The Impact of Biochar Incorporation on Inorganic Nitrogen Fertilizer Plant Uptake; An Opportunity for Carbon Sequestration in Temperate Agriculture

Rebecca Hood-Nowotny 1,*, Andrea Watzinger 1, Anna Wawra 1 and Gerhard Soja 2,3

1 Institute of Soil Research, University of Natural Resources and Life Sciences (BOKU), Peter-Jordan-Straße 82, 1190 Vienna, Austria; Andrea.Watzinger@boku.ac.at (A.W.); Anna.Wawra@boku.ac.at (A.W.)

2 Environmental Resources & Technologies, Austrian Institute of Technology (AIT), Konrad-Lorenz-Straße 24, 3430 Tulln, Austria; Gerhard.Soja@ait.ac.at

3 Institute for Chemical and Energy Engineering, University of Natural Resources and Life Sciences (BOKU), Muthgasse 107, 1190 Vienna, Austria

* Correspondence: Rebecca.Hood@boku.ac.at; Tel.: +43-1-47654-91176

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Abstract: Field studies of biochar addition to soil and nutrient cycling using $^{15}$N fertilizers in temperate agriculture are scant. These data are required in order to make evidence based assessments. This study was conducted to test the hypothesis that biochar application can increase crop yields through improving the nitrogen uptake and utilization of added inorganic fertilizer, whilst sequestering significant quantities of carbon. Results showed that although biochar addition led to significant spring barley grain yield increases in the first year of biochar application, an unusually dry year; this was possibly not solely the result of improved nitrogen uptake, as total crop N was similar in both treatments. Results suggested it was improved water utilization, indicated by the crop carbon isotope values and soil moisture characteristics. In the second year, there were no significant effects of the previous year’s biochar addition on the sunflower yield, N status, fertilizer recovery or any signs of improved water utilization. These data add to a growing body of evidence, suggesting that biochar addition has only slightly positive or neutral effects on crop growth and fertilizer retention but has the potential to sequester vast amounts of carbon in the soil with minimal yield losses in temperate agriculture.

Keywords: $^{15}$N; stable isotopes; isotope ratio mass spectrometry; chernozem; soil; biochar

1. Introduction

Ancient Amazonian soil practices of nurturing Terra Preta through the addition of charcoal to soil and its modern equivalent, biochar production followed by soil incorporation, could provide humanity, one of many carbon farming solutions to global climate change and escalating food demand. Indeed, it has been suggested that biochar addition to soil could sequester 0.7 GtCeq yr$^{-1}$ globally approximately one tenth of the annual anthropogenic fossil fuel flux [1]. However economic incentives will be required to stimulate this transformational change in farming behaviour, this could be in the form of yield increases or compensatory payments, for example, an agri-environment scheme for carbon sequestration. Despite the failures in carbon trading schemes, scholars have suggested the social cost of carbon is in the region of $100/125 t \text{ Ceq}^{-1}$ and that a global carbon tax and coupled negative emission technology (NET) credit, levied at this rate could avoid catastrophic climate change [2]. Exploring the risks and potential of these scenarios requires agro-ecozone specific field data utilizing common or feasible farming practices and determining how these practices effect important aspects of crop production such as fertilizer use.
These scenarios highlight the important role soils could play in balancing global carbon exchanges. Currently, soils account for two-thirds of the terrestrial carbon pool [3] and the flux to the atmosphere from soil respiration is ten times greater than that from fossil fuel combustion [4]. Within this context, biochar addition to soils is a feasible strategy of carbon sequestration, particularly in tropical soils, where biochar has been shown to improve soil properties and increase crop yields [5–8]. Biochar additions have also been shown to reduce greenhouse gas emissions of carbon dioxide, methane and nitrous oxide (CO$_2$, CH$_4$ and N$_2$O) [9–12]. It is these important agronomic properties, in addition to the potential to sequester significant amounts of carbon from centuries to millennia [13–18], that has stimulated a wealth of research into biochar. However relatively few studies have investigated the impact of biochar in temperate agronomic systems; most are focused on C cycling rather than N cycling. Moreover, very few studies have used labelled N fertilizer to investigate its agronomic fate when used in combination with biochar. The inherent fertility, neutral pH and higher organic matter contents associated with temperate soils, suggest that biochar addition would not lead to significant improvements in crop yields [5,19]. Nevertheless, given the changing political and agronomic landscape of European farming there may be opportunities for carbon-farming with associated economic gains for the farmers as discussed above.

Jeffery et al. [20] suggested that the main mechanisms for yield increase following biochar addition were the liming effects and improved water holding capacity of the soils, in addition to improved crop nutrient availability. Given this, we wanted to investigate the impacts of biochar addition on fertilizer use efficiency and related parameters such as yield, whilst also attempting to understand biochar’s impact on soil water availability to the crop, in a temperate farming system.

We set out to trace the fate of fertilizer nitrogen into the soil and crop and to determine whether the addition of significant quantities of biochar to soil had an effect on the plant fertilizer utilization. We hypothesize that biochar does not lead to the significant immobilization of essential crop nutrients. Immobilization of nutrients specifically nitrogen-N, in-part has hampered the wide spread adoption of practices such as no-till, where straw is left on the soil [21]. This is because the untimely incorporation of un-charred carbon can lead to significant immobilization of essential N for plant growth and thus depress yields, unless sufficient inorganic N is added. The reason for this is that the dominant biogeochemical processes of mineralization and nitrification are mediated by the soil microbial biomass (SMB), which requires N for metabolism, maintenance, growth and enzyme production. On addition of high carbon-C containing substrates, such as straw with a high C:N ratio ~100:1, to soil, the microbial biomass will respond to exploit the carbon input as a new energy source. This response requires N to fuel enzyme production, maintenance and growth. In order to sustain its C:N ratio, the SMB will take up available inorganic soil-N, in-turn leaving less N available for plant uptake and consequently temporarily depressing yields, as the dominant SMB constituents, fungi and bacteria have C:N ratios of 15:1 and 5:1 respectively [22].

