MASTER THESIS

Critical levels of $CaCl_2$-extractable soil silicon for rye (*Secale cereale* L.) with and without drought or copper stress

submitted by:

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Abstract

Silicon (Si) is known for its many beneficial effects on stressed plants. It is already used as a fertilizer in some regions and to date no negative impacts of Si on the environment have been found. A pot experiment in a greenhouse was designed to get information on the element's critical/optimal level under drought and copper stress for rye plants (*Secale cereal*e L.). Up to this level, increased addition of the element is expected to increase the crop yield. The experimental setup was as following: Si was added at seven different levels (0, 10, 20, 40, 60, 80, 120 mg kg^{-1}) to a control group (C) (held at 70% of field capacity), a drought stress group (Dr) (held at 30% of field capacity) and a copper stress group (Cu) (added Copper 400 mg kg^{-1}). Silicon was added in the form of wollastonite ($CaSiO_3$) to a soil with naturally low Si content and four repetitions were made. Rye plants were harvested after seven weeks. The following parameters were measured: Shoot and root dry weight (dw), Si concentration in plant shoot and roots, root parameters (length, diameter, volume), leaf water potential, plant-available silicon in soil over time.

The main results were: Si amendment significantly ($p < 0.05$) increased dry matter yield in Dr group but did not affect shoot dw in C and Cu group. A Cate-Nelson model leads to the suggestion of a critical level between 24.5 and 29.6 mg kg^{-1} $CaCl_2$-extractable Si in soil (= 40-60 mg kg^{-1} added Si to soil) for the Dr group, however, as the quadratic model suggests, a higher critical level (> 40 mg kg^{-1}) is possible. Shoot Si concentration increased significantly ($p < 0.05$) in all three groups. The Si concentration was significantly ($p < 0.05$) higher in roots than in shoots in groups C and Dr. Leaf water potential increased evenly in groups C and Dr with increasing amounts of Si.

This thesis states a first derivation of a critical Si level in soil for a crop typically cultivated in temperate regions. Recent studies found that many agricultural soils in Lower Austria have rather low concentrations of $CaCl_2$-extractable Si (ca. 50% of Cambisols, Chernozems and Phaeozems have values < 40 mg kg^{-1}) and could benefit from Si fertilization, especially as in parts of the region droughts are already an issue, that is probable to aggravate due to further climate change.

Zusammenfassung

Silizium (Si) ist für seine vielen nutzbringenden Eigenschaften bekannt und wird in einigen Fällen schon als Düngemittel genutzt. Bis dato wurden keine schädlichen Einflüsse des Elements auf die Umwelt nachgewiesen.

Ein Topfexperiment im Gewächshaus wurde entworfen um Information über den kritischen Wert von pflanzenverfügbarem Si im Boden für gestresste Roggenpflanzen, *Secale cereal*e L., (Kupfer- und Trockenstress) zu gewinnen. Bis zu diesem kritischen Wert erwartet man bei höherer Zugabe des Elements höhere Erträge. Silizium wurde in sieben verschiedenen Stufen (0, 10, 20, 40, 60, 80, 120 mg kg^{-1}) einer Kontrollgruppe (C) (Wasserregime bei 70% Feldkapazität), einer Trockenstressgruppe (Dr) (Wasserregime bei 30% Feldkapazität) und einer Kupferstressgruppe (Cu) (Kupferzugabe von 400 mg kg^{-1}) zugegeben. Silizium wurde in Form von Wollastonit ($CaSiO_3$) einem Boden mit natürlich geringem Siliziumgehalt zugegeben und es wurden vier Wiederholungen gemacht.

Die Roggenpflanzen wurden nach sieben Wochen geerntet. Folgende Parameter wurden erhoben: Biomasse der oberirdischen Pflanzenorgane und Wurzeln, Siliziumkonzentration der oberirdischen Pflanzenorgane und Wurzeln, Wurzelparameter (Länge, Volumen, Durchmesser), Wasserpotential, pflanzenverfügbares Si im Boden.

