The effects of biochar application on nitrogen cycling under different land use and soil management systems

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   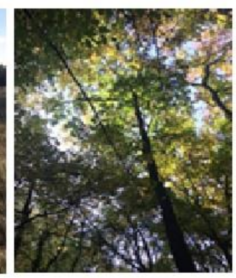

FINAL REPORT

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1. Introduction

Nitrogen cycling processes are very important biological, chemical, and physical transformations that are crucial to soil health. Soil microorganisms need nutrients and carbon source to survive and multiply, which is vital for healthy soil ecosystems (Atlas & Bartha, 1998). Any events that might alter these processes can consequently cause ecosystem changes such as shift in soil microbial communities. Therefore, the soil-plant-water system needs an essential balance to its components to enable nourishing ecosystem.

Biochar, which is made by pyrolysis of biomass such as wood, straw, manure, etc. (Ouyang *et al.*, 2013), without the presence of oxygen, has been used to improve soil quality, for carbon sequestration, might be used for climate change mitigation and to enhance soil hydro-physical properties (e.g. it attracts and retains water), or to reduce soil organic matter mineralization. However, there is limited knowledge on the secondary effect of biochar on soil nutrients and nitrogen cycle processes while being used under different agricultural settings.

The aim of the proposed work was to investigate the effects of biochar application on nitrogen cycling under different land use and soil management systems. The study focused on changes in nitrification, denitrification potentials, nitrogen fixation, and greenhouse gas (GHG) emission such as CO_2 and nitrous oxide (N_2O) in terms of vegetation and soil types while varying biochar types and amounts. Concurrently to soil chemical and microbial changes, the project also investigated in more detail the changes in soil physical and hydrological properties as a result of biochar amendments. Another major area of the project was to study correlations between biochar application and biomass production, changes in leaf area index, and plant health using different techniques, such as spectral reflectance sensors (SRS) or manual measurements. Experiments were carried out in both bench-scale and plot-scale studies. Mathematical models, incorporating the physical and hydrological relationships of the processes in focus were used for making estimations for varying conditions.

The project aimed at accomplishing integrating knowledge on biochar use on the soil-plant-water systems. Therefore, the objectives of the study were i) to investigate the response of potential nitrogen fixation, net nitrification, and denitrification rates to introduced biochar in grass, forest, and cultivated crop dominated agricultural systems; ii) to see if seasonal changes (e.g. temperature, precipitation amount) can influence the rate of the different processes of N cycling in biochar amended soils; and iii) to investigate the rate of influence of different amount and types of biochars on soil hydro-physical properties.

2. Materials and Methods

Laboratory studies

Nitrogen cycling experiments were implemented to measure the effects of biochar addition to soils on nitrogen fixation (N_2 fix), denitrification, and nitrification processes. Potential N_2 fixation was measured as ethylene (C_2H_4) production from acetylene (C_2H_2) reduction. The used method is detailed in Horel *et al.* (2019b; 2018b). Net nitrifications were measured from NO_3 production as described by Kása *et al.* (2016) and Baklanov *et al.* (2019). Potential denitrification rates were measured using the acetylene block technique, which method was also developed for this study and is described in more detail by Baklanov *et al.* (2019).

Pot experiment

Four different treatments were studied in 7 replicates each (with 14 plants per treatment) at the start of the experiment (Figure 1). Out of the four treatments one was used as a control and received no biochar (0%) only plants, while the other three were amended with biochar with the amount of 0.5%, 2.5%, and 5.0% by weight; hereafter referred to as C, BC0.5, BC2.5, and BC5.0, respectively.

a) b)

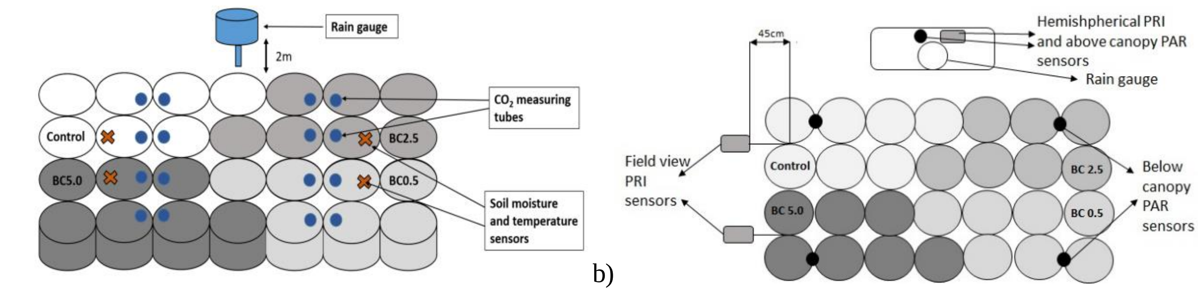


Figure 1. Pot experiment setup schematics for a) soil CO_2 emission, soil water and temperature measurement (Horel *et al.*, 2019d), and b) continuous monitoring instrumentation.

Soil physical and structural studies included aggregate stability, size, hydraulic conductivity, bulk density, surface area, and pore size measurements at different plant phenological phases (Figure 2). Macroaggregate stability (*MaAS*) was measured by a wet sieving apparatus, while microaggregate stability (*MiAS*) was calculated according to Vageler's structure factor from the rate of clay fractions determined with dispersion and without any dispersion (Vageler, 1932). The aggregate size distribution (*ASD*) was determined by shaker and sieve analyses. To quantify the degree of gradation of the structured soils, a modified coefficient of uniformity (U_{ASD}) was defined as the ratio of aggregate diameters corresponding to 60% and 10% (d_{60} and d_{10}, respectively) finer on the cumulative *ASD* curve. Saturated hydraulic conductivity (K_{sat}; cm d^{-1}) was measured with Eijkelkamp permeameter (in a closed system) using disturbed soil samples. All methods used in these studies are further described in the papers written by Horel *et al.* (2019a) and Makó *et al.* (2019).

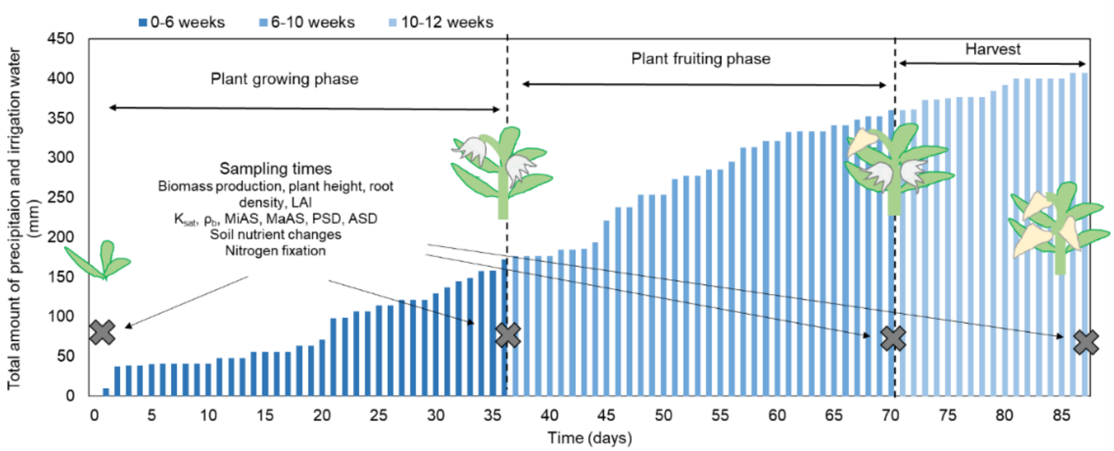


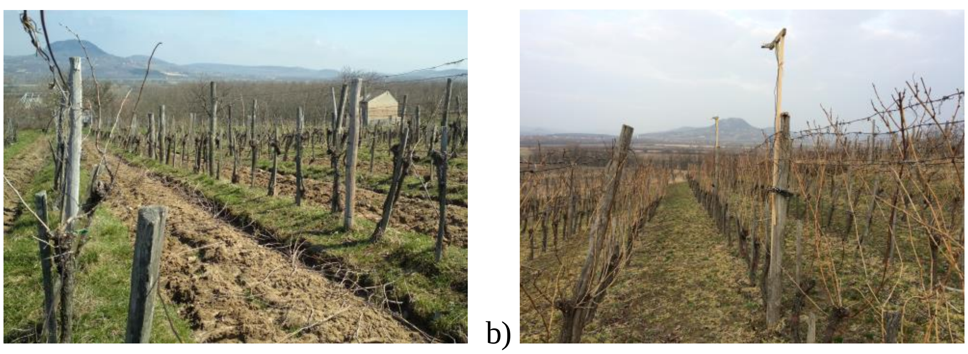
Figure 2. Types of sampling taken at different plant phenological stages.

Field experiment setup and sites

Concurrent to the laboratory and pot-scale studies, two major study sites were selected for the field experiments: i) the Szent György-hegy (Kisapáti) vineyards for investigating different land management systems and biochar amendments and ii) the East-Bakony site where the effects of biochar on plant growth, soil water, and greenhouse gases (GHG) were investigated under different land uses (i.e. maize, grassland, and forest). Based on the findings of the laboratory and pot experiments, which are described below in detail, the best amount of biochar addition was determined for the field studies (i.e. 2.5% by weight of the T600 type of biochar).