Nitrogen immobilization is one of the main problems of nitrogen fertilizer management. The key role that inorganic fertilizer N has played in increasing crop yields, in particular of cereals, is widely recognized [23]. Nitrogen deficiency can have adverse effects on crop development and yield. Stable isotope techniques have played a major role in understanding the fate of applied N fertilizer. Soil processes are notoriously complex and interdependent; stable isotopes facilitate element tracing with minimal perturbation to the on-going agronomic operations. Stable isotope labelling (i.e., producing chemically “identical” fertilizers with different isotopic signatures) allows us to follow the fate of fertilizer nitrogen into the crop, soil, microbial biomass, water and air, giving the opportunity to trace the fate of the fertilizer and make sensible management decisions about fertilizer management [24,25]. This is of particular importance when new agricultural practices are introduced such as the application of biochar to soils, as stable isotope tracing allows us to directly follow the fate and efficiencies of the fertilizer under the new proposed practices.

Nitrogen and water are the main constraints to crop growth in agriculture [26] but it can be difficult to disentangle their impacts. The naturally occurring differences in plant carbon isotope
signatures, as a result of differences in water use efficiency, can offer some insights into crop water usage. Carbon isotope signatures can reveal useful information about the water status of the crop and can assist in untangling the interactions of nutrient and water limitations on crop growth. This study offered the opportunity to investigate if the addition of Biochar with its porous structure influences the soils capacity to store water and deliver water to the crop. Carbon isotope discrimination (Δ) is linearly related to the ratio of the intercellular and atmospheric partial pressures of CO₂ in the C₃ plants [27]. The degree of ¹³C discrimination is directly proportional to the length of time the stomata are open. When open, stomata permit the escape of water (which cools the plant and facilitates the mass transfer of nutrients), ensuring free assimilation of CO₂ and maximum discrimination against the heavier ¹³CO₂. When stomata are closed the availability of CO₂ is limited and plants must take what is there, so discrimination is minimum [27]. As water availability is one of the major controllers of stomatal conductance Δ¹³C serves as a proxy for water availability. Δ is the suggested convention, as the Δ values are the deviation from unity; which basically means the values take into account variation differences in atmospheric isotopic differences in ¹³CO₂ abundance across the globe (see Equation (5)). In effect, Δ normalizes two values into one [27] and therefore makes it easier to directly compare with other results from around the world. Foliar values have been used as an integrated measure of the response of photosynthetic gas exchange to environmental variables such as water availability [28], light [29], humidity [30] and salinity [31] and have been proposed as an indicator of high water use efficiency in C₃ plants (WUE - biomass production per unit water consumed or crop/drop), since a negative correlation between WUE and Δ has been reported for a number of plant species [32]. Conversely high Δ values have been associated with plant water availability in spring barley, with Δ values decreasing as water becomes limited due to stomatal closure and consequent reduction in isotope discrimination against the heavier ¹³CO₂ [27,33,34].

We set out to test the hypothesis that biochar application can increase crop yields through improving the nitrogen uptake and utilization of added inorganic fertilizer. In addition, we investigated the effects of biochar on plant water status using carbon isotope discrimination. Field experiments were conducted in a continental Austrian climate, using local agricultural practices.

2. Materials and Methods

2.1. Experimental Design

The experiments were established to follow the fate of nitrogen fertilizer in the field, amended with biochar under common agricultural practice. This was achieved by applying stable isotope labelled nitrogen fertilizer to sub-plots of an on-going biochar field experiment [35] and harvesting and analysing the plant material obtained from the subplots, to determine the uptake of the ¹⁵N fertilizer added. We did this using elemental analysis isotope ratio mass spectrometry (EA-IRMS). The focus was on comparable rather than absolute values. The rates of Biochar application (<5% W/W based on an approximate 40 cm plough depth) were intended be high concentrations to determine measurable effects of Biochar addition and to determine maximum soil carbon addition using biochar; these rates were comparable with many pot studies of the time. The size and nature of the experiment, which was established to mimic true farmer practice, this meant that it was only feasible to use the labelled fertilizer as a top dressing, as this was a single application and not a standard mixed nutrient formulation as used in the base dressing.

2.1.1. Spring Barley Field Experiment in 2011

The field experiment was established in Traismauer, Lower Austria (48°19′52.6″ N, 15°44′20.5″ E) in March 2011 (Figure 1). The climate is temperate and has a strong continental influence (Pannonian climate), with mean annual precipitation of 550 mm and mean annual temperature of 10 °C. The arable soil was a deep sandy-loamy-silt (18.3% sand, 57.2% silt and 24.5% clay), classified as a calcareous Chernozem on loess. Soil pH of non-biochar treated soil was 7.5 in 10 mM CaCl₂, (1:2.5 soil: CaCl₂,
ratio) the carbonate content was 15.8% and organic matter content 2.4%. Cation exchange capacity of the soil was 22.5 cmol kg\(^{-1}\), dominated by Ca\(^{2+}\) (~90%) and Mg\(^{2+}\) (~7%), with a small contribution by K\(^+\) (2%), available inorganic nitrogen prior to the experiment was approximately 5 \(\mu\)g N g\(^{-1}\) soil. The site has been used for agriculture for >100 years. Hardwood-derived biochar (80% beech, rest derived from other hardwoods) was produced by pyrolysis at 550 °C for two hours and was purchased from S.C. Romchar S.R.L. (Harghita, Romania). This biochar had a pH of 8.6 in water (1:2.5 biochar:water ratio) and 7.5 in 10 mM CaCl\(_2\) (1:2.5 biochar: CaCl\(_2\), ratio) and had the following elemental concentrations: 80.3% C, 9.9% O, 0.4% N and 1.6% H. Volatiles made up 11.1% and ashes 15.2% of biochar dry mass (determined at 550 °C). The Biochar had an H/Corg ratio of 0.3 and a PAH content (\(\sum\) 16 EPA PAH) of 9 mg kg\(^{-1}\) [35]. Further details of the Biochar are described in Sigmund et al., 2017 [36]. Biochar was applied once to the field at a dry mass rate of 0 and 72 t ha\(^{-1}\) in March 2011 and incorporated to a depth of 10 cm using a rotary hoe. There were originally four treatments but only three were used for this study as the forth treatment did not involve matched inorganic-fertilizer applications. The treatments were: control, that is, NPK (no biochar application, NPK added), BCN (72 t biochar ha\(^{-1}\) and NPK added) and for yield and N yield comparison only BC3 (72 t biochar ha\(^{-1}\), no nitrogen fertilizer but 26/31 and 50/100 kg P and K ha\(^{-1}\) in 2011/2012 respectively). The main treatment plots were arranged according to a Latin square design and replicated four times in circular plots of 6.5 m diameter. For yield analysis, net plots of 9.6 m\(^2\) each in the centre of each gross plot were harvested. Harvest products were separated into the different components and weighed, dried and milled separately.

