The same could be seen for ratios $C_{18:2}/C_{18:1.,}$ for ACL and CPI of carboxylic acids. The ratios of alkanes were not strongly affected by lower fertilisation and higher CO_2 condition. For the ratio of long versus short chain carboxylic acids there could be seen lower values under combined effects than under control plot. So a decrease in fertiliser resulted in more short chain acids. As microbial biomass has shorter chains as plant derived carboxylic acids (Hardwood & Russel,1984) it can be assumed that under combined treatment had an effect on soil microorganisms.

$\delta^{13}C$ values were also strongly depleted in plant samples. A difference because of lower fertilisation could not be detected in $\delta^{13}C$ values. For compound specific isotopic composition there could not be seen a clear influence of combined effects on the most abundant n-alkanes, but the $\delta^{13}C$ values decreased by combined effects.

So it could be seen that plant samples and soil samples were influenced by changes in treatment . However due to mixing of soil due to ploughing most influences for plants could not be seen in soils. The main drawback for this thesis is that there were only few replicates. However, there could be seen patterns according to treatment, which indicates that extraction and GC-analysis were conducted properly.

5. Conclusion

In the FACE experiment in Braunschweig an influence of elevated CO_2 and the amount of fertilisation could be detected. An increase in C content in soil could be seen for plots with increased CO_2 concentrations. C/N ratios increased in soils and plants when exposed to higher CO_2 concentrations which influences degradability. Differences in ratios of carboxylic acids could be detected under elevated CO_2 also lower N fertilization could change composition of lipids which can change composition of leave waxes. This can also have influences on soil organisms and also different lipid compostions can have a negative influence on baking quality.

6. Outlook

Although many studies have been conducted about influences of elevation of CO_2 concentration on soil and plants, it would also be interesting how this effect would influence the environment when combined with other stress factors which are predicted as climate change advances. It would be interesting to further analyse the samples obtained through CSIA and look at the influences on different compounds and biosynthesis. It was often argued, that FACE experiments have better results than chamber experiments. However, Allen et al. (2020) argue that the fluctuation of CO_2 in the FACE experiments is responsible for lower CO_2 effects compared to chamber experiments. Because of this new view, it would be interesting to further investigate which experiment setting is most useful.