Die Zugabe von Si hatte eine signifikante ($p < 0.05$) Auswirkung auf die Biomasse der Dr Gruppe, jedoch nicht auf die der Cu und C Gruppe. Mit dem Cate-Nelson Modell kann für die Gruppe Dr ein kritischer Wert von 24.5 bis 29.6 mg kg^{-1} mit $CaCl_2$ extrahierbarem Si im Boden (entspricht der Zugabe von 40-60 mg Si kg^{-1} Boden) nahegelegt werden; wie das quadratische Modell allerdings zeigt, ist ein höherer kritischer Wert (> 40 mg kg^{-1}) möglich. Die Siliziumkonzentration in den Pflanzen erhöhte sich in allen drei Gruppen mit zunehmender Menge an Siliziumdünger und war in den Gruppen C und Dr in den Wurzeln höher als in den oberirdischen Pflanzenorganen.

Das Wasserpotential stieg mit zunehmender Menge an zugegebenem Si in den Gruppen C und Dr gleichermaßen an.

In dieser Arbeit wurde erstmals ein kritisches Level für eine typischerweise in temperierten Regionen angebaute Kulturart abgeleitet. Jüngsten Untersuchungen zufolge ist die Konzentration an $CaCl_2$-extrahierbarem Si in großen Teilen landwirtschaftlich genutzter Böden Niederösterreichs sehr gering (ca. 50% der Cambisole, Chernozeme und Phaeozeme haben Werte < 40 mg kg^{-1}) und könnten von Siliziumdüngung profitieren, zumal Teile des Bundeslands bereits jetzt von Dürreperioden betroffen sind, die mit fortschreitendem Klimawandel zunehmen können.

Resumo

O silício (Si) é conhecido por seus muitos efeitos benéficos em plantas estressadas. Já é usado como fertilizante em algumas regiões e, até o momento, não foram encontrados impactos negativos do Si no meio ambiente.

Um experimento em estufa foi projetado para obter informações sobre o nível crítico do elemento para plantas de centeio (*Secale cereal*e L.) sob um regímen de estresse (seca e cobre). Até esse nível, de um aumento no elemento, pode-se esperar um aumento no rendimento da colheita.

O Si foi adicionado em sete níveis (0, 10, 20, 40, 60, 80, 120 mg kg^{-1}) em forma de wollastonita ($CaSiO_3$) a um solo de teor naturalmente baixo de Si. Um grupo controle (C) (regime hídrico de 70% capacidade de campo), um grupo de estresse por seca (Dr) (regime hídrico de 30% capacidade de campo) e um grupo de estresse por cobre (Cu) (400 mg kg^{-1} cobre adicionado) foram estabelecidos em quatro repetições para cada nível de Si.

As plantas de centeio foram colhidas após sete semanas. Os seguintes parâmetros foram mensurados: a biomassa de brotos e raízes, o teor de Si nos brotos e nas raízes das plantas, parâmetros das raízes (comprimento, diâmetro, volume), potencial hídrico, quantidade de Si disponível para plantas no solo.

A adição do Si afetou significativamente ($p < 0.05$) a biomassa no grupo Dr, mais não foi significativo nos grupos C e Cu. O modelo de Cate-Nelson sugere um nível crítico entre 24.5 e 29.6 mg kg^{-1} de planta-disponível Si (= 40-60 mg Si kg^{-1} terra) para o grupo Dr. No entanto, como mostra o modelo quadrático, também é possível um nível crítico mais alto (> 40 mg kg^{-1}). A concentração de Si nos brotos aumentou nos três grupos e foi maior nas raízes do que nos brotos dos grupos C e Dr. O potencial hídrico aumentou com quantidades crescentes de Si nos grupos C e Dr.

Neste trabalho, um nível crítico para uma cultura que normalmente é cultivada em regiões temperadas foi obtido pela primeira vez.

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1 Introduction

Silicon (Si) is known for its many beneficial effects on stressed plants. It is used already as a fertilizer in some regions and to date no negative impacts of Si on the environment have been found. Even if applied in excess it is suggested, that there are no negative effects on the environment, and water bodies and their food web quality might even benefit (Epstein, 1999; Liang et al., 2015). Current projections of climate models forecast shifts in seasonal weather patterns (ICPP, 2013). This will increase uncertainty as to the availability of water over time and space and more extreme weather events are already happening and will further disturb agricultural production system. Crops have to deal with more severe stresses, as for example droughts can occur more often, last longer or be more severe. Food safety becomes a major challenge. To adopt and not further worsen the situation efforts have to be made on many levels (Institutional government, education; water infrastructure; agricultural management practices, etc.) (Millennium Ecosystem Assessment, 2005; Maria Saleth & Ariel, 2011). This thesis is meant to contribute to one of many approaches on safeguarding food security in the future.