Spring barley (\(\text{Hordeum vulgare}\) L.) was sown on the 12 April 2011. NPK fertilizer (Linzer Star N:P\(_2\)O\(_5\):K\(_2\)O 15:15:15) was applied as a base dressing providing an N input of 60 kg N ha\(^{-1}\) with a P input of 26 kg P ha\(^{-1}\) and K input of 50 kg K ha\(^{-1}\), however the nitrogen application was a split dressing with a further top dressing of nitrogen 60 kg N ha\(^{-1}\) in the form of nitromoncal (ammonium nitrate) applied on 10 May 2011. On the labelled sub-plots the equivalent of 60 N kg ha\(^{-1}\) of 2 atom % \(^{15}\)N labelled ammonium nitrate (labelled on both moieties) was carefully broadcast on a 1 square meter sub-plot of the main plots, substituting the regular May top dressing of broadcast Nitromoncal (ammonium nitrate). Fertilizer
was applied by marking and subdividing the sub-plot into four segments and carefully applying pre-weighed and bagged labelled fertilizer evenly across the plots by hand. Plots were marked with tags for re-identification.

At harvest, on the 21 July 2011, a 40 by 40 cm inner-square (to overcome edging effects) from the sub plot was harvested 2 cm above ground level using shears. Plants were subdivided into shoots and ears (grain), dried (60 °C), chopped and rigorously homogenized. A sub sample of each fraction was finely ground using a Retch ball mill; duplicate subsamples of each treatment were analysed.

To confirm the immobilization hypothesis a greenhouse pot experiment was set-up. Traismauer soil was excavated in 2018 and four treatments were set up with the identical biochar, with five replicates: control, that is, NPK (no biochar application, NPK added), BCN (72 t biochar ha\(^{-1}\), NPK added), SD120N (120 t sawdust ha\(^{-1}\), NPK added), SD1N (1 t sawdust ha\(^{-1}\), NPK added). Both treatments, BCN (80.3% C) and SD120N (48.3% C), added 58 t ha\(^{-1}\) C content to soil. Spring barley was sown on the 21 March 2018, nine seeds were sown in each pot and thinned to six plants after germination. As in the field trial, NPK fertilizer was applied (input of 60 kg N ha\(^{-1}\), 26 kg P ha\(^{-1}\) and 83 kg K ha\(^{-1}\)). A top dressing of 60 kg N ha\(^{-1}\) of 2 atom % \(^{15}\)N labelled ammonium nitrate was applied to each pot on 23 April 2018. The ripe spring barley was harvested on the 4 July 2018. Plant parts were dried (50 °C) and further processed as mentioned above and analysed as mentioned below.

**2.1.2. Sunflower Field Experiment in 2012**

In 2012, sunflower (Helianthus annuus L.) was sown on the 20 April. A base dressing was applied providing an N input of 25 kg N ha\(^{-1}\) with a P input of 31 kg P ha\(^{-1}\) and K input of 100 kg K ha\(^{-1}\) on the 3 May 2012, a top dressing of Nitramonal (ammonium nitrate) was applied at a rate of 50 kg N ha\(^{-1}\) to the main plot applied on the 11 June of 2012. On a different sub plot the equivalent of 50 N kg ha\(^{-1}\) of 2 atom % \(^{15}\)N labelled ammonium nitrate (labelled on both moieties) was applied in a similar manner to that described above on at sufficient distance from the 2011 subplots to avoid cross contamination. Similarly, at harvest on the 26 September 2012 a 50 by 50 cm inner-square from the sub plot was sampled 2 cm above ground level using shears, again the inner was to avoid edging effects. Plants were subdivided into stems, leaves and heads (grain); these were then prepared for isotope analysis as described above. We did not de-hull the seeds, that is, separate seeds from kernels. To calculate back to the seed value, we assumed that kernals account for ~20–30% of the total dry weight, although they only contain an average 0.64% N compared to the seeds which contain 3.3% N. Our average grain values were 2.2% N compared to 3.3% N, the average seed value suggesting a slightly higher kernel: seed ratios ~40:60.

Soil samples were taken from the top 10 cm of the subplot and sieved through a 2 mm sieve and dried and analysed as below.

**2.2. Soil and Plant Analysis**

For measurements of extractable C, fresh soil aliquots of 4 g were extracted for 30 min with 30 mL 0.5 M K\(_2\)SO\(_4\), subsequently filtered through ash-free cellulose filters and extracts kept frozen at −20 °C for later analyses. Dissolved organic carbon was measured as non-purgeable organic carbon by high temperature catalytic oxidation on a Shimadzu TOC-VPCH with TNM-1 unit and ASI-V autosampler (Shimadzu Austria, Korneuburg, Austria).

Soil microbial biomass was determined from 2 g of fresh soil aliquots extracted twice on a rotary shaker for 60 min with 15 mL 0.5 M K\(_2\)SO\(_4\). This slurry devoid of inorganic or soluble organic N was subsequently made up to original volume with mL 0.1 M K\(_2\)SO\(_4\), where upon 0.5 mL of chloroform was added. These samples were subsequently filtered through ash-free cellulose filters. A 5 mL of sample was purged in a stream of nitrogen for 1 h to remove traces of chloroform and then dried at 40 °C, the entire dried residue was placed into a tin cup and analysed for \(^{15}\)N as described below.

For total N, C, \(^{15}\)N and \(^{13}\)C analysis dried ground duplicate plant, K\(_2\)SO\(_4\), or soil samples were placed into 8 by 5 mm tin and analysed at SILVER (Stable Isotope Laboratory at the University of
Vienna) and at the University of Natural Resources and Life Sciences (BOKU), using an isotope ratio mass spectrometer (Delta PLUS, Thermo Finnigan, Bremen Germany) interfaced with an elemental analyser (Flash EA, CE Instruments, UK) [37]. A full complement of internal and external international IAEA standards was run with the samples to calculate the isotopic ratios and % N and % C values, standard error of measurement is typically 0.2‰ for carbon and 0.2‰ for nitrogen.