Field experiment I. – Vineyard, Szent György-hegy (Kisapáti)

During spring of 2017 the vineyard field sites in Szent György-hegy, control plots were chosen and treatment plots were amended with biochar (Figure 3). Calibrated soil water and temperature sensors (Decagon Devices) were deployed for 4 sites: i) control and ii) biochar added Riesling with tilled management practice and organic fertilizer (manure); and ii) control and iv) biochar added Riesling for tilled soil management plots without fertilizer amendment. All four sites were implemented with a PAR (Photosynthetically Active Radiation) sensor below the canopy, a PRI (Photochemical Reflectance Index) and a NDVI (Normalized Difference Vegetation Index) sensor with nadir view collecting data in every 10 minutes. A set of hemispherical sensors (PAR, PRI, NDVI) were also placed to the site along with a pyranometer and a rain gauge (ECRN 100). For CO_2 and N_2O measurements, air sample collecting cylinders were placed into the soils for all treatments. There was an undisturbed site (no-till) chosen as a double control for the air sample measurements. Biochar rate and type was chosen from data on first years' laboratory and pot-scale experiments where optimal biochar amount and type was determined. Hence, the used rate was 2.5% by weight of the T600 biochar.



a) b)

Figure 3. Vineyard sites with a) in-row ploughing with manure and b) no manure amendment.

Field experiment II. – Maize, grassland, and forest, East-Bakony

Similar setup to the vineyard was implemented in the maize study site during the third year of the project. Another full set of SRS sensors were bought from the project's budget and placed above the vegetation enabling to collect spectral data on the second year of the experiment. Two sets of moisture sensors and a rain gauge provided data for two consecutive vegetation periods, while the SRS sensors were placed at the site in April, 2018. In the maize field, plant height changes were measured over time during plant growth, and biomass production at the end of harvest both second and third year of the project. Air samples for GHG measurements were collected from all three East-Bakony sites of maize, grassland, and forest soils. For the maize site, both control and biochar amended experimental plots without plants were also chosen for air samples to investigate the difference between planted and bare soils' GHG emission values. Biochar rate of 2.5% by weight of the T600 was used in all investigated land uses.

Biochars

Three types of biochars were brought from manufacturing factories providing with a European Biochar Certificate. The chemical characteristics of the three types of biochar prepared at three pyrolysis temperatures of 600, 650, and 700°C (hereafter T600, T650, and T700, respectively) used in the present study are shown in Table 1. According to the manufacturers' information, biochar T600 was made from paper fiber sludge and grain husks using Pyreg technology at 600°C; biochar T650 was made from woodchips with Pyreg technology at approximately 650°C; and biochar T700 was made from woodchips using Schottdorf system at approximately 700°C.

Table 1. Chemical characteristics of the three biochar types used in the experiments. TOC represents total organic carbon values. n=3; ±SD

Biochar type	$pH\text{-}H_2O$	$AL\text{-}K_2O$ mg/kg	$AL\text{-}P_2O_5$ mg/kg	Total N %	$NH_4^+\text{-}N$ mg/kg	$NO_3^-\text{-}N$ mg/kg	TOC %
T600	10.3 ±0	13570.3 ±59.1	5031.1 ±32.6	1.01 ±0.1	1.86 ±0	n. d.	47.3[a]
T650	9.6 ±0	4407.5 ±0.9	463.2 ±2.8	0.84 ±0.03	1.81 ±0.07	n. d.	45.7[a]
T700	9.5 ±0.04	1868.2 ±50.9	260.4 ±6.7	0.24 ±0.01	1.68 ±0	n. d.	38.8[b]

[a] Data were based on manufacturers' certificate; [b] Soil organic carbon (SOC; %); n. d. means not detectable. T600, T650, and T700 represent biochar pyrolysis temperatures of 600, 650, and 700°C.

3. Measurement results

3.1 Determination of the best biochar application rate and type for biomass production

During the first year of the study, all three types of biochar were investigated at three different amounts at varying temperatures to determine which type and amount might be best suiting for crop growth and yield, with keeping the economically feasibility in mind as well. To do so, soil physical, hydrological, chemical, and biological changes were investigated, while varying the amount and types of the biochars. The results are detailed in the sections below.

3.2 Nitrogen cycling experiments – types and amount of biochar, land uses

3.2.1 Nitrification

Changes in net nitrification values were investigated by adding different biochar types (T600, T650, and T700) and concentrations to silt loam soil. Using freshly tilled agricultural soil, four treatments were prepared in triplicates (0, 2, 5, and 15% w w^{-1}) at three temperatures (10, 20, and 30ºC). The study showed that temperature, biochar types, and concentrations had significant impacts on net nitrification rates. The largest difference between nitrate productions was observed at low temperature where the nitrification process was relatively slow or even inhibited. Nitrification values at 20ºC and 30ºC were not significantly different, indicating that biochar effects on soil microorganisms are mainly occurring during spring and crop growing periods when temperature rises. In some cases, net nitrification values at 30ºC were three times higher compared to lower temperatures. At this temperature negative effects on nitrate production were also observed, e.g. in the case of T700 biochar. A smaller amount (2%) of biochar already resulted in significantly different ($p < 0.05$, ANOVA) net nitrification amounts compared to control treatments; however, these differences diminished at higher concentrations. Among biochar types, T650 showed the least changes in potential net nitrification values at different concentrations and temperatures. The findings are presented in more detail in the manuscript published by Kása *et al.* (2016).

3.2.2 Nitrogen fixation

The effects of biochar types and concentrations on soil nitrogen fixation (as ethylene (C_2H_4) production from acetylene (C_2H_2) reduction or ARA) measurements at different land use types were investigated. Strong correlations were found between soil chemical parameters and ARA values, especially in the case of soil pH, total N, soil organic carbon, and phosphor contents. In the case of arable soil, the ARA measurements were up to 227 times higher compared to grassland and forest samples. Biochar application affected N_2 fixing microbial responses among land use types, most notably during decreases in arable lands and forest soils. High amount of biochar amendment to the soils greatly suppressed N_2 fixing activities. These results also highlight the strong relationship between soil nutrient changes and the intensity of anthropogenic influence. The research findings and discussions in more detail are presented in the paper by Horel *et al.* (2018b).

Data retrieved from the pot experiment showed that the pepper plant facilitated the biological nitrogen fixation (BNF) rates to triple in the control soils (no biochar added) while plants were in the growing phase (weeks 1–6). This further increased an additional 61% by harvesting (week 12). The high amount of biochar addition suppressed potential BNF rates of the investigated soil, indicating its potentially negative effects on soil indigenous microbial communities if added in excess. More detail about the findings and discussion of this study can be found in the paper by Horel *et al.* (2019b).

3.2.3 Denitrification

Changes in denitrification values were investigated by adding different biochar types (T600, T650, and T700) and concentrations (0.5, 2.5, and 5.0% w w^{-1}, hereafter referred to as BC0.5, BC2.5, and BC5.0, respectively) to soil samples collected from different land uses. Increasing temperature increased the denitrification potentials in most soil samples. The largest potentials were measured in the case of forest soil samples, where regardless treatments the average N_2O production was between 28 and 70 mg kg^{-1} hr^{-1} from 10 to 30°C. In the case of grasslands it was between 8 and 41 mg N_2O kg^{-1} hr^{-1} and for the maize between 2 and 10 mg N_2O kg^{-1} hr^{-1} (Figure 4). Consequently, the most sensitive land use to biochar amendments in terms of denitrification potentials was the forest; however, in most cases the different treatments did not inhibit N_2O production. The most notable N_2O decreases were observed in the case of T600 biochar at 20°C for forest and grass soil samples (Figure 4).

Potential denitrification rates and CO_2 production of four land use types (arable without crops, arable with maize, grassland, and forest soils) at three temperatures (10, 20, and 30°Celsius) were investigated from field soil samples. Soil samples were collected approximately 3 months after biochar application. Similar to the laboratory study, the T600 biochar addition suppressed soil N_2O production in the case of forest and grass soils, while in the arable with and without maize soils showed increases at 20 and 30°C (Figure 5). Similar results were observed with CO_2 measurements (data not presented).

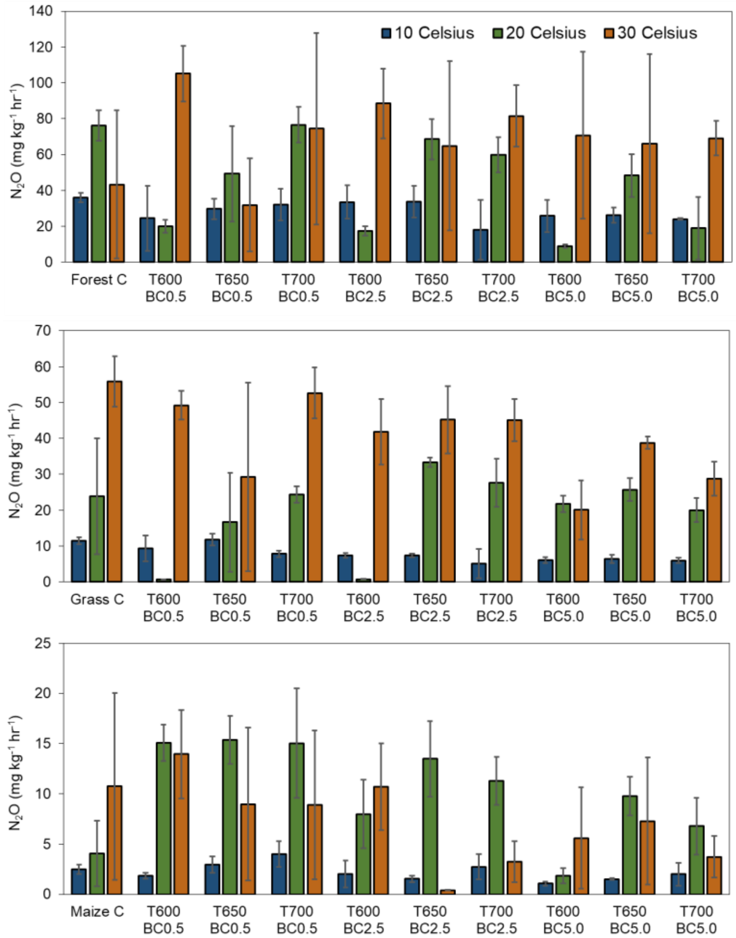


Figure 4. Changes in denitrification potentials for forest, grassland, and maize soils amended with different types and amount of biochars (BC). C represents control. n=3, ±SD