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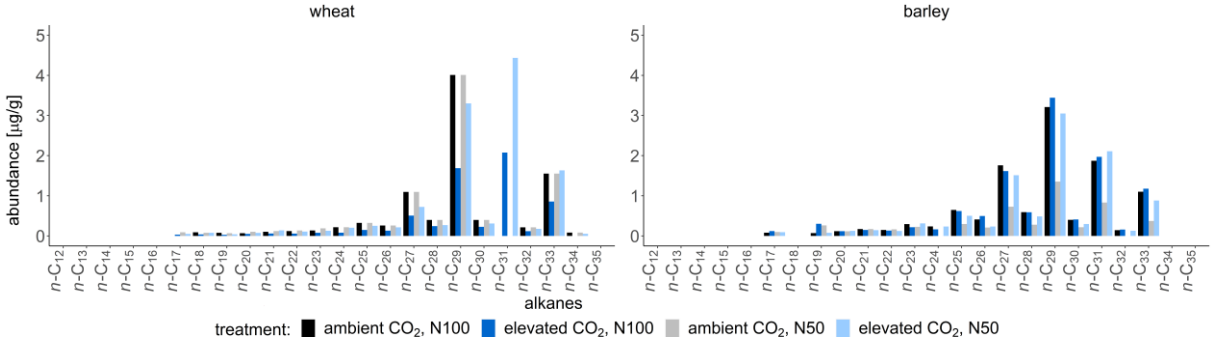
Appendix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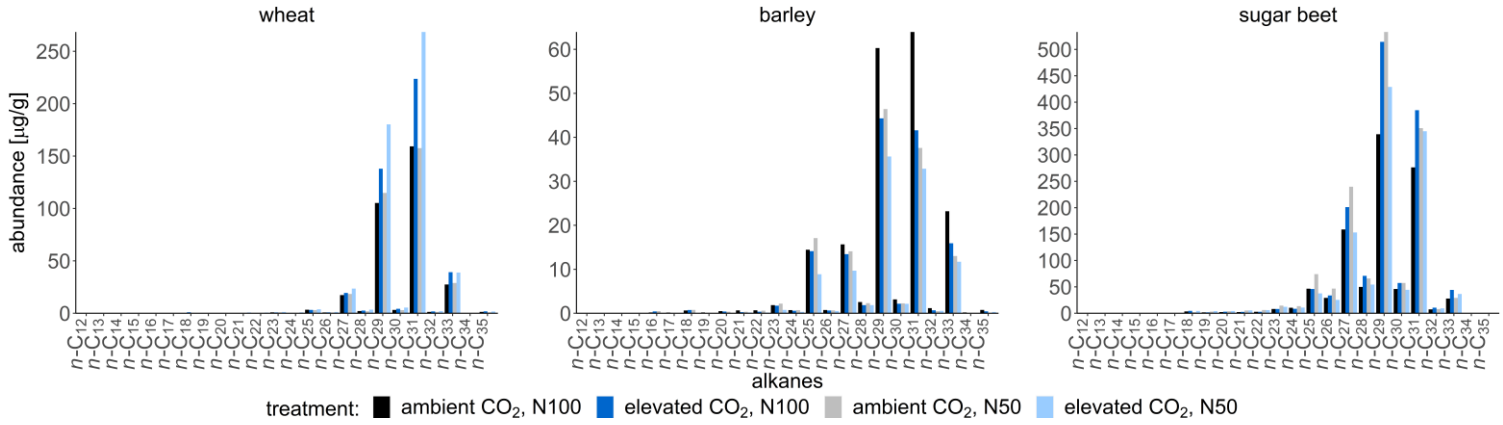
barley
abundance [µg/g]
alkanes
treatment: ambient CO2, N100 elevated CO2, N100 ambient CO2, N50 elevated CO2, N50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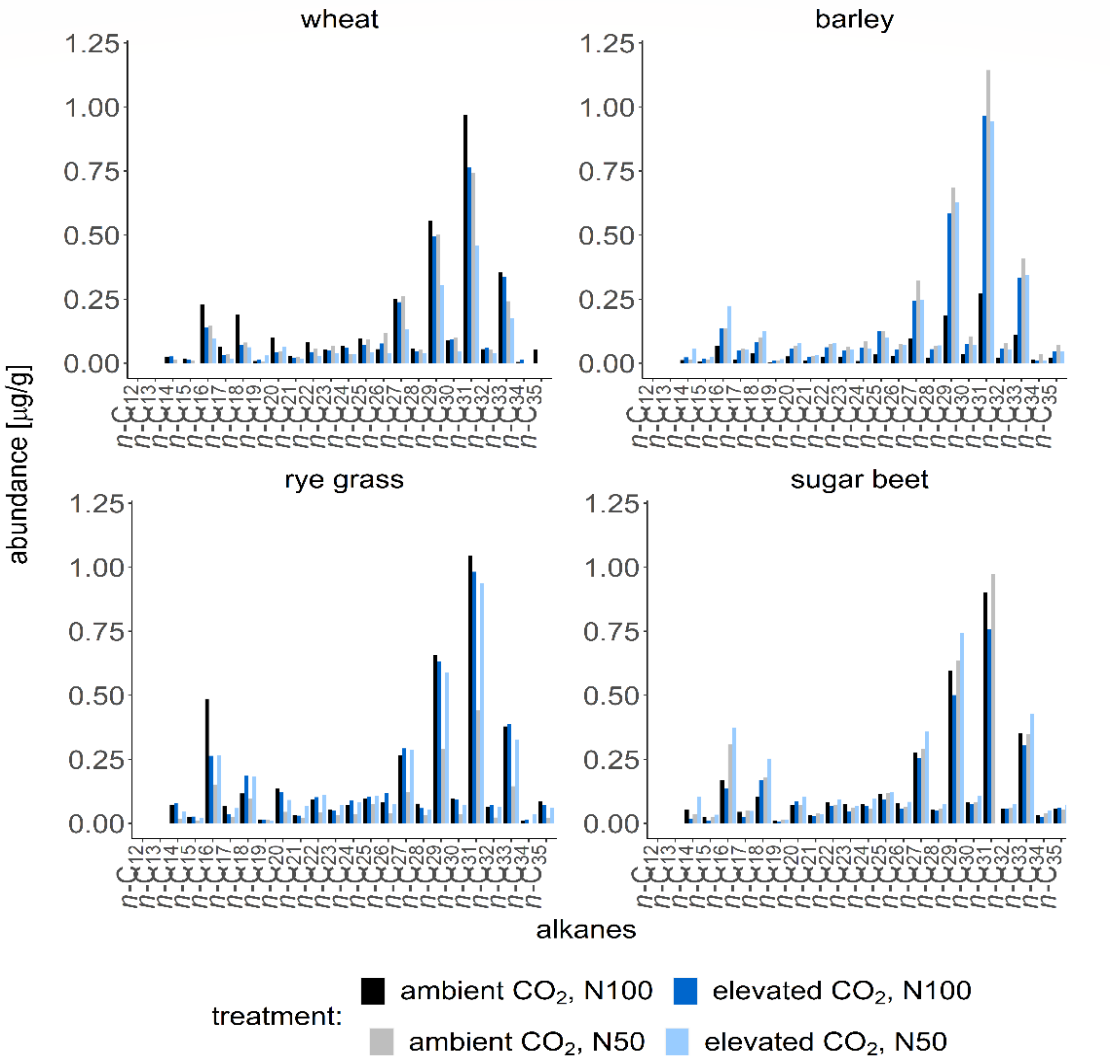
barley
sugar beet
abundance [µg/g]
alkanes
treatment: ambient CO2, N100 elevated CO2, N100 ambient CO2, N50 elevated CO2, N50


