



Limited effects of crop foliar Si fertilization on a marginal soil under a future climate scenario

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ARTICLE INFO

Keywords:

Marginal soil
Climate change
Ecosystem services
Sustainable agricultural practices

ABSTRACT

Growing crops on marginal lands is a promising solution to alleviate the increasing pressure on agricultural land in Europe. Such crops will however be at the same time exposed to increased drought and pathogen prevalence, on already challenging soil conditions. Some sustainable practices, such as Silicon (Si) foliar fertilization, have been proposed to alleviate these two stress factors, but have not been tested under controlled, future climate conditions. We hypothesized that Si foliar fertilization would be beneficial for crops under future climate, and would have cascading beneficial effects on ecosystem processes, as many of them are directly dependent on plant health. We tested this hypothesis by exposing spring barley growing on marginal soil macrocosms (three with, three without Si treatment) to 2070 climate projections in an ecotron facility. Using the high-capacity monitoring of the ecotron, we estimated C, water, and N budgets of every macrocosm. Additionally, we measured crop yield, the biomass of each plant organ, and characterized bacterial communities using metabarcoding. Despite being exposed to water stress conditions, plants did not produce more biomass with the foliar Si fertilization, whatever the organ considered. Evapotranspiration (ET) was unaffected, as well as water quality and bacterial communities. However, in the 10-day period following two of the three Si applications, we measured a significant increase in C sequestration, when climate conditions were significantly drier, while ET remained the same. We interpreted these results as a less significant effect of Si treatment than expected as compared with literature, which could be explained by the high CO₂

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levels under future climate, that reduces need for stomata opening, and therefore sensitivity to drought. We conclude that making marginal soils climate proof using foliar Si treatments may not be a sufficient strategy, at least in this type of nutrient-poor, dry, sandy soil.

1. Introduction

European agricultural land is facing acute challenges brought by rapidly changing climate and demands to quickly adapt the agricultural production for a sustainable contribution to global food security. Among the most promising solutions is alleviation pressure for valuable farmed land by transferring the growth of industrial crops on marginal land. A marginal land is a land that is not farmed because it is not suitable for conventional high-intensity agriculture [1]. This can be due to land abandonment, contamination, or scarcity of nutrients or water [2]. Some of these marginal soils could be mobilized for industrial crop production, for example through a tailored use of sustainable amendments [3].

This process will however be challenged by climate change. Marginal land comprises soils where crops are already facing adverse environmental conditions (e.g., restricted water and nutrient availability, contamination, salinity ...). Consequences of climate change will add an extra environmental pressure: increasing frequency and intensity of droughts will cause yield reduction due to water and heat stress, heat waves and high intensity rainfall combined with storm events may harm crop growth due to wind damage and pests, respectively [4]. In fact, among all these challenges, drought and crop pests will be the most prominent stressors that crops will face in the future [5]. The energy mobilized by plants to fight these environmental stresses cannot be allocated to growth, which will be detrimental to productivity. Therefore, adaptive management strategies for future crop production are one of the most important means to avoid negative climate effects and to benefit from future climate changes [6]. Hence any agricultural practice that could even partially alleviate these stresses could considerably increase the potential of marginal land. Of course, this is under the condition that they are as sustainable and affordable as possible, avoid soil pollution and ensure at least a minimal profitability.

Foliar Silicon (Si) fertilization appears as a promising solution to face this problem. This treatment entails spraying a solution containing Si ions on the leaf surface of the crops. This technique has been found to improve plant tolerance to many stresses [7]. Silicon accumulated on the surface of plant leaves and stems acts as a shield to protect against radiation injury [7], makes plant surface more resistant to penetration by leaf pathogens [8], but also acts as an extra layer altering the cuticle's water permeability, thereby significantly reducing plant transpiration [7,9]. Silicon has even been found to alleviate metal(loid) stress [10]. It can increase plant C content through stimulating the formation of phytoliths, this way enhancing long-term C sequestration potential [11]. Hence Si treatment is expected to have a great potential to improve crop performance on marginal soils, and has been proposed as a large scale sustainable solution to counteract negative impacts of drought on crops [12,13]. However, despite multiple tests of individual mechanisms associated with crop response to Si treatments, an exhaustive assessment of the impacts of Si treatment on soil functioning and associated ecosystem services has to the best of our knowledge so far not been performed, especially in the context of climate adaptation of marginal soils.

In this study, we provide a first exhaustive assessment of the impacts of Si amendments on functioning of a common crop (barley) on a very dry marginal soil, in the conditions where drought is further enhanced to values predicted in 50 years under the most extreme climate projection. By imposing these maximally harsh conditions, we aimed to test (1) whether the Si treatment is effectively improving crop performance under drought stress, and (2) whether and how this treatment does affect ecosystem services related to plant-soil interactions and to soil functioning. Our hypotheses were: i) Si treatment increases crop yield by reducing both crop transpiration (and thus improving water use efficiency WUE and drought tolerance) and pest prevalence by improving plant surface physical resistance; ii) this higher crop yield would translate into more root biomass, and less nutrient leaching because of higher N plant demand; and iii) there will be an increase in C occluded in phytoliths, as previously reported in the literature. To test these hypotheses, we grew spring barley (*Hordeum vulgare* L.) in six 4.7 m³ macrocosms in an ecotron facility and exposed them to a local projection of the 2070–2075 climate, according to RCP 8.5 scenario. All macrocosms received optimal NPK fertilization, but only three were Si-treated by leaf spraying of SiO₂ solution. We monitored hourly the C balance, methane balance and actual evapotranspiration of each macrocosm, and characterized the plant biomass per organ (including crop yield) and soil water chemistry (at different soil depths).