Atom $^{15}\text{N} \text{ excess} = ^{15}\text{N} \text{ abundance } - \text{ background abundance}$. Natural background abundance is generally accepted as 0.3663% and was used in these experiments; as the measured background values of the grain were very close to this value (0.3664). We used isotope labelled fertilizer which for simplicity assumed would have swamped the signal of any small initial natural abundance differences in soil inorganic N values [38].

Delta carbon values ($\delta$) are defined as the isotopic ratio of a sample standardized to the isotopic ratio of the internationally defined reference Vienna Pee Dee Belemnite (VPDB):

$$[(R_S - R_R)/R_R] \times 1000 = \delta \text{ which can also be written as } [(R_S/R_R) - 1] \times 1000 = \delta$$

where $R_S$ is the isotopic ratio of the sample and $R_R$ is the isotopic ratio of the reference standard. The equations used to calculate N uptake and allocations are derived from first principles and have been previously described [39]. They are presented and described here for clarification.

% Nitrogen derived from fertilizer (%Ndff), that is, the percentage nitrogen in the plant has come from the labelled fertilizer application.

$$\% \text{Ndff} = \frac{\text{atom} \ % ^{15}\text{N} \text{ excess in the crop}}{\text{atom} \ % ^{15}\text{N} \text{ excess of the fertilizer added}} \times 100 \ (1)$$

Ndff amount, that is, mass of nitrogen in the plant derived from the labelled fertilizer applied.

$$\text{Ndff (kg)} = \frac{\% \text{Ndff}}{100} \times \text{N(kg) in the crop} \ (2)$$

% Fertilizer use (%FU), that is, the amount of labelled fertilizer applied which ends up in the crop as a percentage of the labelled fertilizer initially applied to the crop.

$$\% \text{FU} = \frac{\text{Ndff(kg)}}{\text{Amount of N added as fertilizer (kg)}} \times 100 \ (3)$$

% Fertilizer nitrogen allocation (%FNA), that is, mass of labelled fertilizer nitrogen allocated to the specific plant part as a percentage of the total above ground fertilizer uptake.

$$\% \text{FNA} = \frac{\text{Ndff(kg)part}}{\text{Ndff(kg)total}} \times 100 \ (4)$$

Carbon isotope discrimination ($\Delta$) was calculated as:

$$\Delta = \frac{\delta_a - \delta_p}{1 + \delta_p} \ (5)$$

where $\delta_a$ and $\delta_p$ are the carbon isotope delta values of source air and plant material, respectively, relative to the international standard Vienna Pee Dee Belemnite (VPDB), note $\delta$ values are usually quoted as $\%_e$, that is, $\delta/1000$. Carbon isotope composition of air was taken as $-8.00\%_{e}$ for Austria [40].

All statistical analysis was carried out using Sigma Plot 12. Systat Software Inc. (Chicago, IL, USA). All data was tested for normality and equal variance and transformed if not conforming. Initially, two-way ANOVA’s to determine influence of block effects, were run. If these were insignificant, which was true in all cases, one-way ANOVA’s were used. If only two treatments were compared
paired student T-tests used to determine significant differences \( (p < 0.05) \). We strived to use the most parsimonious statistical approaches.

3. Results

We set out to investigate the effects of biochar application on crop yield and nitrogen fertilizer usage in a series of field experiments using \( ^{15} \)N labelled fertilizers.

3.1. Results of Spring Barley Field Experiment in 2011

3.1.1. Spring Barley Nitrogen Yields Did Not Differ in Fertilized Treatments

The year 2011 was a particularly dry in Austria with a pronounced precipitation deficit 270 mm compared to the usual 550 mean annual precipitation, with only fifty percent of the long term mean (1971–2000) falling on the site [35]. Under these solely rain fed conditions yields were lower than the mean long-term averages. Grain yields as determined from the whole plot measurements and that have been previously reported, were significantly higher in the BCN treatment \((6065 \pm 427 \text{ kg ha}^{-1})\), than in the NPK treatment \((5488 \pm 756 \text{ kg ha}^{-1})\) and were significantly lower in the BC3 treatment \((4800 \pm 110 \text{ kg ha}^{-1})\) than in both fertilizer treatments \( (p < 0.05) \) [35].

Yet mean total crop nitrogen yields were not significantly different between the fertilized treatments NPK and BCN \((157 \text{ and } 160 \text{ kg N ha}^{-1} \text{ respectively})\), although when biochar was applied without fertilizer (the BC3 treatment), N yields were significantly lower \((98 \text{ kg N ha}^{-1})\) (Table 1).

Table 1. Above ground, total plant biomass-yield, nitrogen uptake and fertilizer usage (%) in barley and sunflower following biochar soil application in 2011 (mean ± 1 standard deviation). Different letters within a column indicate significant differences between treatment means within single years \( (p < 0.05; n = 4, \text{ using Student’s } t \)-test, when only two treatments; ANOVA when 3 treatments; all values based on dry weight basis.

<table>
<thead>
<tr>
<th>Biochar Rate</th>
<th>N Rate</th>
<th>Yield \text{ kg ha}^{-1}</th>
<th>Plant N Uptake in kg N ha}^{-1}</th>
<th>\text{ Fertilizer Usage}</th>
</tr>
</thead>
<tbody>
<tr>
<td>t ha}^{-1}</td>
<td>kg ha}^{-1}</td>
<td>from soil + unlabelled fertilizer</td>
<td>\text{ from labelled fertilizer}</td>
<td>total</td>
</tr>
</tbody>
</table>

2011 Spring barley

NPK 0 60/60 * 5488 ± 756 b 135 ± 17 a 22 ± 18 a 157 ± 17 a 37 ± 28 a
BCN 72 60/60 * 6065 ± 427 a 121 ± 14 a 39 ± 5 a 160 ± 18 a 64 ± 8 a
BC3 72 0 4800 ± 110 c 98 ± 7 b 98 ± 7 b

2012 Sunflower

NPK 0 25/50 * 5145 ± 129 b 126 ± 8 b 25 ± 5 b 151 ± 25 A 51 ± 11 A
BCN 72 25/50 * 5103 ± 393 b 113 ± 16 A 26 ± 4 A 139 ± 26 A 52 ± 7 A
BC3 72 0 4900 ± 300 B 98 ± 12 B 98 ± 12 B

1 Calculated using Equations (1) and (2); 2 Calculated using Equations (1)–(3). * indicates \( ^{15} \)N labelled fertilizer.