1.1 Literature review

A literature review on the element silicon was conducted. It includes information about the cycles it concludes in different systems and to which other cycles it is connected, further information about its role in plants and in soil and its usage in agriculture.

1.2 The element silicon

With an atomic number of 14, a molecular weight of 28,0855 u and melting and boiling points at 1410 °C and 2355 °C respectively, the element Si is surrounded by near neighbors B, C, N, O, P, and S in the periodic chart, which are all recognized as essential elements for plants (Gascho, 2001). Silicon is present in stars and meteorites and with a mean of 28,8% w/w it is the second most abundant element in the earth crust, after oxygen (Struyf et al., 2009). In nature a Si molecule does not appear freely but can form crystals or bind with other elements. Most common forms are oxides or silicates. In a pure form Si crystals are appreciated for solid-state and semi-conductor devices. It is also widely used in cement, glass, fibers and silicones beside a multiple of other applications (Gascho, 2001).

1.3 The global silicon cycle

An overview of the Si cycle as a whole is important to further understand what impact fertilization has on the different pools and to discuss its necessity. The role of Si in soil and plants will be discussed in detail in chapters 1.4 and 1.7 respectively.

Looking at the complex Si cycle different temporal and spatial scales should be considered. Biological, geological and chemical processes interact to transport Si from terrestrial ecosystems to the ocean, interacting with other important biogeochemical cycles (Struyf et al., 2009). Especially its connection to the carbon cycle is of high interest, as at two points in the cycle it shows interaction with CO_2, and therefore has an impact on its global atmospheric concentrations (Conley, 2002). Figure 1 illustrates the four major pools of Si and inter- as well as intra-systemic fluxes and their sizes.