2. Material & methods

2.1. Macrocosms

Six macrocosms with a diameter of 2 m and depth of 1.5 m were extracted monolithically in November 2016 from a dry heathland plot in the 'Hoge Kempen' National Park (50° 59' 02.1" N, 5° 37' 40.0" E) and placed in six separate ecotron units in the UHasselt ecotron facility (Maasmechelen, Belgium, <https://www.uhasselt.be/en/instituten-en/cmk-centre-for-environmental-sciences/infrastructure/ecotron>). This plot is dominated by heather (*Calluna vulgaris*, 70 % cover and *Molinia caerulea*, 10 %) which was in the growing phase at the time of extraction, and has been managed for restoration in 2010. The soil in this plot is a Brunic-Dystric Arenosol [14], with an organic layer of 10–20 cm depth, on top of a sandy matrix containing 5–10 cm clay lenses, and with a ferric iron precipitation horizon at 150–200 cm depth. The soil pH varies from 6 on the top, organic layer and 4 to 5 on the B horizon. The TOC content of the soil varies between 1.9 % in the top, organic layer and 0.5 % below down to 140 cm.

Until January 2020, all macrocosms were exposed to the same artificially re-created ambient climatic conditions. On that date, the macrocosms were “marginalized”: topsoil and vegetation were removed, ending up in a typical marginal land soil: sandy, dry (35 % water holding capacity) and nutrient-poor. Between January 2020 and January 2021, they have been exposed to local projections of the RCP 8.5 scenario in the 2070–2075 period as described in the “climate simulations” section.

Each macrocosm was prepared for the experiment as follows. On the October 13, 2020, mustard was sown as a cover crop, and was harvested on the March 5, 2021. We then corrected soil acidity using 10 t/ha equivalent of CaCO_3 on the same day (DOY 64) to reach a neutral soil pH (pH7). We then applied mineral NPK fertilisation on the March 17, 2021 (100 N 80P 120K, with NH_4NO_3 , $\text{NH}_4\text{H}_2\text{PO}_4$ and KNO_3 , DOY 76), before sowing spring barley on the March 30, 2021 (*Hordeum vulgare* L. cv. FANTEX, AVEVE Belgium, 450 seeds/ m^2 , 3–5 cm deep, DOY 89). Plants were harvested after reaching physiological maturity on the August 18, 2021 (DOY 230). Application of the Si treatment is described below in the “treatment” section.

2.2. Climate simulations

The ecotron was used as a simulator of climate change, where air temperature, air relative humidity, precipitation, air CO_2 concentration, wind speed, bottom soil temperature and bottom soil water potential can be separately controlled for each macrocosm. Additionally, Photosynthetically Active Radiation (PAR), net radiation, lysimeter weight, leachate weight, and air CH_4 and N_2O concentrations were monitored at high frequency (every 1–30min). Soil water chemistry (at depths of 0.10, 0.20, 0.35, 0.60, and 1.40 m, all in triplicates) was measured every 3 weeks. All six macrocosms were separately exposed to the same climate treatment: local climate projections for a typical year in the 2070–2075 period, according to the RCP 8.5 emission scenario [15]. These projections were based on the most accurate combination of large and regional climate models for predicting local (15 km side pixel) conditions. The description of the rationale behind this model choice is detailed in Refs. [16,17]. The model provided 3h-resolution data for air temperature, air relative humidity, precipitation, and wind speed. These data were downscaled to every half-hour (the time scale at which the ecotron operates for most parameters) by linear interpolation. The air CO_2 concentrations were calculated as follows: the difference in yearly average CO_2 concentration between 2020 and 2070–2075 (428 mg/m^3 or 221 ppm) is added as an offset on real-time concentrations measured in a nearby ICOS station [17], resulting in an average concentration of 1280 mg/m^3 (or 661 ppm). Soil water potential and soil temperature were set to follow ICOS field values measured at 1.4 m depth (average values for 2021: 9.3°C and -40 HPa). We also calculated reference evapotranspiration (ET_0) using Penman-Monteith equation with grass as reference crop [18]. To measure the degree of exposure of the plants to drought, we calculated Ri , the ratio between measured evapotranspiration (see below for more details) and ET_0 , on a daily basis for every unit. This ratio is a good indicator of plant exposure to drought stress, with Ri values below 0.6 indicating water stress conditions for the plant [19].

2.3. Treatment

A Si treatment was applied in three out of the six macrocosms; the three other macrocosms were left untreated and will be referred to as “controls” in the manuscript. Optysil (INTERMAG sp. z o.o., Al. 1000-lecia 15G, 32–300 Olkusz, Poland) was applied as leaf spray (rate 0.5 L/ha ; the product had to be diluted in water to allow spraying in a 3.14 m^2 surface) at three growth stages: tillering (April 29, 2021, DOY 119), elongation (June 8, 2021, DOY 159), and heading (June 29, 2021, DOY 180). This commercial product contains 200 g/L SiO_2 and 24 g/L Fe-EDTA . We did not add herbicides but weeded manually at the elongation stage (June 3, 2021) as *Rumex acetosella* L. colonized some macrocosms. We took care during the spraying that the treatment was directed to barley leaves, and only a very limited fraction reached *R. acetosella* leaves and the soil surface.

2.4. Crop properties

Plants were harvested on DOY 231 (August 18, 2021). At that date, we measured plant density by placing three $50 \times 50 \text{ cm}$ quadrats at the surface of each macrocosm and counting the number of barley stems. Then, 10 plants were harvested (shoot and roots) in each quadrat for biomass measurements. The organs of each plant were then separated (grains, chaff, stem, leaves, and roots) and all were dried overnight at 60°C , after which the dry weight of each organ was measured as well as the number of grains and leaves. Grain DW yield was calculated as the number of individual plants multiplied by the grain weight per plant and converted from grams per m^2 to t/ha. The method used in this study for the isolation of phytoliths from leaf and stem samples was the microwave digestion process described in detail by Ref. [20]. The absence of extraneous materials in the samples was checked by optical microscopic examination including cross-polarised light, which can be used to differentiate plant silica from cellulose. The C concentration in phytolith was then analysed on a Thermo Elemental CHNS–O Analyser.