3.1.2. Fertilized Biochar Incorporation Increased C:N Ratio (Grain) and %Ndff Values (Grain & Shoots)

The C:N ratio in the grain was significantly higher \( (p < 0.05) \) in the BCN treatment \( (22:1) \) than in the NPK treatment \( (20:1) \).

In the BCN treatment above ground crop N uptake from the top-dressed labelled fertilizer was almost double that of the NPK treatment \( (39 \text{ and } 22 \text{ kg N ha}^{-1} \text{ respectively})\) but not significantly different (Table 1). Moreover, the average percentage of fertilizer \( \% \text{ FU} \) applied that was taken up by the crop was almost double in the BCN treatment \( (64\% \pm 8\%) \) than that of the NPK treatment \( (37\% \pm 28\%) \) although not significantly different \( (p = 0.06, \text{ Table 1}) \). However, mean %Ndff-values were significantly different between treatments \( (p < 0.05) \); twice as high in the BCN treatment \( (24\% \& 26\%) \), compared to the NPK treatment \( (13\% \& 12\%) \) in both the grain and shoots, respectively (Table 2).
Fertilizer nitrogen allocation was significantly different between the two treatments with the NPK treatment allocating slightly, although significantly more of the labelled fertilizer to grain filling (86%). The % N of the grain was also marginally but significantly higher ($p < 0.05$) in the NPK treatment (2.1 ± 0.13), compared to (1.92 ± 0.15) in the BCN treatment.

Table 2. % Nitrogen derived from top dressing fertilizer (Ndff) and % fertilizer nitrogen allocated (FNA) to the various plant parts. Carbon isotope discrimination in spring barley and sunflower following biochar soil application in 2011 (mean ± standard deviation). Different letters indicate significant differences between treatment means within single years ($p < 0.05$; $n = 8$, using Student’s $t$-test).

<table>
<thead>
<tr>
<th></th>
<th>Stem</th>
<th>Grain</th>
<th>Leaves/Shoot</th>
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<tr>
<td>2011</td>
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<tr>
<td>Spring barley</td>
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<tr>
<td>%Ndff</td>
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<td>NPK</td>
<td>13 ± 8 b</td>
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<tr>
<td>NPK</td>
<td>86 ± 2 a</td>
<td>14 ± 2 d</td>
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<td>BCN</td>
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<td>16 ± 2 c</td>
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</tr>
<tr>
<td>ΔC %‰ 5</td>
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<tr>
<td>NPK</td>
<td>18.6 ± 0.1 a</td>
<td>20.1 ± 0.2 c</td>
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<tr>
<td>BCN</td>
<td>19.0 ± 0.4 b</td>
<td>20.2 ± 0.6 c</td>
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<td>2012</td>
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<td>Sunflower</td>
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<td>%FNA 4</td>
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<td>NPK</td>
<td>10 ± 2 C</td>
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<tr>
<td>BCN</td>
<td>11 ± 1 C</td>
<td>79 ± 10 A</td>
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<td>ΔC %‰ 5</td>
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<tr>
<td>NPK</td>
<td>20.9 ± 0.1 A</td>
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<td>20.8 ± 0.3 A</td>
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<tr>
<td>BCN</td>
<td>20.7 ± 0.2 A</td>
<td>22.0 ± 0.3 A</td>
<td>20.9 ± 0.3 A</td>
</tr>
</tbody>
</table>

3.1.3. Fertilized Biochar Incorporation Increased C Isotope Discrimination (Grain)

There was a significant difference in the carbon isotope discrimination (CID) of the grain between the BCN and NPK treatment ($p < 0.05$), with the BCN treatment exhibiting more discrimination (Table 2). There was a strong and significant correlation between % nitrogen and the carbon isotope discrimination ($r^2 = 0.79$, $p < 0.0001$) with a trend of decreasing discrimination with increasing nitrogen content (for both plant parts). Although this was only a significant correlation for the grain when analysed separately ($r^2 = 0.33$, $p < 0.05$) from the shoots.

3.1.4. Treatment Differences in the Greenhouse Experiment in 2018

Results of the greenhouse pot experiment in 2018 showed significant differences ($p < 0.05$) between the treatments. Significantly lower plant and grain yields were measured in the saw dust treatment (SD120N) compared to the biochar (BC72N) treatment, while the biochar treatment yields did not differ from the control (NPK: 871 ± 157; BC72N: 912 ± 156; SD120N: 513 ± 130 in kg ha$^{-1}$). The amount of nitrogen taken up from the fertilizer was lowest in the SD120N treatment (NPK: 16.33 ± 6.41; BC72N: 19.15 ± 4.14; SD120N: 8.14 ± 2.13; in kg) in the grains, although the %Ndff in the plant was slightly lower in the SD120N treatment (NPK: 58.6 ± 1.5; BC72N: 59.8 ± 2.1; SD120N: 55.3 ± 2.0). The fertilizer use (%FU) did not differ between the biochar treatment and the control but was significantly depressed in the saw dust treatment SD120N (NPK: 41.09 ± 3.24, BC72N 41.93 ± 4.22, SD120N 15.30 ± 1.91 in %).
3.2. Results of Sunflower Field Experiment in 2012

In both 2011 and 2012, additional soil measurements on the top 10 cm were made (Table 3). Total fertilizer N recovery (in plant and soil) was not significantly different between the NPK and BCN treatments and very close to the 50 kg N that was added as labelled fertilizer in 2012, nor was there any difference in the % total N or δ\(\text{15}^\text{N}\) of the soil under the two treatments in 2012. However, in 2011 not surprisingly % total C was significantly (\(p < 0.05\)) higher in the biochar treatment, this was also reflected in the isotope signature of the carbon in the soil and the C:N ratio of the soil, however notably soluble organic carbon was about 10% lower in the biochar treatment. Soil moisture content measured at harvest in 2012 was significantly higher in the BCN compared to the NPK treatment 0.31 versus 0.21 g H\(\text{2}^\text{O}\) g\(^{-1}\) DW, reflecting the significantly higher rainfall in 2012.