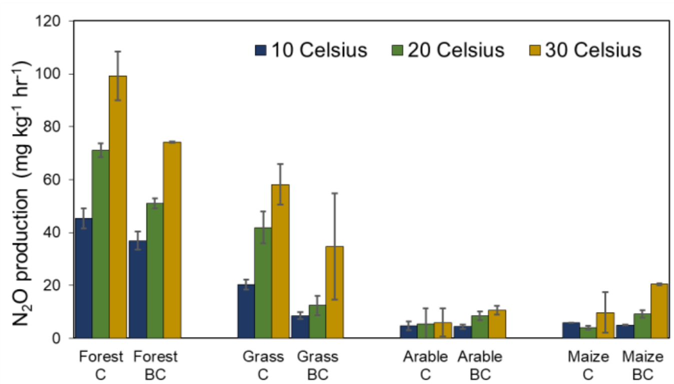


Figure 5. Denitrification potentials based on laboratory experiment for soils collected from different land uses after three months of application. C represents control and BC biochar amended soils. n=3, ±SD

Even though, all three biochar types showed varying benefits and disadvantages on different soil parameters and conditions; overall, the T600 was determined to be the best choice for field application due to its high nutrient contents and economic advantages compared to the other types.

3.3 Soil physical and structural changes as a result of biochar addition

In the pot experiment, different amounts of biochar was added to silt loam soil under natural environmental conditions such as sunlight, and rain with irrigation when it was necessary. Pepper plants (*Capsicum annum* sp.) were planted at 2–4 leaves stage until harvest. During the different vegetation growth period, soil and plant samples were collected to analyze the effects of biochar on the soil physical and structural, chemical, and biological parameters over time, and to determine the best amount of biochar needed to enhance plant growth and fruit yield (Figure 2). The main interest in soil structural characteristics were in aggregate size distribution (*ASD*), micro- and macroaggregate stability (*MiAS* and *MaAS*, respectively), soil bulk density (ρ_b), aggregate size distribution (*ASD*), particle size distribution (*PSD*) and saturated hydraulic conductivity (K_{sat}).

The study found increasing *MaAS* values with increasing biochar addition; however, higher values were also detectable in control treatments over time. Increased *MiAS* values were observed during the plant maturing phase and the decrease, which occurred during fruit development, was more pronounced. The largest *MiAS* value was observed in the case of BC2.5 among all treatments, which corresponded better to plant growth rather than to the amount of added biochar. Strong correlations were observed between *MiAS*, *MaAS*, and aggregate stability (Figure 6). As for materials with low ρ_b, *PSD* measurements are challenging and limited in success; therefore, the laser diffraction method was used in the present study. It was found to be a suitable alternative technique to the sieve-pipette method for analysing biochar and biochar-amended soil particle size distribution and structure. More detail about the study and its results can be found in the paper published by Horel *et al.* (2019a).

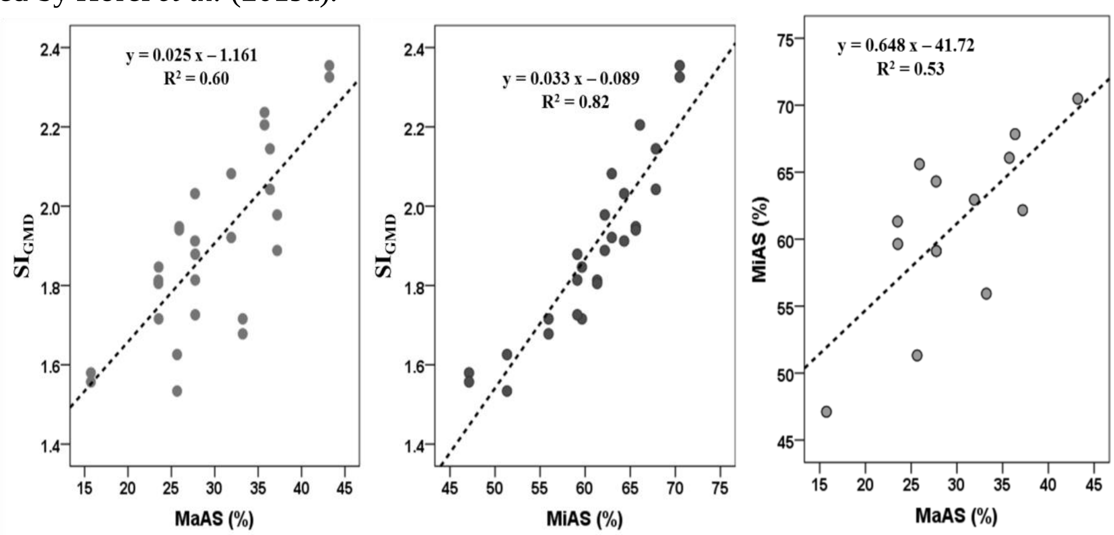


Figure 6. Connections between the different aggregate stability indexes (SI). SI_{GMD} denotes the aggregate stability index based on particles' geometric mean diameter (GDM); *MaAS* – macroaggregates stability; *MiAS* – microaggregates stability. n = 13–26

K_{sat} values showed a general increase with increasing biochar amount, with reducing extent over time. An increase in K_{sat} in control treatments were also found during week 6 and 10, while week 12 data showed even smaller average K_{sat} values than measured at the beginning of the experiment (Makó *et al.*, 2019). The most stable K_{sat} values were measured in the case of BC0.5, where week 6, 10, and 12 showed 5.5, 4.7, and 6.3 times higher values, respectively, indicating a beneficial combination of plant growth and a small amount of biochar in silt loam soil.

The average ρ_b of the artificial soil columns were 1.39±0.03 g cm^{-3} at the beginning of the study. After adding biochar to the soils, the soil ρ_b values showed a significant decrease with increasing biochar amount. At week 6 these values ranged between 1.25±0.03 g cm^{-3} and 1.16±0.01g cm^{-3} for C and BC5.0, respectively. When comparing week 12 results, further decrease in soil ρ_b was observed at high biochar additions (1.38±0.03 g cm^{-3} and 1.14±0.01 g cm^{-3} for C and BC5.0, respectively). However, these changes did not reflect a clear trend related to plant phenological phases.

Examining the possible causes of various degrees of compaction, the modified coefficient of uniformity (U_{ASD}) was used which showed a close relationship (R^2 = 0.82–0.91) with both ρ_b and K_{sat} values of the samples. Similarly, a good correlation between ρ_b and the measured K_{sat} values were found (R^2 = 0.78). More detail on the findings are presented in the paper written by Makó *et al.* (2019), currently in press.

3.4 Soil water and temperature changes as influenced by biochar addition

During the pot experiment, the effects of different amount of biochar (T600 only) were investigated on soil water and temperature. Soil water content (SWC) measurements showed two distinct time periods when biochar addition notably affected the water status of the soils. The first period was during the vegetation growing phase, until approximately the 6th or 7th week of the experiment. During this time relatively similar trends in soil water content changes were observed as a response to both irrigation and rain, but the SWC differed among treatments (all but BC0.5 and BC2.5 treatments' SWC values showed significant differences; $p < 0.01$; ANOVA). After the plants reached their maturity (around day 40 – 45), the BC0.5 and BC2.5 treatments showed substantial decrease in SWC during hot periods in contrast to control and BC5.0 (between 12.2 and 36.0% less SWC), where no such drying trend was observed. Among the four treatments, the highest SWC amount throughout the experiment was observed in the case of BC5.0 (38.4% average SWC compared to 33.3% SWC in the case of control). In general, between the treatments statistically significant differences ($p < 0.001$) were found. All treatments' overall SWC were significantly different from each other ($p < 0.0016$), except between control and BC2.5 ($p = 0.073$). Soil temperatures were monitored concurrently with the SWC measurements; however, their values showed very minimal changes between treatments due to the pot setups (unlike field soil planting), which enabled fast soil temperature adjustments to changes in air temperatures. Further data and discussions are presented in the manuscript published by Horel *et al.* (2019d).

There were enough soil moisture sensors and loggers purchased from the project's budget to use for continuous monitoring at two experimental field sites. One set was placed at the vineyard, while another set was placed to the maize field, for two consecutive vegetation periods. Even though the figures presented in this report include continuous monitoring data based on availability, data collected during winter periods – when soil moisture sensors are less reliable below 0°C – were omitted from the analyses.