2.5. Carbon sequestration

Carbon sequestration has to be understood here as the amount of $\text{CO}_2\text{--C}$ exchanged between the atmospheric compartment and the ecosystem (specifically, the macrocosm). It was calculated as follows. The CO_2 concentration in each ecotron chamber was controlled to automatically follow ambient concentrations plus a 398 mg/m^3 (or 221 ppm) offset with a time step of 30 min. If the CO_2 concentration was below the required value, CO_2 was injected from a bottle; if above, the air was scrubbed for CO_2 by exposure to lime. Since i) every macrocosm sits in a gastight chamber, and ii) we previously established the relationship between injection or scrubbing time and CO_2 change in absence of a macrocosm, it is possible to estimate its C sequestration through a simple model. For this, we

calculated the delta in CO₂ concentrations at every half-hour interval, subtracted the change in CO₂ concentration due to injection and/or scrubbing, and converted from ppm CO₂/30min into grams of C/m²/d using the perfect gas law and correcting for macrocosm surface. To reduce noise and fill up data gaps, this output is fitted to a moving average function (ALMA function from the TTR package [21] in R).

2.6. Methane emissions

The Methane (CH₄) concentrations in the growth chamber were measured every half-hour with an LGR gas analyser. CH₄ concentrations in each unit remained stable in absence of a macrocosm and were not affected by CO₂ scrubbing, but immediately reached the concentration in the main corridor after opening of the access door. The latter event was easily detectable as the absolute value in CH₄ concentration change in the chamber was several orders of magnitude faster than normal. We verified that the CH₄ budget was not affected by bacteria living in the pipe system linked to the evacuation sink on the chamber floor by hermetically clogging it with plastic foil for two days, which did not affect methane budget (DNS). The methane budget was therefore calculated as the delta CH₄ after cancelling out the difference due to door opening, and converted from ppm/30 min to g/m²/d using the perfect gas law, using air temperature and pressure data recorded in the same chamber at the same time.

2.7. Soil C balance

In the previous sections, we described measurements of the following fluxes: C sequestration, CH₄ C emissions, plant biomass C production, C leaching losses, and C deposition through rainwater, all across the whole season and for every unit. The only unknown to close up the C budget of the ecosystem was delta soil C, which is the difference between C sequestration and all other fluxes mentioned above.

Soil C balance was calculated in two steps: first, the net ecosystem balance was estimated by calculating the difference between incoming (CO₂-C balance – also referred to as C sequestration above in the manuscript, CH₄-C balance, rainwater-C) and outgoing (C leaching) C fluxes. CO₂-C balance was C sequestration numbers aggregated per unit over the growth season (01/03/2021-01/10/2021). Note that the CO₂-C balance already accounted for the difference between photosynthesis and respiration. CH₄-C balance was methane emission numbers aggregated (sum) per unit over the growing season, corrected by methane C content (75 %) and expressed in g/m². Rainwater-C was estimated by multiplying the non-purgeable organic carbon (NPOC) concentration of the tap water (g/L) at a given date by the cumulated precipitation between this sampling and the previous one (obtained from the weighable lysimeter at each unit) and aggregated (sum) per unit over the growing season. Carbon leaching was estimated by multiplying the NPOC concentration of the soil water samples (g/L, after quality control to remove too high values and considering only depths below 0.60 m; in case of absent values because of sample contamination, we used the average NPOC concentrations of this unit) with the drainage between this sampling and the previous one, and aggregated (sum) per unit over the growing season. The drainage was determined from the weight changes of the leachate tank and therefore accounts for the water reinjected from the tank to the lysimeter (to adjust for soil water potential, see the “climate simulations” section). The losses in C from the system due to soil water sampling were estimated considering a pooled sample size of 6.3 (high estimate) and 4.5 (low) liters per unit, an average C concentration of 10 mg/l, a surface of 3.14 m², and a total of 9 sampling events during the growing season.

The net C balance of the soil was then calculated as the difference between plant biomass and the net C balance of the ecosystem. We estimated C in plant biomass as follows. For barley, we multiplied the average dry weight (DW) of the sum of all plant organs by stem density in every unit, and corrected by the C content of barley (we assumed 40 % [22]). For weeds, we measured the difference in lysimeter weight before and after weeding, corrected by a water content of 10 % (this value was found after drying on a representative sample of weeds), by the surface area of the lysimeter to get values in g/m², and for both barley and weeds, given a C content of 40 %. Values are in g/m² and integrated over the growing season (March 1, 2021 to October 1, 2021).

2.8. Water balance

Each macrocosm was located into weighable lysimeter, where soil water potential at the bottom of the lysimeter (soil depth 1.4 m) is regulated by a lower boundary control system. It consists in 10 suction cups placed at the bottom of the lysimeter, which are activated by a bidirectional pump that either feeds or drains a leachate tank; this way, water can be pumped in or out of the lysimeter, and thus the soil water potential can be increased or decreased, to follow field values measured at the ICOS site (see “macrocosms” section above). Soil water potential at the bottom of the lysimeter and in the field were measured by tensiometers (TS1 METER Group, USA). The lysimeter and the leachate tank were weighted every second and values were averaged per minute with a resolution of 0.001 kg and accuracy of 0.1 kg (corresponding respectively to 3.1e10⁻⁴ and 0.3 mm of ET or precipitation). Increases in weight are due to precipitation or water injected at the lysimeter bottom and decreases are due to actual evapotranspiration (ET) or seepage. Accurate estimation of the water budget from noise prone lysimeter data, in particular ET and precipitation, required the application of a filter algorithm to reduce the noise [23].

2.9. Water quality

Soil water samples were collected via suction cups installed at 10, 20, 35, 60 and 140 cm depth and in triplicate in each lysimeter. The tension in the suction cups was adjusted by a vacuum pump (VS Pro, METER group, USA) applying a constant –50 hPa and –150

hPa to the upper (10, 20, 35 cm) and lower (60, 140 cm) cups, respectively, as upper soil layers are usually drier. The water extracted from the cups was then stored in 1L-bottles sitting in a temperature-controlled cabinet (+10 °C). Every three weeks, the water contained in the bottles was filtered at 0.5 µm and analysed for total organic Carbon (TOC), total Nitrogen (TN), anions and cations. Four water samples taken from the main pipe feeding the rain system were also filtered and analysed in the same way, as well as water samples from the leachate tank (aliquot of the drainage).