Table 3. Soil data. C:N ratio, carbon content and isotope ratios of the soil following sunflower harvest. Total nitrogen derived from top dressing fertilizer (Ndff) recovered in sunflower and soil following harvest. % moisture content of soil at following harvest in 2012 (all values mean ± standard deviation, \(n = 4\)). Different letters indicate significant differences between treatment means within single years, \(p < 0.05\); based on Student’s t-test).

<table>
<thead>
<tr>
<th>Soil C:N Ratio</th>
<th>NPK 23.9 ± 5 (^b)</th>
<th>BCN 35.8 ± 2 (^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil carbon content (mg C g soil(^{-1}))</td>
<td>NPK 37 ± 7 (^b)</td>
<td>BCN 64 ± 13 (^a)</td>
</tr>
<tr>
<td>15N-labelled sub-plot following sunflower harvest 2012.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil δ(\text{13}^\text{C}) (‰)</td>
<td>NPK −13.9 ± 1.8 (^b)</td>
<td>BCN −18.9 ± 2.0 (^a)</td>
</tr>
<tr>
<td>Soil (\text{15}^\text{N}) (atom %)</td>
<td>NPK 0.3819 ± 0.005 (^a)</td>
<td>BCN 0.3826 ± 0.003 (^a)</td>
</tr>
<tr>
<td>Total soil + plant Ndff (kg ha(^{-1}))</td>
<td>NPK 47 ± 13 (^a)</td>
<td>BCN 53 ± 7 (^a)</td>
</tr>
<tr>
<td>Soil moisture content at harvest (g H(\text{2}^\text{O}) g dry soil(^{-1}))</td>
<td>NPK 0.21 ± 0.09 (^a)</td>
<td>BCN 0.31 ± 0.03 (^b)</td>
</tr>
<tr>
<td>Soil soluble organic carbon (µg C g(^{-1}) DW)</td>
<td>NPK 123 ± 45 (^a)</td>
<td>BCN 111 ± 45 (^b)</td>
</tr>
</tbody>
</table>

3.2.1. Sunflower Nitrogen Yields Did Not Differ in Fertilized Treatments

No additional biochar was added in 2012 and it was a mildly dry year, with a cumulative precipitation in the first nine months reaching 93.1% of the long term mean (420 mm—2012 total annual rainfall, 550 mm a\(^{-1}\) long-term average). Again, labelled fertilizer was applied as a top dressing, in late spring on new sub-plots which had previously received no labelled fertilizer. Seed-head yields (referred to from herein as grain) as determined from sub plot measurements, were not significantly different between the fertilized treatments, in the NPK treatment mean grain yield was 5145 ± 129 kg
ha$^{-1}$ and in the BCN treatment 5103 ± 393 kg ha$^{-1}$, although they were both significantly higher than the un-fertilized biochar BC3 treatment (4900 ± 300 kg ha$^{-1}$) and conferred well with whole plot data [35]. Again, mean total crop nitrogen yields were not significantly different between the fertilized treatments NPK and BCN (151 and 139 kg N ha$^{-1}$ respectively) but in the BC3 treatment, N yields were significantly lower (98 kg N ha$^{-1}$) and extremely similar to the previous year’s spring barley 2011-N yield data for the BC3 treatment (Table 1). An average of ~26 kg N ha$^{-1}$ derived from the added top dressing of fertilizer (Table 1).

3.2.2. Fertilized Biochar Incorporation Did Not Affect Mean %Ndff Value and C Isotope Discrimination

Mean %Ndff was not significantly different between plant parts or treatments with an average of around 17% of the nitrogen in the plant being derived from the labelled fertilizer added (Table 2). Ndff, the % of nitrogen in the plant that was derived from the fertilizer, was on average ~18% and for each component plant part, ~18% of the nitrogen in that part was derived from the second fertilizer application (Table 2).

Above ground crop N uptake from the labelled fertilizer was very similar and not significantly different between the BCN and NPK treatments (25 and 26 kg N ha$^{-1}$ respectively, Table 1). Accordingly, mean fertilizer recovery from the applied labelled fertilizer was also not significantly different and very similar in both the NPK (51%) and BCN (52%) treatments but slightly higher than the average recovery in spring barley in 2011 (Table 1). Fertilizer nitrogen allocation showed a slight, difference ($p = 0.051$) between the two treatments with the NPK treatment allocating slightly less of the labelled fertilizer taken up to grain filling (75%) compared to the BCN treatment (79%) (Table 2). There were no significant differences in the % N of the grain or in the carbon isotope discrimination of the grain, shoots or leaves between the BCN and NPK treatments but minor differences between plant parts (Table 2).

4. Discussion

4.1. Spring Barley Field Experiment in 2011

4.1.1. Yield Response after Biochar Incorporation Minimal in Temperate Soils

Contrary to our 2011 spring barley results, significant yield increases in European temperate arable systems with inorganic fertilizer applications have not often been observed following biochar applications in field trials, although substantial but not significant increases of greater than 20% have been observed in German sandy soils amended with biochar [41], highlighting the difficulty in overcoming heterogeneity in field experiments [42–45]. Although many positive increases in yield have been shown in tropical and oceanic cropping systems [46–48], these increases have not been so dramatic in temperate soils as they generally have sufficient inherent fertility, high CEC and neutral pH [5].

The yield responses for biochar reported herein were lower than to those found for winter barley grown on volcanic soils in an oceanic climate near Tumuco, Chile, with an annual rainfall of around 1100 mm a$^{-1}$. In that experiment, the addition of biochar at the rate of 20 mg ha$^{-1}$ significantly increased grain yield by 31.3% and 21.8% in the Inceptisol and Ultisol, respectively, relative to the control [47]. Although they did not measure N yields, chlorophyll measurements indicated biochar addition had a significant negative impact on N yields.