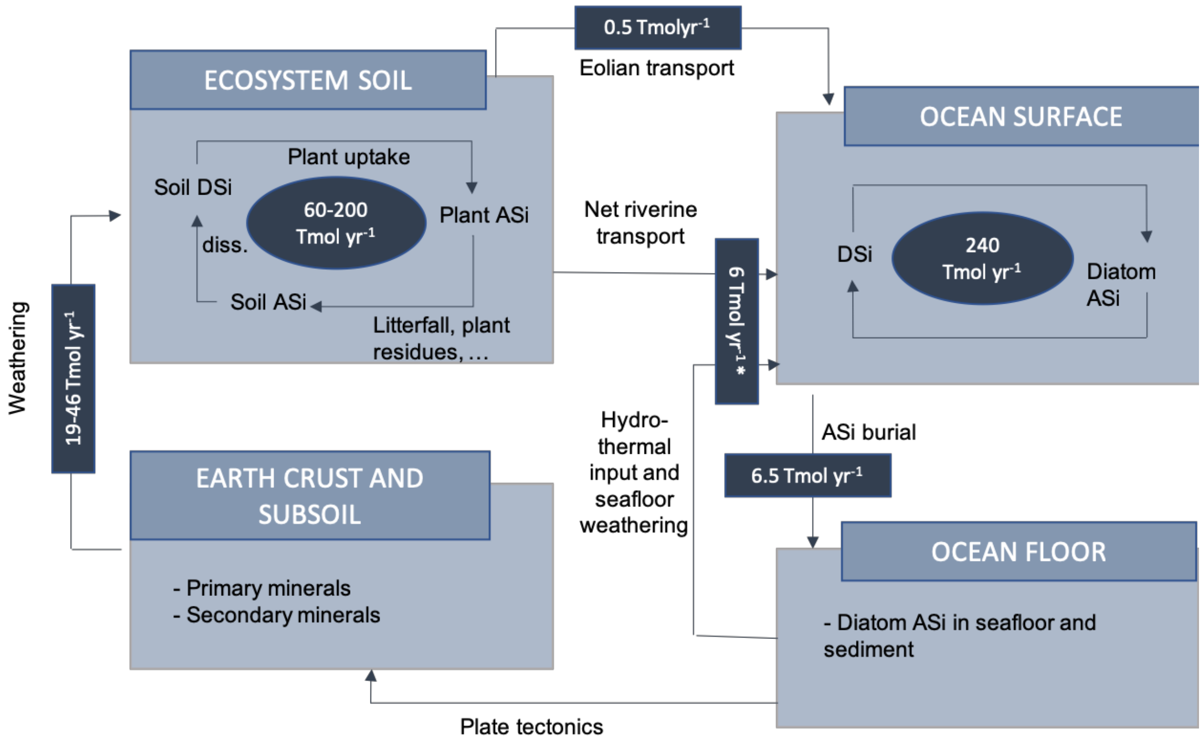


*Figure 1: The global biogeochemical cycle of Si, showing the main continental and oceanic Si pools. Rectangular dark boxes represent Si fluxes between the primary Si pools, circular dark boxes represent the fluxes within the pools. Diss.= dissolution. ASi = amorphous Si. DSi = dissolved Si. * The 6 Tmol yr^{-1} flux includes net riverine transport and fluxes from hydrothermal activity and seafloor weathering. (modified from Struyf et al. 2009)*

Silicon, so abundantly existing in the earth crust in form of silicate minerals, is released mainly due to chemical and biological weathering processes. In a reaction with dissolved soil CO_2 orthosilicic acid (H_4SiO_4) is dissolved from the crystalline structure of the minerals (Struyf et al., 2009; Conley, 2002). On geological timescales, the weathering of silicate minerals constitutes an important sink for atmospheric CO_2 (Berner, 1983). The CO_2 consumption by this process globally is estimated to be approx. 0.26 Gt C yr^{-1} on long term average (Hartmann et al., 2010). Also, enhanced silicate weathering was reported by Andrews and Schlesinger (2001) at elevated atmospheric CO_2 concentrations.

The dissolved Si (DSi) is transported to rivers and thereby reaches the ocean. This flux however is buffered by terrestrial systems, where in a biogeochemical cycle uptake, storage and recycling of the element take place (Conley, 2002). In aquatic systems DSi plays an important role in primary production. This constitutes another important link to the carbon cycle, as it is involved in creating a CO_2 flux towards the ocean floor (Ragueneau et al., 2006). For diatoms Si is an essential nutrient, as they need it to build up their cell wall, called frustule. As the alga dies, Si reaches the ocean floor through sedimentation (Biological Si-pump). This process is coupled to the biological carbon pump, as diatoms use CO_2 to build the frustules, hence creating a net CO_2 flux towards the seabed (Ragueneau et al., 2006; Struyf et al., 2009). Plate-tectonic processes recycle the Si again to minerals of the earth crust (Struyf et al., 2009). Other groups of phytoplankton are able to create a CO_2 flux towards the ocean floor too. However, there exists an important group of non-siliceous plankton, the coccolithophores, that create a carbonate counter pump, as they create CO_2 during calcite formation, which they need for their calcite shells (Rost & Riebesell, 2004). Changes of the input amount of Si to marine and especially coastal systems may therefore have an impact on the species composition of primary producers and influence the balance between diatoms and non-siliceous phytoplankton species (Harrison, 2000). If such a nutritional situation would favor the dominance of coccolithophores it would mean a decrease in the net sequestration of CO_2, as the counter carbon pump would reduce the net CO_2 flux from the atmosphere towards the ocean floor enhanced by diatoms (Tréguer, 2002). Not only the Si input alone may be responsible for this influence, as also the availability of other nutrients and their ratios to Si play a role. Especially the N:P:Si ratio is an important factor (Struyf et al., 2009). It has been observed that increasing amounts of N and P (from agricultural fertilization activities) in coastal waters, nutrients that are usually limiting factors in these systems, led to eutrophication, and potentially serious ecosystem changes (Conley et al, 1993). The relative scarcity of Si leads to a change from diatom-dominated ecosystems to non-diatom-based ecosystems (Officer & Ryther, 1980). Consequences can be algal blooms where toxic compounds are released, anoxic conditions and increased turbity. Moreover, diatoms are the base of the trophic food chain in coastal ecosystems. They are considered to have a high nutritional value and with their decline an economically unfavorable, non-diatom-based food web would be established, leading to problematic situations for many fisheries worldwide (Doering et al., 1989; Struyf et al., 2009).