Based on the water balance components mentioned above, we knew both the volume and concentration of water inputs (wet deposition by the precipitation from rain system, injection from leachate tank) and output (seepage into leachate tank), we could calculate the budget for all measured chemical species by multiplying concentration per volume and computing the difference between inputs and outputs. We assumed that ET water had a negligible concentration of TOC, TN and ions, as shown by Ref. [24] for N, and that therefore losses of these chemical species through volatiles were negligible.

2.10. Characterization of bacterial communities using metabarcoding

The soil samples were collected on the 15h of May 2021. In each unit, we randomly selected 3 positions and took 3 soil samples per position using a 1 cm diameter, 5 cm long soil corer. The soil samples were immediately stored at 4 °C. Once in the lab, the samples (including the roots) were sieved using a 2 mm mesh size and aliquots of the sieved soil were labelled and stored at −20 °C. The samples were then pooled per position. Soil DNA was extracted using the Dneasy® PowerSoil® Kit (QIAGEN, Venlo, Netherlands). Each soil DNA sample was then subjected to bacterial 16S rRNA gene sequencing. In the first round of 16S rRNA gene PCR, an amplicon of 290 bp was generated, using primers 515F and 806R [25]. Using the Q5 High-Fidelity DNA Polymerase system (M0491, NEB), a reaction volume of 25 µl per sample was prepared containing 1 µl of extracted DNA (final DNA-concentration per reaction 1–10 ng), 1x Q5 Reaction Buffer with 2 mM MgCl₂, 200 µM dNTP mix, 0.2 µM forward and reverse primer, and 1.2 U Q5 High-Fidelity DNA polymerase. The PCR program started with an initial denaturation for 3 min at 98 °C, followed by a 30 s denaturation at 98 °C, a 30 s annealing at 53 °C, and a 1 min extension at 72 °C, all three steps were repeated for a total of 35 cycles. The reaction was ended by a final 7 min extension at 72 °C. The amplified DNA was purified using the AMPure XP beads (Beckman Coulter) and the MagMax magnetic particle processor (Thermo Fisher, Leuven, Belgium). Subsequently, 5 µl of the cleaned PCR product was used for the second PCR attaching the Nextera indices (Nextera XT Index Kit v2 Set A (FC-131-2001), and D (FC-131-2004), Illumina, Belgium). For these PCR reactions, 5 µl of the purified PCR product was used in a 25 µl reaction volume, and prepared following the 16S Metagenomic Sequencing Library Preparation Guide. PCR conditions were the same as described above, but the number of cycles reduced to 20, and 55 °C annealing temperature. PCR products were cleaned with the Agencourt AMPure XP kit, and then quantified using the Qubit dsDNA HS assay kit (Invitrogen) and the Qubit 2.0 Fluorometer (Invitrogen). Once the molarity of the sample was determined, the samples were diluted down to 4 nM using 10 mM Tris pH 8.5 prior to sequencing on the Illumina MiSeq. Samples were sequenced using the MiSeq Reagent Kit v3 (600 cycle) (MS-102-3003) and 15 % PhiX Control v3 (FC-110-3001). For quality control, a DNA-extraction blank and PCR blank were included throughout the process, and also the ZymoBIOMICS Microbial Mock Community Standard (D6300) to test efficiency of DNA extraction (Zymo Research). Obtained sequences were clustered into operational taxonomic units (OTUs) and annotated using Qiime within the DADA2 package, with the SILVA database for taxonomy assignments.

2.11. Statistics

For data on crop properties, we tested the effect of treatment on plant organ DW using a mixed model ANOVA with crop property as the response variable, treatment as a fixed variable, and mesocosm unit as a random variable. For ecosystem processes, calculated every day on each unit separately (C balance, ET, seepage, CH₄ emissions, dissolved organic C in soil water, total N in soil water and nitrate-N in soil water), and repeatedly on each same unit across the whole season, we considered that a model accounting for repeated measurements had more statistical power than one aggregating all values over the whole growing season. Hence, we ran a mixed linear model with the process as response variable, treatment as a fixed variable, and date plus mesocosm unit as random variable. For WUE, we could only aggregate data at the level of the unit (crop weight was measured only at harvest and not across the season, and there were too few data points for soil water samples); hence we ran a Wilcoxon test with WUE calculated as the cumulated ET (mm/ha) divided by the total crop biomass (kg/ha). The effect of Si treatment on phytoliths and C occluded in phytoliths was evaluated by comparing means of Si and control treatments in both straw and grain *via* a one-sided *t*-test. Microbial diversity was calculated as species richness (number of ASVs), Shannon and Simpson indices in each sample; the effect of treatment was evaluated by comparing indices means between treatments using a Student *t*-test. Effect of the treatment on the microbial community structure was done using analysis of similarities (ANOSIM). Statistics were done in R [26] using nlme [27], dplyr [28], lubridate [29] and vegan [30] packages.

3. Results

3.1. Environmental conditions

The characteristics of the climatic conditions applied to the six macrocosms are displayed in Fig. S1. It was characterized overall by a fresh early spring and a warm summer, with overall low precipitation: a cool month of March (but no negative temperature), a gradual increase in temperature through spring, culminating in a warm day in early June (daily maximum air temperature >25 °C and minimum >20 °C), and a long warm period in August (in the first 25 days of August only three days had a maximum daily temperature below 25 °C); in terms of precipitation, most rain events were happening in the second half of April and mid-May, with a storm in

August; all these rain events were mild and almost never exceeded 2 mm/day. This translated into a soil surface water content gradually decreasing from field capacity (35 %) in early March to already 10–15 % in mid-May, and to very low values (between 5 and 10 %) for the whole summer. Plants experienced significant water stress in summer, with Ri around 0.7 for the whole spring, but dropping below 0.6 in the first half of July, down to 0.2–0.3 at harvest (end of August) (Fig. 1).