At the vineyard site, soil water content measurements showed that the driest upper layer (15cm) occurred in the case of fertilizer added treatment (two year average SWC = 11.4%), while the highest average soil moisture content was in the fertilizer + biochar amended treatment (16.5%

SWC, sandy loam soil). SWC was the lowest in the case of Tilled+BC treatments, while the highest water contents were found in the upper 15 cm for the shallow tilled soil. However, these differences, were not significant ($p = 0.214$). When analyzing SWC at the lower 40 cm of the soil layer, the changes were more pronounced between treatments (Figure 7). For instance Tilled+BC treatment had 15% less SWC compared to Tilled+Manure treatment during the vegetation period, while compared to Tilled+Manure+BC the difference was even higher (20.9%). These differences were relatively substantial, as compared to the SWC measured at the 15 cm soil layer, where the highest observed difference was around 13% during vegetation growth (Figure 7). Through grapevine dormancy to bud break, the biochar amended treatments showed higher water holding capacities during rain events in the upper soil layers; however, at later times these capacities seemed to diminish, and similar responses to precipitation were noticed in the treatments. In general, continuous changes to precipitation or drier time periods showed that biochar amended soils also dried out faster than non-amended soils in the upper layers. After analyzing the lower layers' SWC, the results showed that manure amended soils could retain more water compared to non-fertilized soils (Figure 7).

Soil temperature changes were not significantly affected by any of the treatments (Horel *et al.*, 2018e).

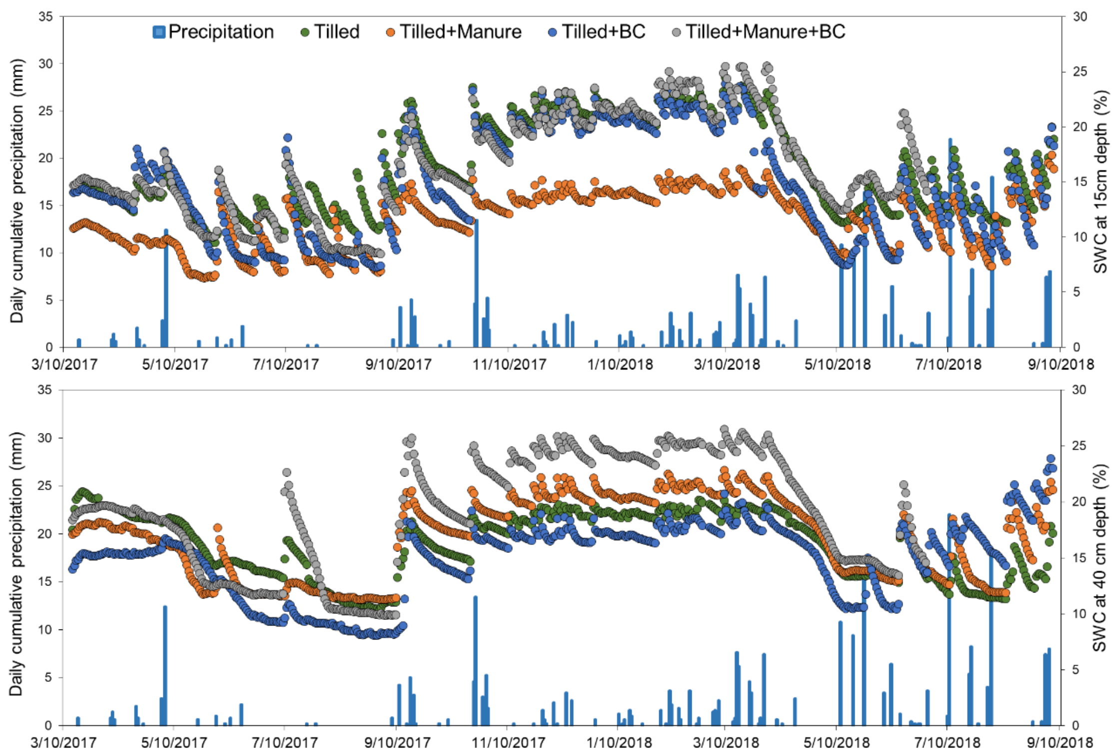


Figure 7. Daily average soil water content (SWC) and precipitation at the vineyard field site for tilled, manured, or biochar (BC) amended soils for 2017 and 2018.

The data in the maize field showed that during the first vegetation period there were no major differences between the treatments' SWC. During the second year the biochar treated soil showed a substantially higher SWC at precipitation events with much faster drying periods between rain events compared to control at both investigated depths (Figure 8). Similar to the pot experiment's results, biochar did not influence significantly soil temperature fluctuations during growing season of maize.

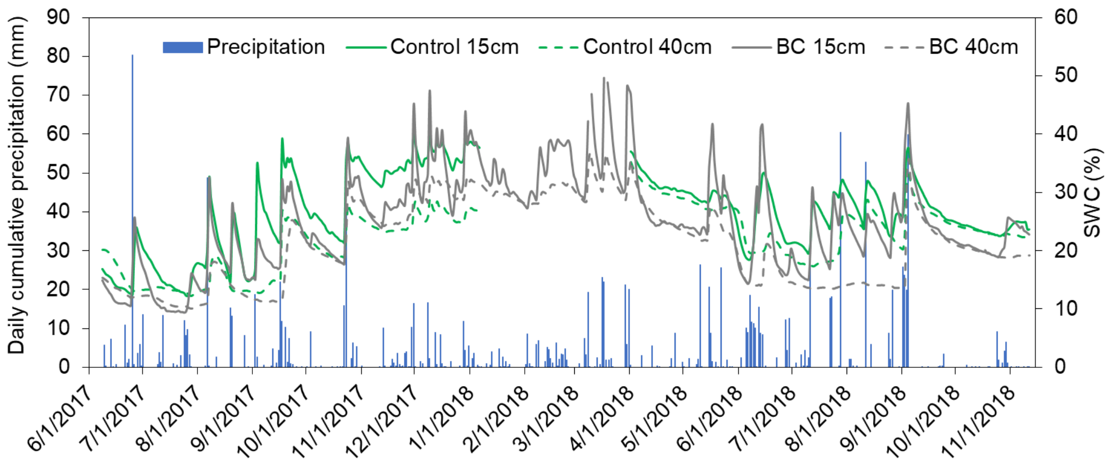


Figure 8. Daily average soil water content (SWC) and precipitation at the maize field site for control and biochar (BC) amended soils.

3.5 Greenhouse gas (GHG) emission changes – two years data on soil CO_2 and N_2O

The effects of different biochar amount on soil respiration was measured during the pot experiment three times a week. Sudden increases in CO_2 amounts were noticed after adding biochar to the soils during the first few days, which was followed by periods of more and later less intense CO_2 productions. The most pronounced differences between CO_2 concentrations were detected in the cumulative measurements, where the control treatment showed a steadier increase of CO_2 values during the first two weeks, while at the same time all other treatments CO_2 concentration increases were much smaller. In general, at the end of the experiment, the control treatments showed the highest and BC2.5 treatments showed the lowest cumulative CO_2 concentrations, with 15.1% differences observed between the two treatments. This finding supports the possible carbon sequestration potential of the biochar, even after an initial increase in CO_2 values (Horel *et al.*, 2019d). Overall, daily soil respiration values did not differ significantly among treatments ($p > 0.05$, ANOVA). However, when the different treatments' CO_2 concentrations were investigated over time, significant differences were observed ($p < 0.001$, ANOVA), indicating that soil temperature was a more influencing factor than biochar amendment alone (Horel *et al.*, 2019d). These findings are presented in more detail in a paper published by Horel *et al.* (2019d).

The comparison of the two investigated years' CO_2 and N_2O data are presented in Tables 2 and 3, respectively.

Table 2. Soil CO_2 respiration values for the different land uses and management systems. C represents control, while BC biochar amended treatments. n=76-99 (2017), n=84-93 (2018)

CO_2 (mg m^{-2} s^{-1})	2017	2018	2017-2018
Treatment types		**Average**	
Forest C	0.0920	0.1140	0.1045
Forest BC	0.0856	0.1240	0.1073
Grassland C	0.1158	0.1330	0.1256
Grassland BC	0.0973	0.1256	0.1134
Maize C	0.1000	0.1038	0.1018
Maize BC	0.1092	0.1118	0.1105
Grape C	0.0791	0.0704	0.0746
Grape, Tilled	0.1065	0.1035	0.1050
Grape, Tilled, Manured	0.0887	0.1122	0.1008
Grape, Tilled, BC	0.1078	0.1175	0.1128
Grape, Tilled, Manured, BC	0.0869	0.0893	0.0881