In our experiment, overall the amount of fertilizer recovered in the plant from the fertilizer added in the NPK treatment was in a similar range to previous studies of wheat, in which around 40% of the total fertilizer N added as ammonium sulphate was recovered in the above ground crop [48]. The higher than average fertilizer recoveries in the biochar treatment (~64%) need to be further verified, as this could be extremely useful biochar induced effect as N pollution from fertilizer is a major
contributor to agricultural pollution, through greenhouse gas emissions and nitrate leaching, therefore any improvements in fertilizer use efficiency should be investigated thoroughly.

Similar N allocation patterns were also observed in the Garabet study [48], with 79% of the N accumulated in grain and 21% in straw, in comparison to ~85 and ~15% in the grain and shoots respectively in our barley experiments. These slight differences could be attributable to differences in wheat and barley or to different sampling methods but overall they show plant nitrogen is preferentially allocated to grain filling.

In our soil it was clear in the first year after biochar application that there was insufficient N in the native soil for optimum crop growth, evident from the BC3 biochar-no-fertilizer N treatment, however there was no significant difference in the total crop N uptake in both the NPK and BCN treatments, suggesting that fertilizer application was adequate to overcome the plant nutritional constraint. This similarity in crop N uptake after biochar and mineral fertilizer addition has been observed in American temperate maize cropping systems [44] and again highlights the need for identifying soil productivity constraints if biochar is to be used to improve crop yields [10,45]. Our results are in line with Glaser et al. [41] although they observed 20% average increases in maize yields in sandy German soils with the addition of biochar but they also failed to observe significant differences in either grain yield or N uptake when inorganic-fertilized and non-fertilized treatments were compared.

4.1.2. Stabile Isotope Analysis Indicates Biochar Incorporation to Overcome Water Constraints and Increase Fertilizer Efficiency

Although %Ndff values were significantly different in the NPK and BCN treatments in the field experiment, this did not translate into significant differences in the amount of fertilizer N uptake, this could be attributable to the high combined error generated when calculating both crop N uptake and fertilizer recovery, as they are calculated from a combination of %N, dry matter yield and \(^{15}\)N enrichment data. %Ndff, however, is based on a single isotope measurement of the plant material and the fertilizer used which is constant for all treatments. However, what it does highlight is that there was sufficient soil N mineralization or basal fertilizer to compensate for the short fall of labelled fertilizer in the NPK treatment and it suggests in that dry year that water was constraining crop growth to a greater degree than N deficiency in both the NPK and BCN treatments. There is evidence for this from the \(^{13}\)C carbon isotope discrimination data.

The carbon isotope discrimination (CID) was significantly higher in the BCN treatment, which in turn suggests it was less water stressed. As under water stress there is increased stomatal closure this leads to less discrimination against the heavier \(^{13}\)C containing carbon dioxide molecules [40]. In addition, the C:N ratio in the grain was significantly higher (p < 0.05) in the BCN treatment (22:1) than in the NPK treatment (20:1) again suggesting, water was constraining photosynthesis and carbon accumulation rather than nitrogen constraining growth. It is possible that the discrimination is seen more profoundly in the grain rather than the shoots as it is an integrated measure over many weeks of carbon export during grain filling. Leaf carbon values more likely reflect the initial water status of the plant in terms of structural leaf production and the current status of photosynthate and may explain why we only saw the effects in the grain. Craufurd et al. [49] demonstrated that isotope discrimination was a good indicator of yield in dry land barley and it has been attributed to improved water use efficiency [34].

The average increase in fertilizer efficiency could be linked to the improved water availability and increased mass flow of nitrate into the crop in the biochar treatments. Mass flow has been shown to be responsible for the majority of nutrient uptake in agronomic crops, for example in Zea mays grown in a fertile loam soil accumulated 79% of its nitrogen (N), by mass-flow, the rest being taken up by diffusion and interception by the roots [50]; moreover, nitrate has higher mobility and is obviously more available than ammonium, which is generally bound to clays or organic matter. We have previously demonstrated that nitrification rates were very high in these soils and higher in the BCN treatment than the NPK treatment [51] and that there were significant differences in the soil volumetric
water contents between the two treatments throughout the growing season, with average g H$_2$O g$^{-1}$ DW, 0.17 and 0.14 for the BCN and NPK treatments respectively [51]. Indeed, Karer et al. [35], had observed significantly greater water holding capacity at low water tension (0 pF) and field capacity (1.8 pF) but not at higher water tensions in the biochar treatments, suggesting there may have been more plant water available water as a consequence of higher soil water storage in the biochar treatment over the whole growing season, which could account for the higher carbon isotope discrimination in the biochar treatment crop.

4.2. Sunflower Field Experiment in 2012

We conjecture that the biochar treatment yield increases we observed in 2011 were more linked with the increased availability of water consequently affecting nitrogen uptake rather than nitrogen effects per-se. This is supported by the 2012 observations. In that mildly dry year there were no significant nitrogen effects of biochar treatment on sunflower grain yield or total above ground biomass accumulation, when it was added with fertilizer; although yields were significantly lower in the un-fertilized biochar treatment. Overall grain yields were much higher in the fertilizer treatments (5100 kg ha$^{-1}$, equivalent to 3060 kg seeds ha$^{-1}$) than the average for Austria in 2012 (2270 kg ha$^{-1}$) [52]. These values are much lower than the values for sunflower grown in in Argentina and reported by Scheiner et al. [53], of 51% Ndff but reflect the fact that in these experiments we are only looking at the fate of the second top dressing of N fertilizer and not the total fertilizer application of 150 kg N ha$^{-1}$ as they did in Argentina.

The significantly higher %N recovery in the NPK 2012 treatment compared to the previous year was possibly a result of the improved water availability in 2012. Atta & Van Cleemput [54] had studied the fate of labelled fertilizer ammonium-N applied to sunflower in a sandy soil and concluded that only 30% of the fertilizer nitrogen was recovered in the plant, a lower value than in our experiments and possibly the result of the sandy soil type with a high leaching potential in their experiments. Our soil however was a sandy loam with 25% clay which possibly prevented excessive leaching as evident from the overall fertilizer recovery data in the above ground and soil components, as the amount of fertilizer recovered was 47 and 53 kg N ha$^{-1}$ in the NPK and BCN treatments, respectively and which was similar to the 50 kg N ha$^{-1}$ that was added as the N top dressing which was labelled. The allocation data again shows N is dominantly allocated to grain filling with ~80% of the fertilizer nitrogen taken up ending up in the seed tissues, similar to the cereal allocation and an obvious consequence of the combined effects of breeding selection and evolution.