3.2. Crop properties

3.3. Effect of treatment on crop properties

Treatment had no significant effect on the DW of any organ (Fig. 2); root biomass was lower by 28 % (124 and 97 mg/plant on average in control and Si treatments, respectively) in the Si treatment, but this was not significant ($p = 0.24$). The density of stems was also lower under Si treatment (–23 %: 600 and 460 plants/m² on average in control and Si treatments, respectively), but this was again not significant according to our model ($p = 0.08$). Total plant biomass, calculated by multiplying total biomass per plant (sum of all organ's individual biomass) by the plant density, was 40 % higher under control (809 g/m²) than under Si treatment (582 g/m²). Two plants (out of an estimated 5652 in the three control macrocosms) were attacked by a pathogen, each in a different macrocosm belonging to the control treatment (Fig. S2). In both cases, symptoms were the same: a black, powdery mass replacing grains in each kernel, on a slightly stunted plant, which resembled false loose smut or covered smut. As the number of infected plants was too low, we consider this result as anecdotal and will not discuss it further. The number of phytoliths and C occluded therein was not significantly affected by Si application, neither in straw, nor in grains (Table 1, Fig. S3).

3.4. Effect of treatment on C balance

We found that C balance was significantly more negative in the Si treatment than in the control: hence C sequestration was higher with Si treatment (Fig. 3, flux number 1). When aggregated over the whole season, C balance went from in average –300 to –400 g/m², with most of the sequestration happening in June, and a significant amount of C release in July (Fig. S4). However, at a finer time scale, the Si treatment had a significant effect on the C balance in the days following the Si application (Fig. S5). In fact, the cumulated C balance for the 10 days post-application was significantly lower for application 1 (April 23, 2021) and 3 (June 23, 2021) and significantly higher for application 2 (June 2, 2021) (Table 2). For the first application at the end of April, the C balance was 0.1 g/m²/d lower under Si treatment; at that moment PPT was low (5.2 mm/d) but the soil water content was relatively high (10–15 % depending on the unit; soil water capacity is 35 % in this sandy soil). For the second application, at the beginning of June, when C balance was the most negative, it was 0.5 g/m²/d higher in Si treatment. At that moment PPT was average (15.3 mm/d) and soil water content was average too (around 10 %). Finally, for the last application, at the end of June, when C balance was back to almost neutral

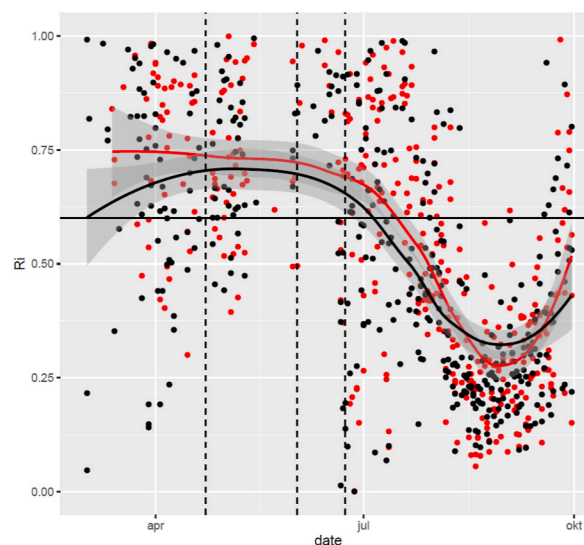


Fig. 1. Evolution of the actual/potential evapotranspiration ratio throughout the growing season. Red: Si treatment, black: control. The lines are obtained by a loess smoothing function per treatment per day and the grey areas correspond to the confidence interval. Every full circle represents a value of this ratio in one unit for one day. The three vertical, dashed lines materialize the three dates at which Si treatment was applied. The black, full horizontal line represents the threshold value under which plants experience water stress. Actual evapotranspiration was calculated from changes in the weight of the lysimeter at a temporal resolution of 1-min using the AWAT noise filter [31]. Potential evapotranspiration was calculated based on the Penman-Monteith equation [18].

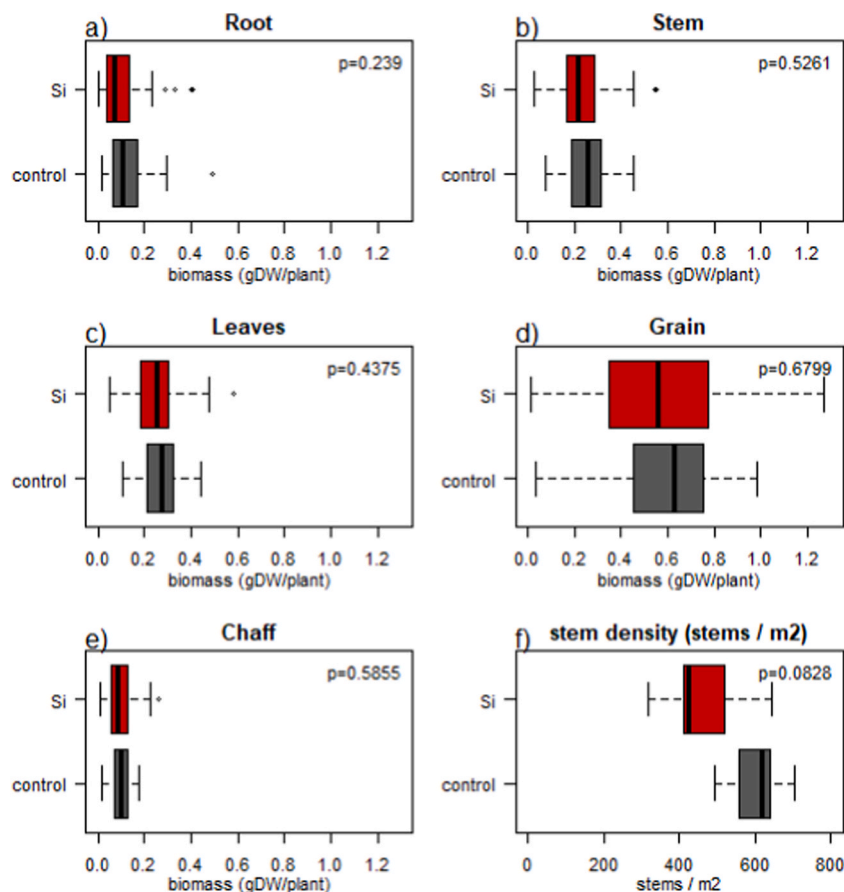


Fig. 2. Effect of foliar Si treatment on the dry weight (DW) of each organ (a: roots, b: stem, c:leaves, d: grain, e: chaff, f: stem density) and plant density (f) of barley individual plants at harvest. The p-value was obtained using a mixed model ANOVA with crop property as the response variable, treatment as a fixed variable, and mesocosm unit as a random variable.