In terrestrial systems, weathered Si is translocated horizontally as well as vertically and can be temporarily or permanently immobilized. Terrestrial Si can be classified as mineralogical or biogenic. As it is present in different chemical-mineralogical compositions it can vary in its water solubility and reactivity (Sommer et al., 2006). The solid fraction in soil is the reservoir of mineral elements, thus also of nutrients that plants need for growth. The immediate source of those elements however is the liquid phase in the soil, where nutrients are in a dissolved status (Epstein, 1994). Plants can take up DSi in the form of monomeric orthosilicic acid (H_4SiO_4) from the soil solution. Uptake can happen passively or with active uptake mechanisms (Ma et al., 2006). Silicon is accumulating primarily in parts of the plant where water loss is high. It is deposited irreversibly as hydrated, amorphous silica structures (ASi), called phytoliths ($SiO_2 \cdot nH_2O$) (Struyf et al., 2009). Through litterfall and plant residues these phytoliths recycle back into the soil, contributing to the pool of amorphous Si (ASi). The concentration of ASi is usually highest in the upper soil layer and decreases with increasing soil depth. The Si release from biogenic Si constitutes an important source for DSi and is twice as high as the release of Si from weathering processes (Conley, 2002). It can be taken up again by plant roots, translocated within the soil or exported to aquatic systems. Phytoliths dissolve very fast in the saline ocean water and therefore become available for phytoplankton in marine ecosystems (Sommer et al., 2006; Struyf et al., 2009).

In agricultural systems desilification is often enhanced due to the removal of Si-containing crops and leaching of the plant-available Si fraction (Berthelsen et al., 2003). Especially in intensive agricultural cropping systems the continuous harvest of Si-accumulator plants can reduce the amount of plant-available Si in soil drastically (Meunier, 2003) and the pool can be exhausted in only a few years of cultivation (Desplanques et al., 2006).

Deriving from this cycle the following points, which are relevant for further discussion on fertilization, can be stressed:

- Plant removal depletes the system from its major source of plant-available Si. Although abundantly present in the earth crust, it can become a scarce resource in soil layers accessible to plant roots.
- The high abundancy of Si in the earth crust suggests, that an appropriate fertilizer might be available at low expense.
- To date, no negative effects (as observed for other fertilizers, eg. eutrophicational effects of P and N) of Si on the ecosystems have been found. Even if applied in excess no negative effects on the environment have been observed, and water bodies and their food web quality might even benefit.

1.4 Silicon in soil

In this chapter the role and components of Si in the ecosystem soil will be analyzed in detail. Being the second most abundant element in the earth crust makes Si a major constituent of most rocks in parent material. The concentration of Si in rocks can differ and usually ranges from 50 to 400 g kg^{-1} (Matichencov & Bocharnikova, 2001). Very small amounts of Si can even be found in carbonaceous rocks such as limestones and carbonites (Tubaña & Heckman, 2015). Looking at soil Si two fractions can be distinguished, as there are the solid phase and the aqueous phase (solution) in which Si is dissolved, as depicted in Figure 2 (Matichencov & Bocharnikova, 2001; Sauer et al., 2006; Tubaña & Heckman, 2015). The adsorbed Si and the aqueous phase of Si comprise of quite similar components, with the difference that those in the aqueous phase are dissolved in the soil solution, whereas the adsorbed fraction is held by a variety of soil particles including clay particles, iron- and aluminum- oxides and -hydroxides (Tubaña & Heckman, 2015).