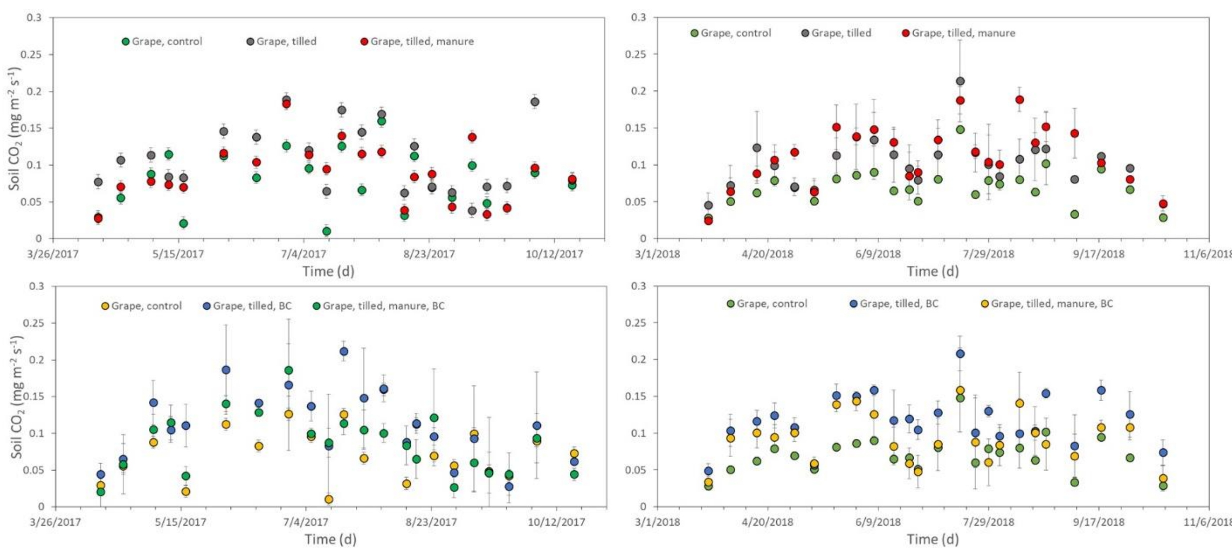


Figure 9. Soil respiration as CO_2 emission (mgCO_2 m^{-2} sec^{-1}) changes over time for the grapevine sites with and without tillage, manure, or biochar (BC) addition for 2017 and 2018. n=4; ±SD

The first year vineyard measurements of CO_2 and N_2O over time, and the effects of soil water content and/or temperature on these greenhouse gases were published by Horel *et al.* (2018e), titled “Soil CO_2 and N_2O emission drivers in a vineyard (*Vitis vinifera*) under different soil management systems and amendments”.

CO_2 fluxes increased in the second year in the vineyard samples in all treatments but the absolute control (no till, no fertilizer addition, and no biochar amendment) and the control (tilled with no fertilizer and no biochar addition; Figure 9). During the two year-long study at the vineyard the highest overall CO_2 production was observed in the case of the tilled, not fertilized, and biochar amended soils, while the lowest in the undisturbed control plots (C; Table 2).

Data from maize, forest, and grassland sites on soil CO_2 emissions show that during the second year of CO_2 production, the biochar amended treatment had 8% higher emission compared to control treatment in the case of maize and 5.9% in the case of forest, while in grassland the biochar amendment reduced the overall CO_2 emission by 6.3% (Figures 10 and 11). While during the first year in the forest soils biochar amendment reduced overall CO_2 emission (7% reduction), the two year average showed a small increase (2.3%) compared to control treatment (Table 2). In general, consistent reduction in soil CO_2 production was only found in the case of grassland when soils were amended with biochar.

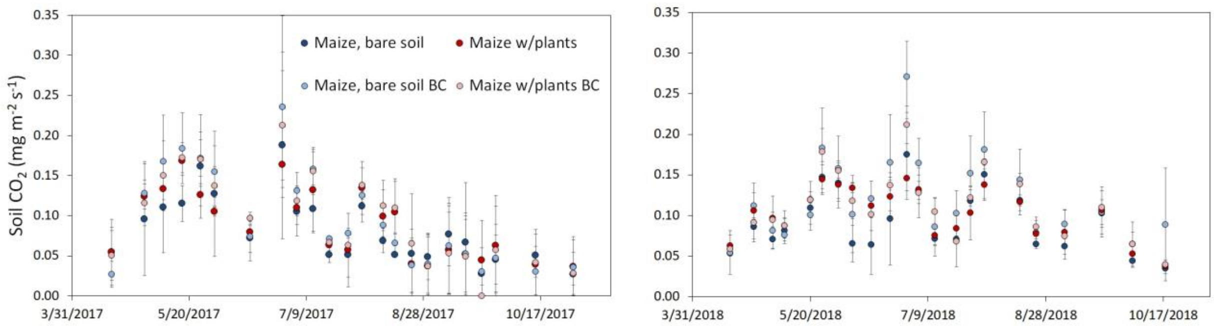


Figure 10. Soil respiration as CO_2 ($mgCO_2$ m^{-2} sec^{-1}) changes over time for the maize sites with and without vegetation or biochar (BC) amendment for 2017 and 2018. n=4; ±SD

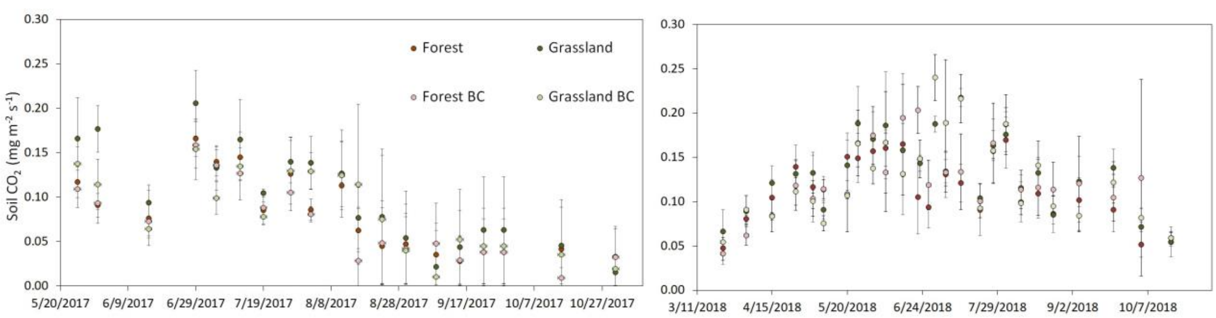


Figure 11. Soil respiration as CO_2 ($mgCO_2$ m^{-2} sec^{-1}) changes over time for the forest and grassland sites with and without biochar (BC) amendment for 2017 and 2018. n=4; ±SD

N_2O production in the vineyard showed similar or even lower emission values with biochar amendment; however, during the second year and consequently over the overall two year period, the N_2O fluxes increased in the biochar added site with manure amendment compared to the control treatment. Similar findings were observed in the maize field (Table 3). Overall, N_2O production decreased in the case of vineyard samples, while increased emission values were observed in the case of forest, grassland, and maize samples from 2017 to 2018 (Table 3). During the first year biochar amendment resulted in a decrease in N_2O emission all but the forest samples, while during the second year all but in the case of tilled + biochar amended vineyard samples compared to their non-amended controls. In the maize, grassland, and manure added vineyard sites, both first and second year of the experiment biochar amendment decreased the overall N_2O fluxes.

Table 3. Soil N_2O respiration values for the different land uses and management systems. C represents control, while BC biochar amended treatments. n=76-99 (2017), n=84-93 (2018)

N_2O ($\mu g\ m^{-2}\ s^{-1}$)	2017	2018	2017-2018
Treatment types		**Average**	
Forest C	-0.0348	-0.0006	-0.0162
Forest BC	-0.0339	-0.0108	-0.0222
Grassland C	-0.0273	0.0038	-0.0102
Grassland BC	-0.0348	0.0020	-0.0147
Maize C	-0.0178	0.0006	-0.0088
Maize BC	-0.0185	-0.0080	-0.0134
Grape C	0.0108	0.0032	0.0071
Grape, Tilled	0.0126	0.0040	0.0084
Grape, Tilled, Manured	0.0112	0.0092	0.0102
Grape, Tilled, BC	0.0111	0.0074	0.0093
Grape, Tilled, Manured, BC	0.0085	0.0017	0.0052

3.6 Biomass production, leaf area index, and spectral reflectance studies on plant growth

Plant growth and maturing were examined weekly for the pot and the maize experiment. The results of the pot experiment showed that plant biomass varied similarly in all treatments, but some small differences attributable to biochar addition were also detected. Based on temporal changes of stem biomass values, regardless of treatment, all stages were significantly different ($p < 0.033$) from each other, indicating a continuous plant development. Looking at plant parts separately as stem and leaf, the growths of leaves and, consequently their biomass values, showed similar tendencies to that of stems, although both leaf biomass and leaf numbers reached their maxima earlier around the 6th week. The compared stems in all treatments remained approximately constant afterwards. Maximal stem biomass occurred in the 10th week in all treatments, when BC2.5 presented the highest stem biomass; however, the difference among treatments was not statistically significant ($p > 0.05$). Plant leaf biomass showed significant differences from the other growth stages' values only at the third week ($p < 0.001$, ANOVA) in the early stage of plant development (Horel *et al.*, 2019b). There was a plateau in plant biomass production that after reaching an optimal (2.5%) biochar amendment in the soils, and excess biochar addition did not result in significant changes in the soils' pH to achieve better nutrient (potassium, nitrogen, phosphorous) use or crop growth.

During the first three weeks, stem thickness and plant heights were increasing at rates between 0.24 (control) and 0.42 mm d^{-1} (BC2.5), which showed an exponential increase in all treatments until week 6, where the growth slowed down reaching a plateau. Even though biochar addition showed larger plant heights and stem thickness values, the amount of biochar added to the soil showed no linear response, e.g. BC2.5 treatment had the highest average values in all investigated plant growth phases. Based on the 12 weeks averages, BC 2.5 treatments showed 15.44% higher stem thickness compared to control treatment, while BC0.5 and BC5.0 had 3.77 and 3.56% increase, respectively. These changes showed no substantial influence on stem thickness of low or high biochar amendment to the soil. When comparing plant heights during the experimental period, BC2.5 and BC5.0 treatments had 13.58 and 12.10% increase, while BC0.5 had only 6.98% increase in height compared to controls. Plants leaf and tiller numbers were also measured and data presented in manuscripts by Horel *et al.* (2019b) and Horel *et al.* (2019d), respectively. The weight of the plants' roots showed similar results, the BC2.5 had the highest value with 45.5% increase compared to control, though, BC5.0 and BC0.5 also had higher root density and weight compared to control (20.0 and 8.1%, respectively). These results indicate that biochar addition can help plant growth during the maturity phase; however, at later times, in this study around 6 weeks, the plants might reach a plateau value and the difference between treatments might stay relatively constant.