Table 1

Effect of foliar Si fertilization on ecosystem properties. Evapotranspiration was calculated from changes in the weight of the lysimeter at a temporal resolution of 1-min using the AWAT noise filter [31]. Drainage was calculated from changes in the weight of the drainage tank. Water use efficiency was calculated from plant biomass measurements at the end of the experiment and evapotranspiration aggregated to the growing season. The effect of Si treatment on evapotranspiration, drainage, methane budget, total N concentration in soil water, and nitrate-N concentration in soil water were tested using a mixed linear model with treatment as fixed variable and date and unit as random variables. The effect of Si treatment on WUE was tested using a Wilcoxon test, and on C occluded in phytoliths (both straw and grain) using a *t*-test.

Process	Unit	Si treatment		Control		p-value		
Evapotranspiration	mm/day	4.49	+/-	2.46	8.38	+/-	4.56	0.43
Drainage	mm/day	-0.08	+/-	0.13	-0.01	+/-	0.12	0.68
Water use efficiency	kg/Ha/mmET	8.23	+/-	3.76	6.70	+/-	4.07	0.40
Methane budget	g/m2/day	-0.05	+/-	<0.01	-0.05	+/-	<0.01	0.31
Total N concentration in soil water	g/l	6.53	+/-	0.59	8.05	+/-	0.77	0.19
Nitrate concentration in soil water	g/l	6.20	+/-	0.64	7.51	+/-	0.92	0.35
C occluded in phytolith (straw)	mg/gDW	2.49	+/-	0.60	3.44	+/-	0.73	0.19
C occluded in phytolith (grain)	mg/gDW	0.61	+/-	0.30	0.58	+/-	0.25	0.47
soil bacterial species richness	number of species	381	+/-	92	357	+/-	49	0.53
soil bacterial shannon diversity index	-	5.28	+/-	0.20	5.19	+/-	0.18	0.34
soil bacterial simpson diversity index	-	0.989	+/-	0.002	0.986	+/-	0.008	0.25

levels, it was 0.1 g/m²/d lower under Si treatment; at that moment PPT was average (13.5 mm/d) and soil water content was low (around 5 % or lower). During these three periods, PET was roughly the same (18.0–22.3 mm/10d). The dissolved organic C sampled in soil water at down to 35 cm (pooled 10, 20 and 35 cm) went from very high early in the growing season (21–24 mg/l end of April) to low in the mid-season (4–6 mg/l end of June) and very low at harvest (1 mg/l mid of August), but was not affected by the Si treatment (Table S1).

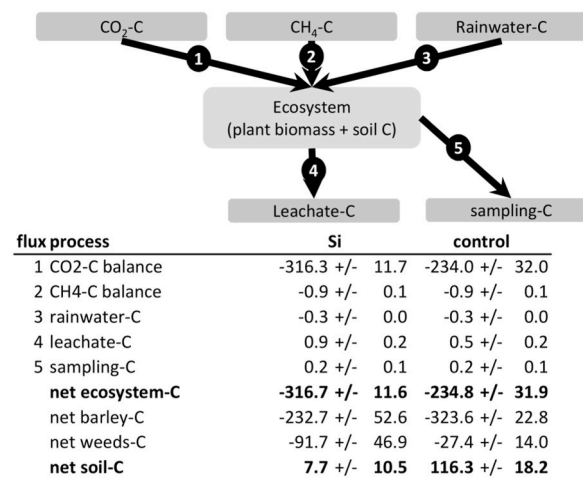


Fig. 3. Detailed C budget of the effect of foliar Si fertilization. Values are in g/m^2 and integrated over the growing season (01/03/2021-01/10/2021). Negative values indicate C sequestration (net C gain in the macrocosm) and positive values C emissions. Soil C budget was calculated in two steps: first, the net ecosystem balance was estimated by calculating the difference between incoming (CO_2 -C balance, CH_4 -C balance, rainwater-C) and outgoing (leachate-C, sampling-C) C fluxes. Note that the CO_2 -C balance already accounts for the difference between photosynthesis and respiration. The net C balance of the soil was then calculated as the difference between plant biomass (barley, estimated based on stem density and average weight per stem; and weeds, estimated as the difference in lysimeter weight before and after weeding, corrected by water content of 10 %, found on a representative sample of weeds, by the surface area of the lysimeter to get values in g/m^2 , and for both barley and weeds, given a C content of 40 %) and the net C balance of the ecosystem.

Table 2

Short-time effect of Si treatment on the cumulated C balance. The C balance was measured in each of the six units (3 under Si treatment, 3 controls) every hour based on a model using CO_2 concentration, air temperature, air pressure, CO_2 injection time and CO_2 scrubbing time as an input, and aggregated on a daily basis. The C balance was then cumulated for the 10 days after each treatment for each macrocosm and a student's t-test was performed on these cumulated values ($n = 3$).

Treatment	Date	Average C budget Si ($\text{g/m}^2/10\text{d}$)	Average C budget control ($\text{g/m}^2/10\text{d}$)	p- value	Sign of the Si effect	Cumulated PPT ($\text{mm}/10\text{d}$)	Cumulated PET ($\text{mm}/10\text{d}$)
1	April 23, 2021	-3.50	-2.40	0.049	-	5.2	22.3
2	June 02, 2021	-12.55	-17.55	0.015	+	15.3	18.0
3	June 23, 2021	-1.73	0.51	0.025	-	13.5	22.3

All measurements made in the previous sections of this manuscript allowed us to i) make a detailed C budget of the crop ecosystem (Fig. 3, diagram) and ii) evaluate the effect of treatment on this budget (Fig. 3, table). Overall, macrocosms C inputs were 235 (control) and 317 (Si treatment) g/m^2 throughout the whole season, 99.6 % of which came from net CO_2 exchange (Fig. 3). The remaining came from methane absorption (0.3 %) and rainwater (0.1 %). As for outputs, a marginal amount of C (less than 1 g/m^2) was lost by leaching (C in soil water) and sampling.