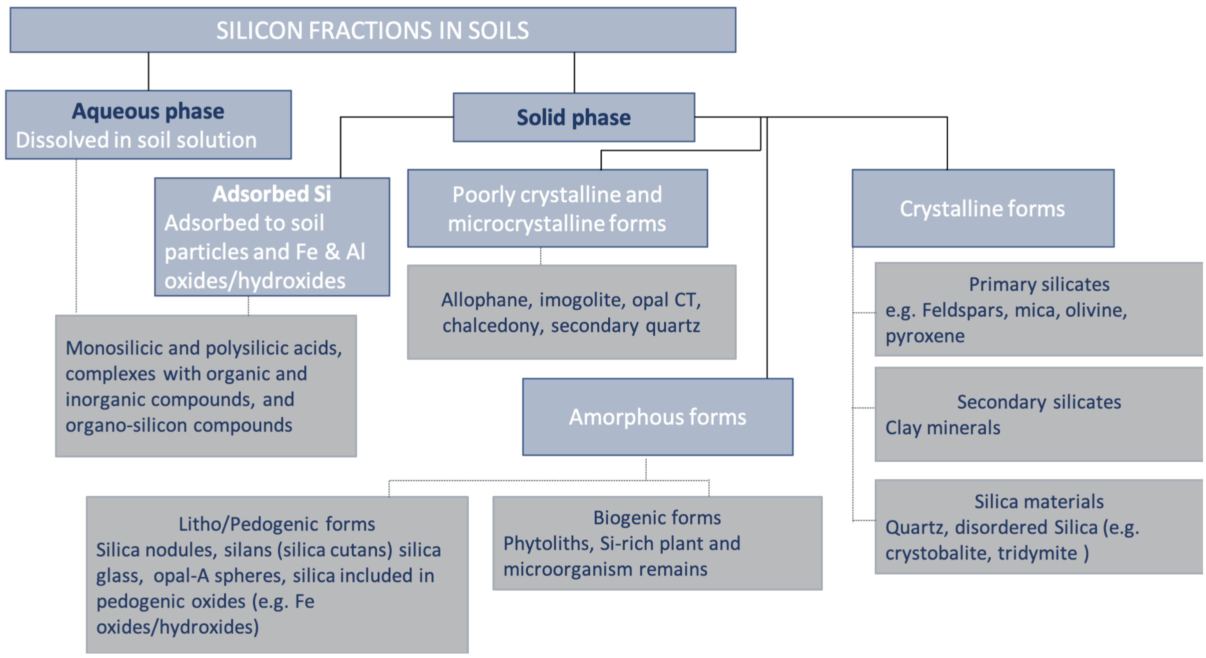


Figure 2: Different Si fractions in soil (Modified from Tubaña & Heckman, 2015)

1.4.1 The solid phase

Within the solid phase there are crystalline forms, poorly crystalline forms, microcrystalline forms and amorphous forms. The largest fraction therein are crystalline forms, which can be primary minerals, secondary minerals and secondary poorly to non-crystalline disordered silica materials (Tubaña & Heckman, 2015; Drees et al., 1989). Primary minerals are usually classified as parent material, whereas the other two groups develop during processes of soil formation (Struyf et al., 2009). The poorly crystalline and microcrystalline forms of the solid phase accrue from orthosilicic acid (H_4SiO_4). Allophane and imogolite are precipitates of the acid with aluminum hydroxides (Doucet et al., 2001). When the concentration of H_4SiO_4 in the soil solution exceeds the solubility of ASi the formation of opal-CT is promoted (made up of cristobalite and/or tridymite). Secondary quartz is formed by re-precipitation of opal-CT from dissolved opal-A (amorph) (Chadwick et al. 1987). Several reports led Tubaña and Heckman (2015) to the conclusion that the concentration at which polymerization begins is at about 2 mM. Amorphous silica can be of biogenic or litho/pedogenic origin. Biogenic forms derive from plant and microorganism residues. The amorphous phytolits that had been formed and deposited in plant parts are recycled to the soil sphere. Litho/Pedogenic ASi is formed of Si complexes with Al, Fe, heavy metals and soil organic matter (Matichencov and Bocharnikova, 2001; Farmer et al., 2005). And again, if the concentration of H_4SiO_4 in the soil solution exceeds the solubility of ASi, opal-A can be formed (Drees et al., 1989).

The adsorbed Si is represented by fractions of the dissolved silicic acid from the aqueous phase that are adsorbed onto components of the solid phase, including clay particles and Fe- and Al-hydroxides. The amount of silicic acid removed from the soil solution due to adsorption is minimal for clay particles, but quite high for Fe- and Al- hydroxides. Also, the amount of adsorbed monosilicic acid is influenced by pH, soil redox potential and the type of metal. Adsorption increases from pH 4 to pH 9 and Al-based oxides have been shown to be more effective than Fe-based oxides. However, Fe oxides are more commonly found in soils. The OH group of the oxide surface is replaced with the H_4SiO_4 through ligand exchange (Tubaña & Heckman, 2015).