The leaf area index (LAI) was measured with different methods. One method used the complete plant disassembly and measuring using a plant scanner (Delta-T Devices). This method was applied for the pot experiment only. For the field studies, photosynthetically active radiation (PAR) sensors were placed below and above canopy and normalized difference vegetation index (NDVI) and photochemical reflectance index (PRI) sensors for hemispherical and field view (nadir). Placing these sensors enabled continuous monitoring, concurrent with occasional LAI measurements taken using a handheld AccuPAR LP-80 instrument.

The investigated pepper plants during the pot experiment showed that the highest leaf area and consequently LAI was measured in the case of BC2.5 treatment, corresponding to the amount of biochar chosen for the field experiment. The measured LAI were the following: 1.58, 1.59, 2.03, and 1.89 for control, BC0.5, BC2.5, and BC5.0, respectively. However, during earlier plant development stages, higher biochar amendment (BC5.0) also resulted in the highest LAI.

The PRI value, which might reflect the stress response from the plants mainly to drought or nitrogen deficiency (Zhang *et al.*, 2016), showed decrease in the case of BC5.0 treatment compared to control during the entire measuring time (from week 4 through 12; Figure 12). The differences between the treatments were significant ($p < 0.001$, ANOVA), indicating changes in light use efficiency when biochar is used in excess amount.

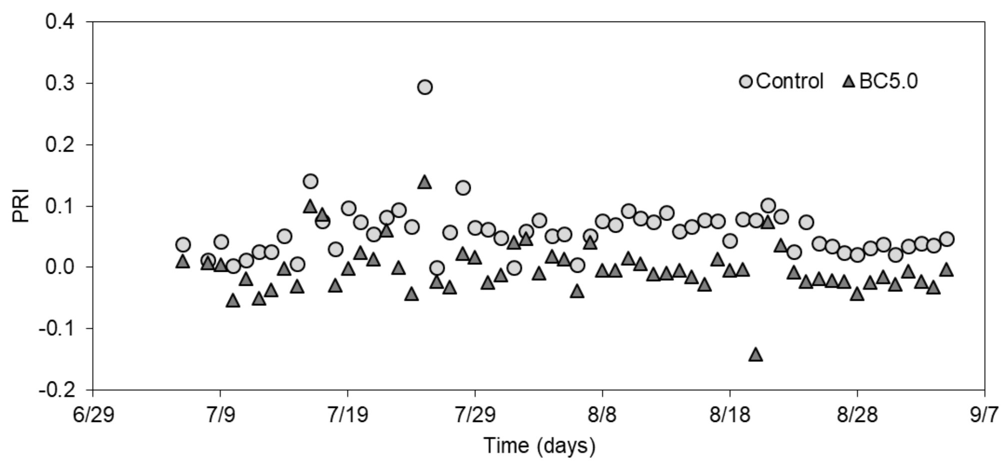


Figure 12. Average midday PRI values for the pepper plants in control and BC5.0 treatments.

After two years of data on grape production from all four treatments in the vineyard, biochar addition to the tilled and fertilized (with organic fertilizer) site resulted an increased overall fruit yield by 53.8 and 23.9% for 2017 and 2018, respectively. In the case of no fertilizer treated plots, biochar addition did not increase the fruit yield, 14.1 and 15.6% decrease in yield values were observed in 2017 and 2018, respectively, compared to control plots.

Data retrieved from the PAR sensors at the vineyard site showed that the highest biomass production occurred in the case of biochar and organic manure treated plots; however, biochar addition without fertilizer also showed much lower PAR values compared to the non-amended treatments ($p = 0.749$ between the two biochar amended treatments), especially during the second year of the experiment, which can signify for denser vegetation (Figure 13). The collected PRI data showed that both fertilizer added and fertilizer + biochar amended treatments had lower PRI values (often negative) compared to control, indicating that there might be significant effects of the soil moisture differences between treatments, causing water stress related changes to the plants. NDVI values increased in biochar amended treatment compared to control; however, the organic manure and biochar amended sites had lower NDVI values compared to fertilized only plots (Figure 13). Similar to the PAR data, these NDVI data indicate healthier and denser vegetation in fertilized plots (i.e. greener, higher biomass).

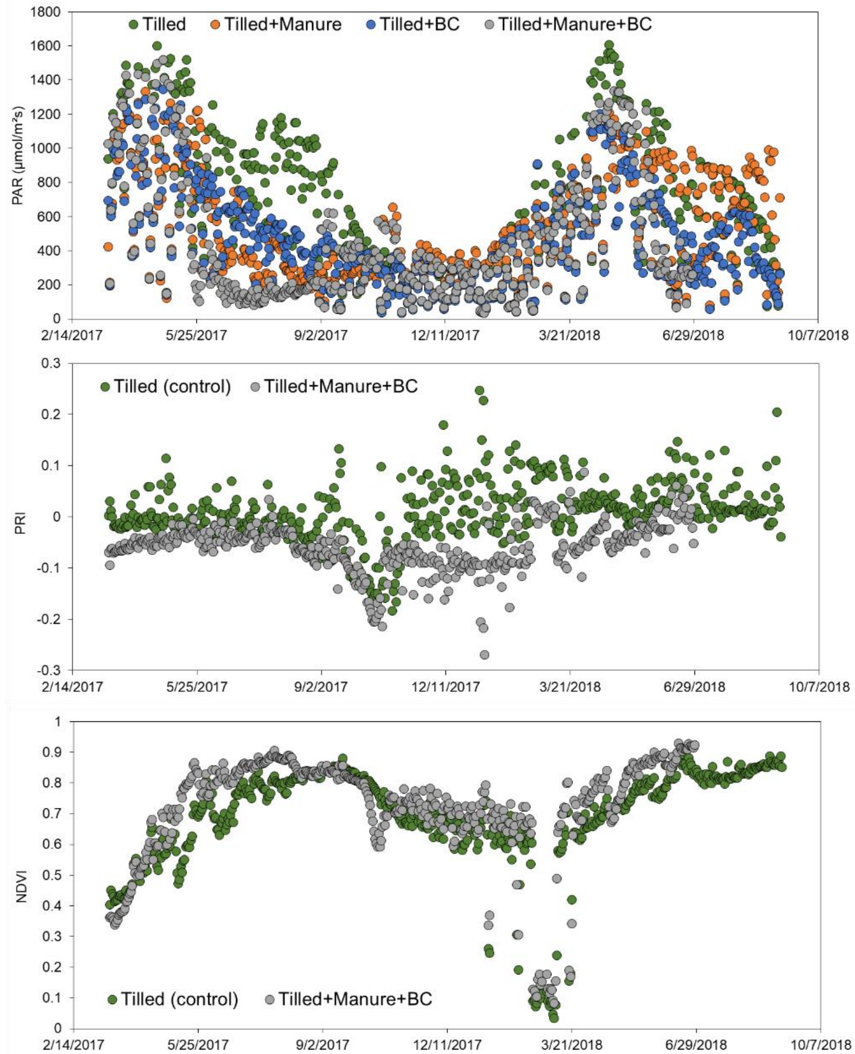


Figure 13. Midday average PAR, PRI, and NDVI data of the vineyard plots for tilled, manured, or biochar (BC) amended treatments. Presented values are raw, unfiltered for snow or weeds data.

The first year biomass production in the maize field showed higher root and lower above ground biomass in the case of biochar amended soils; however, these differences were not significant ($p > 0.05$). Similarly, during the second field year, the biochar amended treatment showed 4.8% less average aboveground biomass, while the below ground biomass (roots) showed 43.1% increase compared to the control. When analyzing the plant heights over time, the control showed minimally higher plants (9.3%); however, these differences were statistically not significant ($p = 0.905$, ANOVA). For the maize experimental site, LAI measurements were done using the handheld ceptometer. The average LAI in 2017 for the plants after full plant maturation were 1.32 and 1.45 measured within rows, and 1.50 and 1.76 measured in rows for the control and biochar amended treatments, respectively. However, after harvest the maize plants in the biochar amended plots dried out faster resulting in lower LAI numbers compared to control. Interestingly, during both 2017 and 2018 the number of maize grown for the investigated area were approximately 8.75% less in the biochar amended plots than in the controls. Data based on the PAR sensors showed, that during the second year of the maize treatments, control had somewhat lower but similar biomass and consequently LAI compared to biochar amended treatments (Figure 14), highlighting the possible diminishing positive effect on biomass production of biochar application and the necessity of studies based on different agricultural crops at different time scales. Comparable data were observed in the case of the PRI measurements, biochar amended plots showed lower values compared to controls (Figure 14). This might indicate the possible negative effect of soil moisture changes on plants, as there were no nitrogen deficiency observed in the soils.