The C gained by the ecosystem could be split in two general compartments: 1- plants, itself separated in two pools: barley and weeds, and 2-soil. The soil was the unknown of the equation and was calculated as the difference between C ecosystem gain and the sum of C in weed and barley biomass. We found that the amount of plant C always exceeded the ecosystem C gains, and therefore the soil lost C in both treatments. Soil C balance was however significantly less negative under Si treatment than under control conditions ($p=0.01$, data not shown), while other processes did not significantly differ. In fact, soil C losses were near inexistent under Si treatment (-7 g/m^2) but rather high under control (-116 g/m^2). Ecosystem C gains were not significantly correlated to barley stem density (Pearson's correlation, $p = 0.18$), but soil C balance was (Pearson's correlation, $p = 0.03$).

3.5. Methane balance

We found that the Si treatment had no significant effect on the crop ecosystem's methane balance (Table 1, Fig. S6). All units behaved as net methane sinks (on average $-50 \text{ mg CH}_4/\text{m}^2/\text{day}$).

3.6. Evapotranspiration and water use efficiency

There was no difference in evapotranspiration nor drainage between Si and control treatments (Table 1, Fig. S7). We also did not find any significant effect of the treatment on WUE (Table 1, Fig. S7), despite the fact that plants had lower density in Si treatment (and hence lower total biomass) for a similar ET. As for NEE, we did not observe any transient effect of Si treatment on daily ET (Fig. S8).

3.7. Nitrate and N balance

The Si treatment had no significant impact on the N and nitrate balance of the soil water extracted from each microcosm (Fig. S9).

3.8. Soil bacterial communities

There was no effect of treatment on soil bacterial species richness, nor diversity calculated as Shannon or Simpson index (Table 1). We did not see any effect of the treatment either on soil bacterial community structure using ANOSIM ($R = 0.056$, $p = 0.21$) (Fig. S10). Many ASVs were not identified to the species level, but among the ones for which it was possible, we could identify the following: *Leifsonia xyli* (1070 counts, 14 samples), *Sphingomonas melonis* (2 ASVs: 979 counts, 6 samples and 910 counts, 5 samples), *Pseudomonas viridiflava* (34 counts, 1 sample), and *Acidovorax aveniae* (275 counts, 4 samples). Only the latter was significantly affected by the treatment, and was more abundant in the Si treated units (Fig. S11).

4. Discussion

Overall, we found that Si treatment had very little influence on crop performance, pathogen presence and ecosystem functioning, despite many otherwise promising results reported from field trials in literature. We speculate that a combination of high CO₂ levels under future climate, dilution of the spraying product and variability in environmental conditions at application times may have reduced its efficiency time window to only a few days after application.

Silicon treatments are mostly reported as beneficial to crops under drought-stress conditions [7,32]. We therefore first wanted to ensure that barley plants have been exposed to drought conditions in our experiment. This was the case, with most days in July and August showing low to very low actual/potential evapotranspiration ratios. These values of Ri and soil water content are low but not unrealistic for this area and the same soil [33], especially considering that we did not irrigate to ensure that plant experienced drought (as Si foliar sprays are known to protect plants against drought stress).

We found that the Si treatment had no effect either on the biomass of any aboveground organ, under the specific marginal soil and future climate conditions of this experiment. Aboveground biomass production nor grain yield were not affected by the Si treatment, contrary to what has been observed on wheat [34]. There was no effect on phytolith density either. As a side note, Si treatment also did not influence the competitiveness of barley as weed colonisation was not significantly different between treatments. We were not able to make conclusions on potential impact on pathogen prevalence as so few plants were contaminated overall. Unsurprisingly, as plant biomass, plant roots and soil water chemistry was not affected by treatment, we did not see an effect either on the diversity of microbial communities nor on their structure. Hence, even in conditions where Si treatment are the most expected to bring a critical advantage (dry marginal soil, arid climatic conditions), Si treatment did not potentially bring a direct benefit for the farmer in terms of irrigation needs, grain yield or weeding requirements. We tried to investigate deeper on the reasons potentially explaining this lack of effect.

The reason behind the effectiveness of foliar Si treatments has been attributed to the sprayed product settling at the surface of the leaves, generating a film that alter plant permeability and reduces transpiration, which is especially beneficial in dry conditions, by allowing the plant better maintain stomata conductivity and hence photosynthesis under drought stress [9,35]. Based on these findings we expected that Si treatment would have a most prominent effect on evapotranspiration and ecosystem water balance. But we did not detect any effect of Si treatment on evapotranspiration, whether aggregated over the whole growing season, nor even transiently, immediately after treatment. This suggested that the treatment did not affect transpiration; this did not rule out, however, that the photosynthesis was not affected. A large scale study of the impact of 2018 drought on ecosystems functioning showed indeed a significant decrease of net photosynthesis while ET remained the same [36].

We therefore examined the effect of the Si treatment on the ecosystem C balance. We found that even though treatment did not affect C sequestration in the plant biomass, C losses from soil were almost completely offset in the Si treatment. The Si-treated macrocosms had higher values for net ecosystem C gains than control ones, while at the same time having less plant biomass production. Nevertheless, it is very likely that plant density was a confounding factor here, as it was significantly lower in the Si-treated units (despite being sown at the same density), and was significantly correlated to soil C balance. We speculate that the higher germination observed in the control units resulted in higher biomass production, and deriving more C from the soil pool. This higher germination is an introduced confounding factor, independent from the treatment as plants germinated before the first Si application.

Beyond the effect of Si treatment, these results indicated that crops in such a marginal land were inducing a net C loss from soil, suggesting increased soil organic matter decomposition (net soil C loss) in presence of barley. This was expectable as the land management we tested here was a conversion from a natural, marginal soil to a crop system, which is known to induce soil C release from increased decomposition [37].