There were no significant differences in the carbon isotope discrimination between the NPK and BCN treatments, although previous studies have shown that in field grown sunflower plants is strongly correlated with stomatal conductance ($r_g = 0.64$, $p < 0.01$) and the ratio of net assimilation rate (A) to growth ($r_g = −0.86$, $p < 0.001$), an instantaneous measure of transpiration efficiency [55]. The lack of difference was probably due to the lack of water constraint during 2012, as it was a much wetter year than 2011 particularly in the growing season and evident from the soil moisture contents at harvest.

It is difficult to completely reject our original hypothesis that biochar application can increase crop yields through improving the nitrogen uptake and utilization of added inorganic fertilizer, as we did see increased yield and improved fertilizer nitrogen uptake in the biochar treatment in the first year. However, it did not translate to increased overall N status in the crop at harvest suggesting the water rather than nitrogen was the yield limiting factor. In the second year, we can reject our hypothesis for this soil type, crop and agro-ecosystem.

It can be clearly seen from these experiments that biochar additions of up to 72 tonnes ha$^{-1}$ did not cause significant N yield or plant yield depression as a consequence of N immobilization when mineral N fertilizer was added. In contrast, Recous et al. [56] showed that a year after wheat straw application of only 4000 kg C ha$^{-1}$ to a loamy soil that immobilization of nitrogen was in the order of 380 kg N ha$^{-1}$; three times higher in the straw amended soils compared to the non-amended controls.
Moreover, when one tonne ha$^{-1}$ of un-charred maize carbon was added to a temperate Austrian soil with similar climatic conditions to this experiment, it resulted in a significant 20% decrease in maize N yield compared to controls over similar time scales [57]. Glasshouse experiments herein confirmed when similar quantities of un-pyrolyzed carbon was added to the soils as sawdust that grain yields were halved as a consequence of immobilization. This was evident from the significantly higher $^{15}$N enrichment of the soil microbial biomass in the sawdust treatment (615$\pm$ 115) which was twice that of the both the NPK (332$\pm$ 128) and BCN (278$\pm$ 23) treatment. Indeed, these experiments suggest that immobilization of nitrogen on the addition of $\sim$58,000 kg of C ha$^{-1}$ in the form of biochar resulted in similar if not improved rates of plant fertilizer nitrogen uptake, with a potential maximum carbon credit value of around $7000$ ha$^{-1}$ based on values stated above.

Biochar addition appeared not to have any immobilization impacts over and above those of the native soil, as confirmed by the results of our greenhouse pot experiment. In their recent meta-analysis, Liu et al. [58] found soil N mineralization to remain stable after biochar addition. Despite the fact that we have previously shown that there is a microbial response to biochar addition, with soil microbial DNA concentrations being significantly higher in the biochar treatment (18.0 to 12.4 $\mu$g DNA g$^{-1}$ soil BCN and NPK, respectively) [51], this increase in microbial biomass appears to have had little impact on the inorganic nitrogen availability. Taken together with some experimental results from our detailed nitrogen cycling measurements on the same plots, we suspect that the addition of carbon in the form of biochar does not induce the primary signalling of the eukaryotic nitrate reductase activity (EukNR) in the soil microbial biomass, in the same way that addition of other carbon based carbohydrates have been shown to Gorfer et al. (2011), as biochar is not a readily available energy source for the microbial biomass. Indeed, over the long term, biochar could further reduce the EukNR signalling response, as it was shown that there was significantly lower availability of soluble organic carbon in biochar-amended soils. In this context, $^{13}$C labelling of biochar revealed that soil microorganisms took up only little amounts of biochar-C in the study of Watzinger et al. [13] and consequently no biochar C degradation within two years was observed [59].

Moreover, further evidence comes from the detailed N experiments, where the gross nitrate consumption: production rates were 3:1 and 1:1 in the NPK and BC3 treatments respectively in 2011 [51], again suggesting lower nitrate consumption in the biochar treatments and are confirmed by SMB N uptake data in these experiments. This nascent novel “no-EukNR” hypothesis, which suggests biochar does not induce primary signalling of nitrate reductase activity, offers some explanation for the observed effects of biochar on immobilization in contrast to other uncharred carbon sources but needs further verification based on experimental and molecular analysis.

5. Conclusions

Massive biochar additions to soil did not cause significant N yield or plant yield depression as a consequence of N immobilization when mineral N fertilizer was added in both field and glasshouse experiments. This is of paramount agronomic importance as addition of non-charred carbon sources often leads to significant microbial immobilisation of inorganic N and causing a depression in plant growth and yield [60]. As previously stated, it is this aspect of non-charred soil carbon incorporation for sequestration that makes it a difficult proposition to sell to profit-focused farmers. If biochar addition has minimal impacts on inorganic nitrogen immobilization but improves soil nitrogen retention as has been suggested here and recently by Güereña et al. [44], then it becomes an attractive option for increasing the profitability of marginal cropping lands. Here we offer some mechanistic explanation for these observations allowing us to further understand and manage the role of biochar in an agro-economic system.

In the context of agronomy for sustainable development this could mean that the peripheral benefits associated with soil carbon addition such as, carbon sequestration, improving cation exchange capacity and water holding capacity, could be reaped without the negative effects of yield loss due to nutrient immobilization. Indeed, another factor that these experiments emphasised was the positive
influence of biochar on soil water status with its inherent implications for the biogeochemical processes. The impact of biochar on soil water status during the extremely dry year and evaluated using the described carbon isotope discrimination approach is an area worthy of further research. Especially following global research, which suggests that cereal yields are reduced by as much as 10% in climate change driven, hot and dry extreme weather years in developed countries [61].

Given this and the changing landscape of European agriculture biochar-based carbon farming may become a viable agribusiness for marginal cropping lands in temperate agricultural climates.

Author Contributions: R.H.-N. designed, conducted and analysed the experiments and wrote the first draft of the paper. A.W. (Anna Wawra) conducted and analysed the greenhouse pot experiment, revised and edited the manuscript. G.S. designed and implemented the original biochar field experiments and edited the manuscript. A.W. (Andrea Watzinger) oversaw the sample analysis in the stable isotope lab.

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References