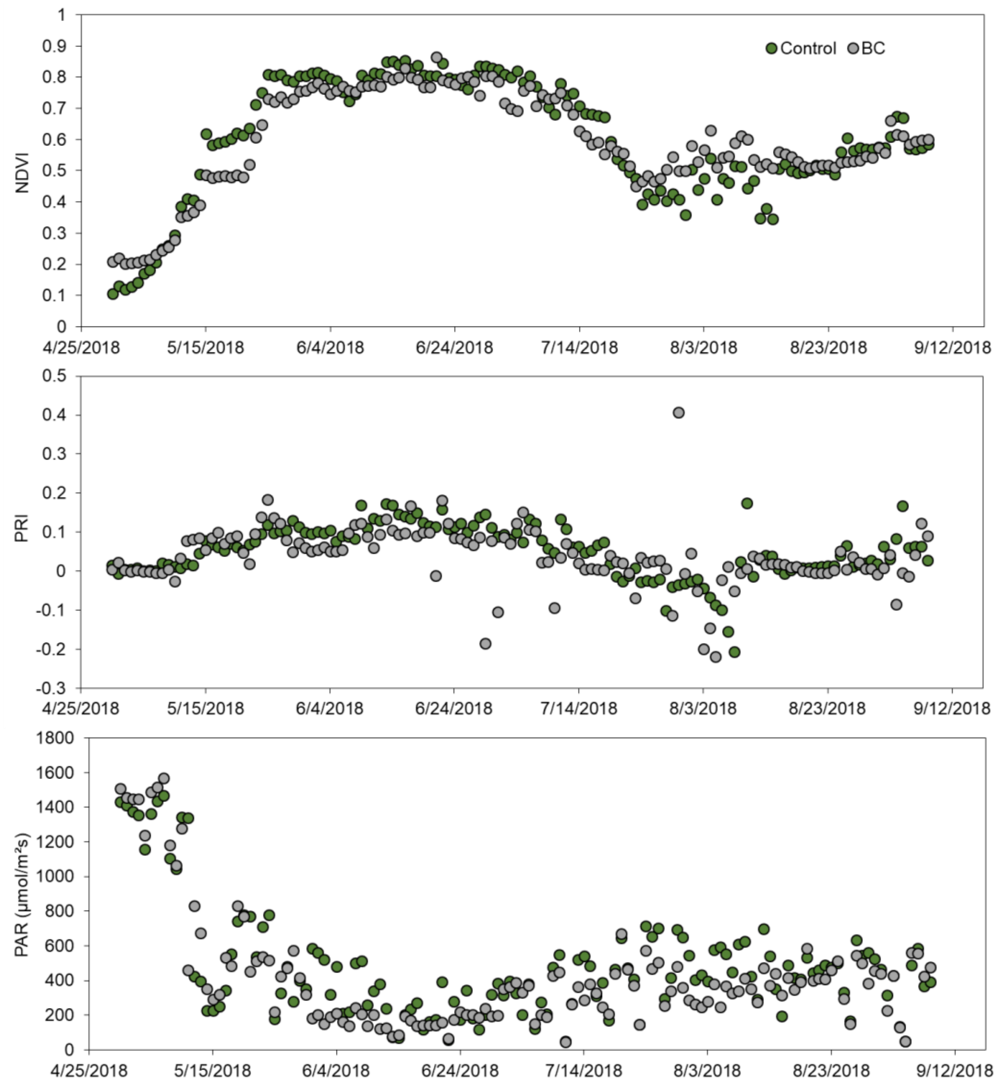


Figure 14. Midday average NDVI, PRI, and PAR measurements for maize field with (BC) and without (control) biochar addition during 2018 vegetation period.

3.7 Mathematical modeling – HYDRUS 1D

Mathematical modeling on soil water changes was completed on an arable land using HYDRUS 1D where different biochar amounts were applied to silt loam soil. As some of the meteorological data, especially rainfall amounts, were locally monitored, the collected data could be used to mathematically model the changes. The control and low (BC0.5) biochar amended treatments were used to calibrate the chosen hydrological model (HYDRUS 1D) and the medium and high amount (BC2.5 and BC5.0, respectively) of biochar added treatments were used to validate the model. Model parametrization and exact input/output information can be found in Horel *et al.* (2019d).

In general, the model was slightly underestimating changes in SWC when high biochar amount was added to the soils, especially during higher rain events (Figure 15). These changes were also concurrent with plant biomass amount, as BC2.5 and BC5.0 had the highest plants and denser roots, which were important input parameter differences in the biochar amended models. Simulated BC0.5 SWC showed more overestimation of moisture content compared to the measured data and to the simulation results of the other treatments, moreover, the model performed the least accurately in this case (RSR = 0.5361) compared to the other simulations (Horel *et al.*, 2019d).

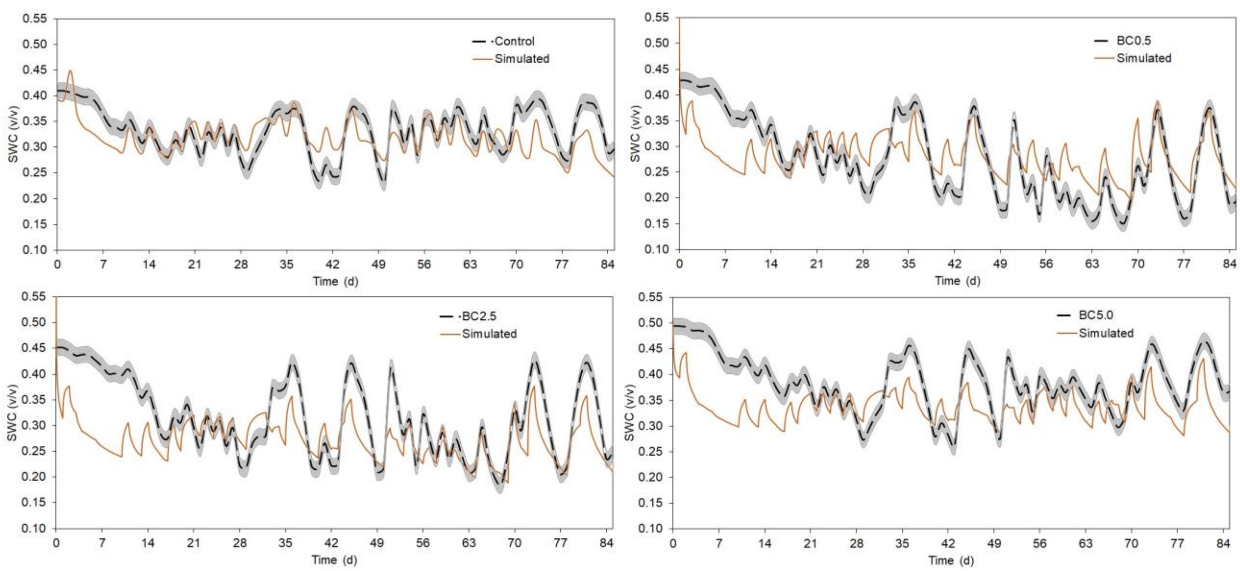


Figure 15. Observed and simulated SWC changes for the different treatments. i) control, ii) BC0.5, iii) BC2.5, and iv) BC5.0. Grey area represents measured SWC uncertainty of 1.5%.

Using data retrieved from the hydrological models, potential evaporation, transpiration, and evapotranspiration (ET) changes for all four treatments were also simulated over time during the different plant growth phases. All four treatments showed similar trends of potential evaporation, transpiration, and ET changes driven by varying air and soil temperatures. Potential evaporation values were larger in the treatments during the first three weeks of the study (average evaporation was 0.3745 mm d^{-1} during the first three weeks), especially at precipitation or irrigation events. Potential evaporation values leveled out during the third to sixth weeks, and increased during the last four weeks (from 8^{th} to 12^{th}) of the study (average potential evaporation was 0.4148 mm d^{-1}). Overall potential evaporation (PET) values were continuously increasing during the course of the study simultaneously with plant growth and fruit maturity. The highest PET was estimated in the case of BC2.5 (average PET was 1.6953 mm d^{-1}), while the lowest in the case of control and BC5.0 (average PET was approximately 1.6065 mm d^{-1}). These results, among the plant growth differences, were also related to the soil moisture data, as higher average soil moisture contents were observed in the control and BC5.0 treatments (Horel *et al.*, 2019d).

Overall, the model was able to accurately estimate the effects of different biochar amount additions to soil hydrological processes (RMSE=0.0405, RSR=0.139, NSE=0.732); therefore, this model can be a great tool to better approximate the best amount of biochar that optimizes soil water content for plant use.

4. Conclusions

The nitrogen cycling studies highlight that soil biological and chemical differences can be developed over time between land use types due to human interferences such as tillage, fertilizer addition, crop rotation or biochar application. The human impact on soils were found to be significant between land uses, e.g. arable land had the lowest denitrification potentials, while the highest were observed in the forest soils, with or without biochar amendments. Nitrogen fixation were highest in the most agriculturally disturbed soil (i.e. arable land), where biochar addition restricted N_2 fixation. Biochar amendment at high amount resulted in a reduced N_2 fixation potentials in all land uses.

The present study emphasizes strong connections between soil structural changes and plant development phases, and enhances the importance of soil and site specific analyses prior to application of biochar. There were distinct connections between the physical and structural properties of the investigated soils. Biochar can positively influence aggregate formation and hydraulic conductivity, and can reduce the bulk density of the soils. These connections emphasize soil biotic health. Good correlations were found between the investigated soil structural parameters and their changes due to biochar addition, including changes during different plant phenological stages. There were variations in aggregate stability without the addition of biochar to silt loam soil; however, these changes were less pronounced when compared to the biochar amended treatments, indicating the benefits of biochar amendments on soil structural strength. The amount of biochar addition can influence the rate of aggregate stability increase, such that too much biochar addition may not provide optimal results.