At a finer time scale, however, the Si treatment had a significant effect on the ecosystem C balance which we could not attribute to a confounding effect of plant density. Indeed, after every Si application, the C balance of the system was significantly different between the Si treatment and the control, though the sign of the effect changed. The environmental conditions differed between the three

applications. For the period after first application, end of April, where hydric stress was moderate (average soil water content, mild temperature but almost no precipitation), the ecosystem was a slightly larger C sink in the Si treatment. For the period after the second application, early June, where hydric stress was low (low soil water content, but mild to fresh temperature and average precipitation), the ecosystem was overall a much stronger C sink overall, but significantly less so in the Si treatment. Finally, the period after the third application, end of June, which was characterized by a higher hydric stress than the two previous ones (very low soil water content, warm temperature, average precipitation), saw the units in the Si treatment behaving as a low C sink and the control units as low C source. We did not measure any effect on dissolved organic C in soil water, hence this increase in C fixation did not lead to increased rhizodeposition. At all times, CO₂ levels were about two times higher than ambient conditions, as we were simulating 2070 local climate projections. In other words, Si treatment transiently increased C sequestration by the ecosystem under moderate to relatively high hydric stress and high CO₂ levels, while ET remained the same. During drought, and at high CO₂ levels, stomatal conductance decreases [38], reducing photosynthesis. In our experiment, we see that plants under Si treatment were able to photosynthesize more for a short period of time during dry periods. Even though we did not measure a significant effect of Si treatment on water use efficiency, there was a clear upward trend, which is congruent with higher photosynthesis under hydric stress. We speculate that we do not see a significant effect over the whole season because of the high CO₂ levels in the experiment, which already reduces stomatal opening, and decreases the potentially beneficial effect of the treatment during plant hydric stress. These effects were averaged out by inverse effect during period of low hydric stress.

A couple of other factors stemming from the experimental setup may have artificially increased noise on the effect of the Si treatment. First, the soil water tension at the bottom of the lysimeter varied a lot between units, because of the inherent macrocosm spatial soil heterogeneity, leading to very different amounts of water pumped back from the leachate tank to the lysimeter to adjust to field values, and therefore different access to water for pivot roots of plants in each unit. In other words, because we used one single field value to adjust bottom soil water tension in the lysimeters, but at the same time every macrocosm had a slightly different texture at the bottom, some plants may have been exposed to less arid conditions than expected based on only soil surface water content and precipitation/evapotranspiration, which both affect mostly topsoil. Second, we had to significantly dilute the product (see material and methods section), to ensure a reliable spraying on such a small surface, which may have decreased its efficiency. The commercial product used as Si spray also contains EDTA, which has been shown to have toxic effects on plants [39]. We however assume that this effect was negligible in our study, as the concentration used in the final spray was very low (200 µM, instead of 65 mM in the cited study).

The Si treatment had no effect on methane emissions; we did not expect any effect as methane budgets are mostly affected by microbes themselves functionally highly depending on soil water saturation level [40], which had no reason to be different under leaf Si treatment. In fact, these cropland macrocosms behaved as a constant methane sink in the ecotron setting, at a relatively high rate (0.9 g/m², which corresponds to 12 kg CH₄/ha/year). This matches the higher end of the range of forests, which are the strongest methane sinks [41]. It may be that methane concentrations are overestimated in the ecotron, as CH₄ inputs originate almost exclusively from the air inside the building while opening units. Negative methane balance is however not surprising in such highly draining soil [40].

Finally, we did not measure any effect of the treatment on bacterial pathogen abundance, nor on diseases on barley. The ecotron is a closed system, which limits colonisation by pathogens from the environment to the ones already present in the macrocosm at the beginning of the experiment. We however had evidence of the presence of some potential bacterial pathogens for barley in the soil throughout the experiment, and the treatment had no effect on their abundance. This suggests that bacterial pathogens were not particularly thriving under control conditions, and therefore does not allow us to derive strong conclusions on the protective effect of Si treatment against barley pathogens in our system.

5. Conclusion

In summary, our results show that Si foliar sprays may not always be as efficient as hoped, and may not always be a viable mitigation solution against climate change in marginal soils. In fact, the only transient effects of the treatment we observed were detected thanks to a combination of highly controlled and realistic climate simulation and high-frequency ecosystem monitoring. There was a strong, significant impact on soil bacterial diversity. We therefore would like to encourage approaches at the ecosystem level to test the effectiveness of climate change mitigation treatments.

Data availability statement

Data will be made available on request.

CRedit authorship contribution statement

Francois Rineau: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Jannis Groh:** Writing – review & editing, Software, Resources, Methodology, Data curation. **Julie Claes:** Formal analysis. **Kristof Grosjean:** Methodology. **Michel Mench:** Writing – review & editing, Funding acquisition, Conceptualization. **Maria Moreno-Druet:** Formal analysis, Data curation. **Virmantas Povilaitis:** Writing – review & editing, Conceptualization. **Thomas Pütz:** Writing – review & editing, Software, Resources, Formal analysis. **Beata Rutkowska:** Writing – review & editing, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Peter**

Schröder: Writing – review & editing, Funding acquisition, Conceptualization. **Nadejda A. Soudzilovskaia:** Writing – review & editing. **Xander Swinnen:** Methodology. **Wiesław Szulc:** Writing – review & editing, Investigation, Funding acquisition, Conceptualization. **Sofie Thijs:** Methodology, Investigation, Formal analysis. **Jan Vandenborgh:** Writing – review & editing, Software, Resources, Methodology, Investigation. **Jaco Vangronsveld:** Writing – review & editing, Investigation, Conceptualization. **Harry Vereecken:** Writing – review & editing, Software, Resources, Methodology, Investigation, Data curation. **Kasper Verhaege:** Formal analysis. **Renaldas Žydelis:** Writing – review & editing, Conceptualization. **Evelin Loit:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The study was supported by the FACCE-SURPLUS project BiofoodonMars. Jannis Groh was supported the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation-project no. 460817082). Michel Mench is member of the COST Action PlantMetals (<https://plantmetals.eu/plantmetals-home.html>) and the Ecotox INRAE network (https://www6.inrae.fr/ecotox_eng/The-network). We thank AVEVE Belgium for providing us with barley seeds.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.heliyon.2023.e23882>.

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