wheat
barley
rye grass
sugar beet
abundance [µg/g]
1.25
1.00
0.75
0.50
0.25
0.00
n-C12
n-C13
n-C14
n-C15
n-C16
n-C17
n-C18
n-C19
n-C20
n-C21
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n-C29
n-C30
n-C31
n-C32
n-C33
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alkanes
treatment:
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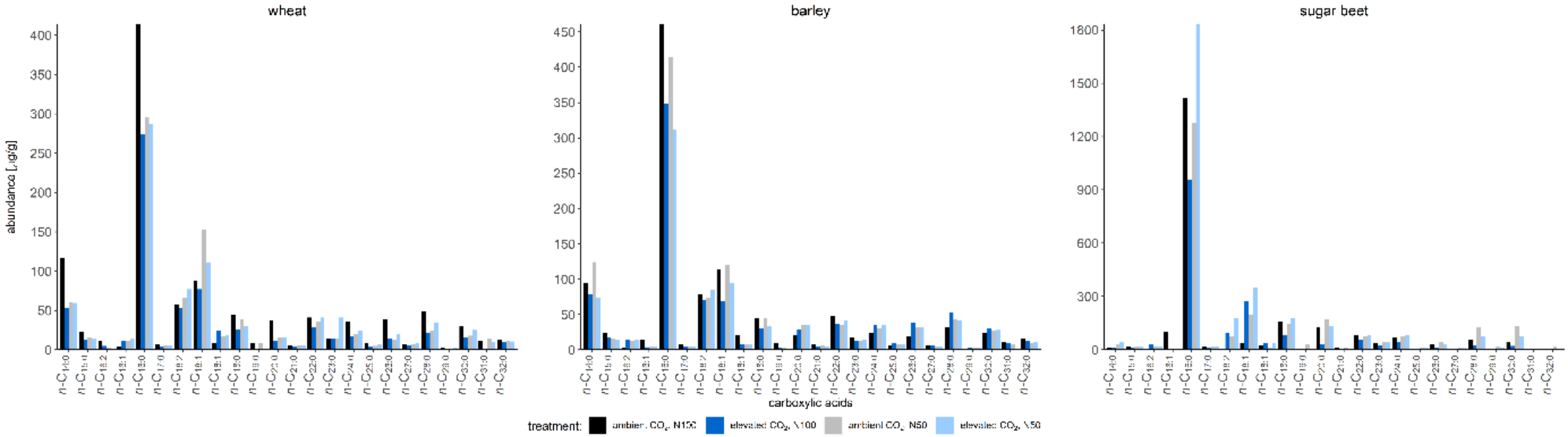
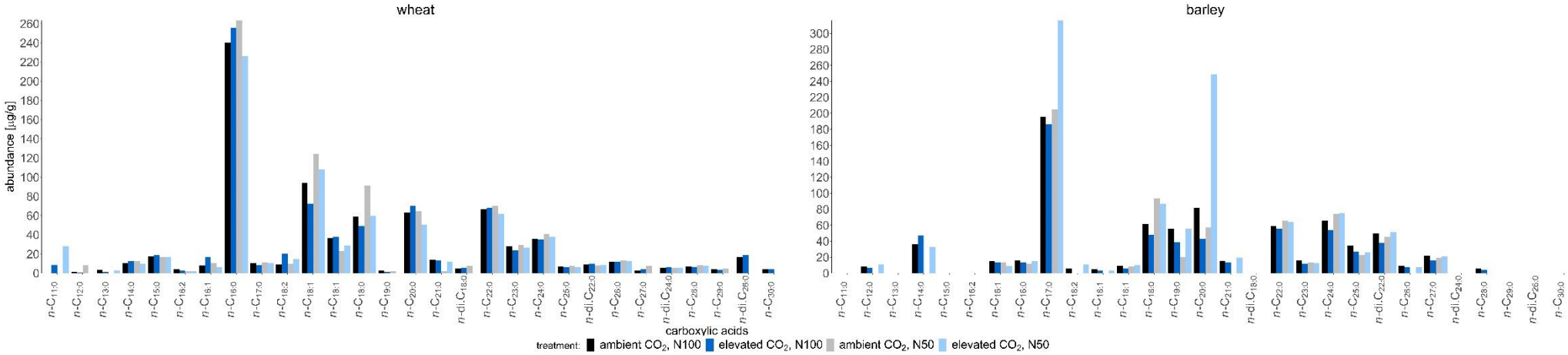
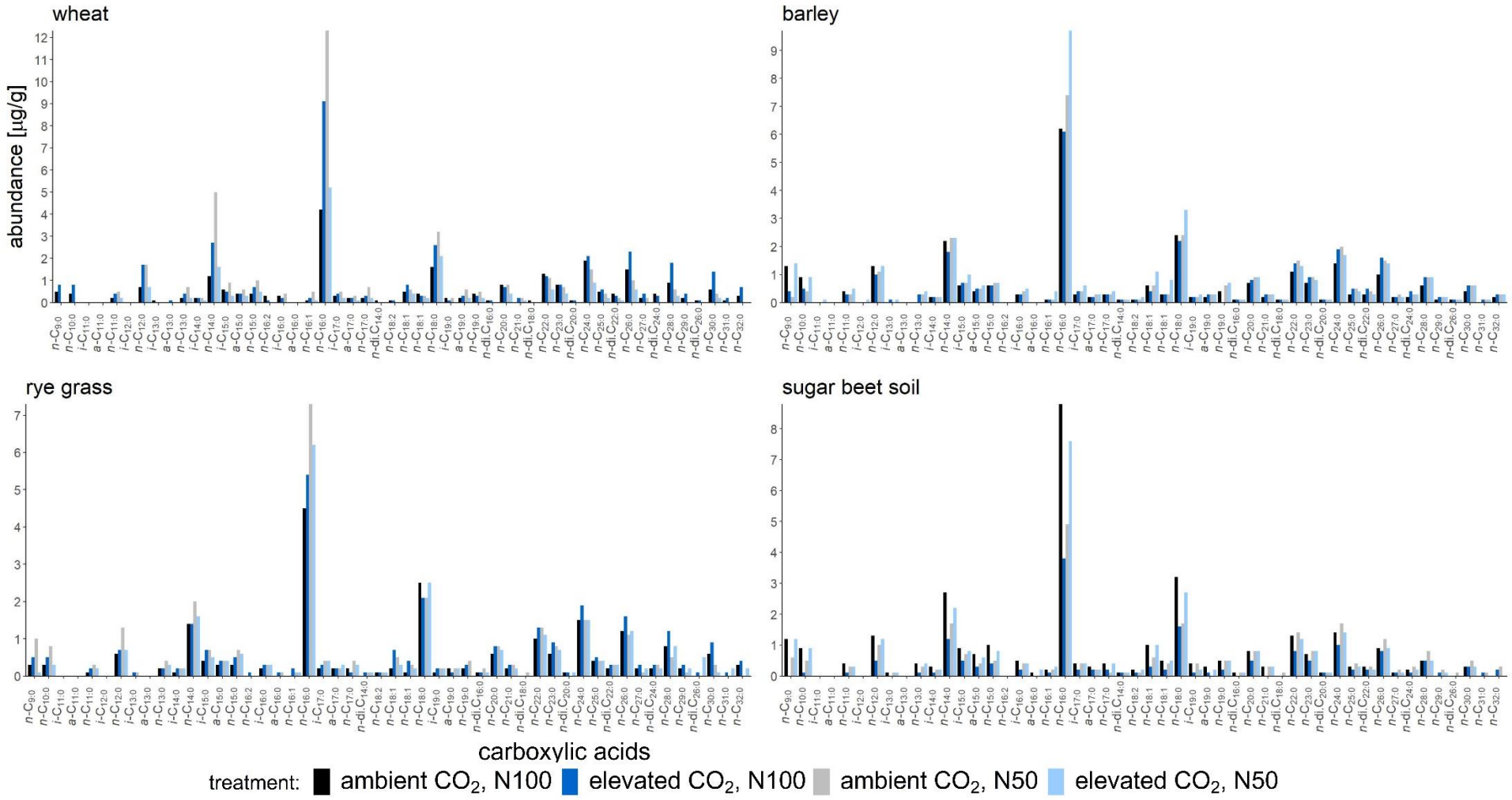
elevated CO2, N100
ambient CO2, N50
elevated CO2, N50


wheat
barley
sugar beet
abundance [µg/g]
carboxylic acids
treatment: ambient CO2, N100 elevated CO2, N100 ambient CO2, N50 elevated CO2, N50


wheat
barley
abundance [µg/g]
carboxylic acids
treatment: ambient CO2, N100 elevated CO2, N100 ambient CO2, N50 elevated CO2, N50


wheat
barley
rye grass
sugar beet soil
abundance [µg/g]
carboxylic acids
treatment: ambient CO2, N100 elevated CO2, N100 ambient CO2, N50 elevated CO2, N50

Personal declaration

I hereby declare that the submitted thesis is the result of my own, independent work. All external sources are explicitly acknowledged in the thesis.

Herznach, 31th of August 2020

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