The most notable result in soil water changes was the faster drying period with biochar amended soils compared to controls in most field sites. This was especially the case at the top portion of the soil columns, even though biochar helped retain more water at precipitation events.

The CO_2 emissions increased in most land use types and soil management systems, but the grassland and at vineyard with tilled, manured, and biochar amended soils, indicating that soil chemical and water availability for the microorganisms were site specific. In term of N_2O emissions; however, all East-Bakony land uses and the vineyard with manure and biochar addition resulted in lower emission values compared to their non-amended controls. This finding highlights the GHG reducing potentials of biochar use in agricultural soils.

Leaf area indexes (LAI) showed that biochar addition increased LAI values for maize, while in the vineyards, the tilled only soil management resulted in higher LAI compared to tilled + manured soils. In the vineyard, during leaf development both PRI and NDVI values were higher for control plots, while after fruit production, NDVI and PRI values increased in biochar treated plots. These findings indicate short-term changes in photosynthetic light-use efficiencies and no major differences in physiological stress between the treatments during fruiting stage.

While these results are confirmed for specific types of biochar and soil types during a given plant growing phase, there are still many areas with research gaps present in the study of biochar amendments to soils. Therefore, further investigations should be implemented to draw larger-scale conclusions.

5. The Project's achievements in the light of the expected deliverables

According to the proposal, the main objectives (O), deliverables (D) or the expected practical and scientific impacts of the project were the following:

- ***O1:*** *to investigate the response of potential nitrogen fixation, net nitrification, and denitrification rates to introduced biochar in grass, forest, and cultivated crop dominated agricultural systems;* this objective was met during the first year (nitrogen fixation and nitrification) and a half of the project, as denitrification experiments had to be delayed due to instrumentation difficulties.
- ***O2:*** *to see if seasonal changes (e.g. temperature, precipitation amount) can influence the rate of the different processes of N cycling in biochar amended soils*; seasonal changes on N cycling were investigated both in laboratory and field-scale studies. Findings are detailed in the report.
- ***O3:*** *to investigate the rate of influence of different amount and types of biochars on soil hydro-physical properties*; this objective was achieved using both pot- and field-scale experimental data. Findings are detailed in the report.
- ***D1:*** *Better understanding of the effects of biochar on soil nitrogen cycle processes in different agricultural systems, including different land use and soil management systems.* The findings present in this study help filling in the research gap on the influence of biochar on agricultural ecological systems when applied to different soil types, land uses, and management systems.
- ***D2:*** *Enhancement of our current knowledge on soil property improvement techniques that could be used on determining the best biochar application method based on soil properties.* While the present study did not come up with a definite answer on how generally biochar application rates should be determined, the study did highlight the necessity to carefully investigate site specific applications prior to biochar use.
- ***D3:*** *Supporting ecosystem improvements and the additional knowledge would help in both short-term and long-term decision making processes in regards to agricultural applications and agro-ecosystem sustainability.* Several findings of the present project help ecosystem improvements, economical decision making; however, for the longer term benefits, the present experimental time frame might not be sufficient enough. To better address this shortcoming, the research sites should be revisited in the upcoming years.
- ***D4:*** *The study would result in numerous publications in national and international peer-reviewed scientific journals.* The outcomes of the study were presented in several national and international peer-reviewed journals, detailed below in the "Publication activity" section of this Final Report.

6. Publication activities

During the first year of the study the main focus was on laboratory studies and analyzing the findings to plan the field studies for the second and third year of the project; therefore, the publications were mostly including conference proceedings and poster presentations. Studies with methodical similarities on soil water and respiration under different land management systems (Gelybó *et al.*, 2015) and results of soil hydrological processes (Kása *et al.*, 2015) were presented at the Transport of water, chemicals and energy in the soil-plant-atmosphere system conference in Bratislava, Slovakia, 2015 and published as conference papers/book chapters. In the European Geophysical Union (EGU) meeting in Vienna, Austria a poster was presented on soil hydrological processes in 2016 (Horel *et al.*, 2016). The first peer-reviewed paper from this project's outcome was published in the journal of Agrokémia és Talajtan (Q3) in 2016 in Hungarian, explaining the effects of temperature and biochar on soil nitrification processes while varying land uses and biochar types and amount (Kása *et al.*, 2016).

At the EGU a presentation was delivered on soil nitrogen dynamics changes as a results of biochar amendment in 2017 (Horel *et al.*, 2017b). Peer-reviewed papers (in Hungarian) were published in 2017 in the journal of Agrokémia és Talajtan (Q3), where compost and biochar treatment effects on soil moisture and respiration were analyzed (Dencső *et al.*, 2017). Also some methodologically related papers were published on soil hydrological processes in the journals of Agrokémia és Talajtan (Q3) (Horel *et al.*, 2017a) and Biologia (impact factor: 0.696, Q3) (Kása *et al.*, 2017).

Based on the pot experiment, conference proceedings/abstracts and posters were presented on changes in soil physical properties of a biochar amended soil at the International Conference on Agrophysics: Soil, Plants & Climate in Lublin, Poland, 2018 (Barna *et al.*, 2018). Greenhouse gas emission changes at the vineyard sites under different soil management systems and biochar amendments, emphasizing on emission driving forces such as soil water and temperature were presented at the EGU conference in 2018 (Horel *et al.*, 2018c). A methodologically related, peer-reviewed book chapter on soil CO_2 emission and soil moisture changes was published by Academic Press, Elsevier in 2018 (Tóth *et al.*, 2018). A review article was published in 2018 by Agrokémia és Talajtan (Q3) in English where the potential impacts of climate change on soil properties were analyzed in greater detail (Gelybó *et al.*, 2018). A paper on potential nitrogen fixation changes under different land uses as influenced by seasons and different types and amounts of biochar amendments were published in 2018 in the Arabian Journal of Geosciences' Special Issue on "Implication of biochar application to soil environment under arid conditions" (impact factor: 1.141, Q2) (Horel *et al.*, 2018b). The first year's data collected in the vineyard experimental site was published in the journal Sustainability (impact factor: 2.592, Q2) in 2018, where soil CO_2 and N_2O emission drivers were investigated under different soil management systems and amendments (Horel *et al.*, 2018e). Several posters were presented at the Hungarian Soil Science Society's biannual meeting in Pécs, Hungary in 2018 (in Hungarian). One highlighted the soil hydro-physical changes such as hydraulic conductivity or micro- and macro aggregate stability, while another the specific surface area and bulk density changes as influenced by biochar addition to silt loam soil at different amounts (Horel *et al.*, 2018a; Horel *et al.*, 2018d).

As the project's deadline was extended by 7 months to include the entire second vegetation season for field experiment, there were also more time to process the gathered data and publish the findings in peer-reviewed journals and present them in conferences/meetings. During this extended time, two additional presentations were prepared and four manuscripts were accepted in journals with impact factors. The data retrieved from the spectral sensors under different cultivation methods and biochar amendments were presented at the EGU meeting (PICO presentation – Presenting interactive content) in 2019 (Potyó *et al.*, 2019). Results based on two consecutive vegetation years' on greenhouse gas emissions for all four land uses (i.e. vineyard, maize, grassland, and forest) were also presented at the EGU conference in 2019 (Horel *et al.*, 2019c). A paper examining changes in soil nutrient dynamics as influenced by biochar amendment was published in the journal of Agronomy (impact factor 2.259, Q1), which paper also focused on nitrogen fixation changes resulting from different amount of biochar addition at different vegetation growth periods (Horel *et al.*, 2019b). Part of the present project aimed at using mathematical modeling to simulate the effects of biochar amendment on soil hydrology. Successful calibration and validation of the HYDRUS 1D model was accomplished, where soil water changes were simulated while varying biochar amounts. These findings were published in the journal of Agronomy (impact factor 2.259, Q1) (Horel *et al.*, 2019d). Prior to the mathematical modeling, especially for model input parameters, soil structural and hydro-physical changes needed to be investigated, measured, and analyzed, which results were published in the journal of International Agrophysics (impact factor 1.227, Q2), in two parts. The first paper is already published and available online (Horel *et al.*, 2019a), while the second manuscript was also accepted and is currently in press (Makó *et al.*, 2019). The methods developed for the nitrogen cycling measurements during this project's framework were used to investigate nitrogen cycling changes under different land uses at a Balaton Upland study site and the results were published in Agrokémia és Talajtan journal (Q3) in Hungarian (Baklanov *et al.*, 2019).

Even though this OTKA/NKFIH project has finished, there are still several manuscripts being currently written and expected to be published in the upcoming years, including manuscripts with international collaboration.

7. Main changes in the course and budget of the study

One major instrument upgrade originally considered in the research plan was financed by another project; therefore, the unused money was spent on different sensors such as spectral sensors, and additional data loggers for continuous field work monitoring after acquiring permission from the OTKA/NKFIH.

Due to optimization of time and cost associated with fieldwork some of the site locations originally planned were changed. This change did not influence the research outcomes, as the sites provided similar research environment, providing all information for the study to succeed. This change also enabled more frequent sample collections.

The duration of the project was expanded with seven months with permission from OTKA/NKFIH. During these additional months, the last field year was measured throughout the entire vegetation season, and data gathered were analyzed and manuscripts prepared or submitted.

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