The solubility of Si from the solid phase differs with its various forms and therefore has a significant impact on the concentration of Si in the aqueous phase – the soil solution. It is affected by pH, temperature, particle size, packing density of the silica tetrahedral, water and organic matter contents, and redox potential (Savant et al., 1997; Iler, 1979; Drees et al., 1989). Overall it is the pH that is responsible for the regulation of solubility and mobility of Si, as it also affects the adsorption/desorption processes, which are acting between the solid and aqueous phase. Between pH values of 2 and 8.5 the solubility of both the crystalline and the amorphous silica stays nearly constant, but increases rapidly at higher values, as the H_4SiO_4 dissociates to $H_3SiO_4^- + H^+$ which promotes the dissolution of the solid forms of Si to replenish or buffer the H_4SiO_4 pool in the soil solution (Knight & Kinrade 2001).

1.4.2 The aqueous phase

In the aqueous phase, also called the soil solution, Si exists as orthosilicic acid (H_4SiO_4). It mainly occurs as a monomeric molecule, which is the form in which it can be taken up by plants (Tubaña & Heckman, 2015). Oligomeric silica can be formed by chains of H_4SiO_4; they eventually play a role in soil aggregation, water holding capacity and buffering capacity. Some dissolved silicic acid can form complexes with organic or inorganic compounds (Berthelsen & Korndörfer, 2011; Tubaña & Heckman, 2015). At pH values lower than 8 silicic acid occurs in an uncharged state (H_4SiO_4). At pH 9 it dissociates into $H^+ + H_3SiO_4^-$ and further into $2H^+ + H_2SiO_4^{2-}$ at pH 11 or higher. The amounts of oligomeric and polymeric silica and organic complexes with orthosilicic acid depend on the pH and increase as the pH increases (Tubaña & Heckman, 2015; Keeping, 2017). The concentration of orthosilicic acid in the soil solution usually varies between 0.1 mM and 0.6 mM (Epstein, 1994). The maximum solubility is at 2 mM. At higher concentrations SiO_2 silica gel is formed (Coskun et al., 2018).

The source of the dissolved Si in the aqueous phase are the various forms of silicate minerals and plant residues of the solid phase. The amount of H_4SiO_4 released to the soil solution is influenced by several physico-chemical properties such as pH, particle size, the presence of aluminium, iron and phosphate ions, temperature, soil moisture and adsorption/desorption reactions on soil colloids (Berthelsen et al., 2003; Keeping, 2017).
Hence, the overall Si content of soils does not necessarily correlate with the concentration of soluble Si in soils, the component which is crucial for plant availability. Three aspects about plant available soil Si levels should be considered. The intensity gives the concentration of Si that is available for immediate use. The capacity refers to the reserve supply stored in the solid phase and the buffer capacity describes the ability of the solid phase to replenish the aqueous phase (Berthelsen & Korndörfer, 2011).

1.5 Scarcity of silicon in soil

Although Si is known to be the second most abundant element in the earth crust and Si dioxide takes up 50-70% of the soil mass (Adrees et al., 2015), certain soils experience shortage of the element, especially in its plant-available form (Tubaña & Heckman, 2015). In the wet tropics soils are exposed to high weathering and desilication as an immediate consequence of high temperatures and high rainfall (Berthelsen et al., 2003). The solubility of silicic acid, the dominant and plant-available form in soil solution, is pH-dependent and also influenced by adsorption/desorption reactions of the silicic acid with the solid phase, especially Al- and Fe-hydrous oxides. The solubility and concentration of Si in the soil solution is highest at low pH values and decreases up to a pH of 9.8, where the adsorption of the silicate anion reaches a maximum, hence reducing the Si concentration in the soil solution. This strong relation between soil pH and the solubility of Si is one of the major factors accounting for the Si depletion in weathered, acidic soils and is even aggravated in intensive, long-term agriculture, where Si is taken out of the system with each harvest (Sommer et al., 2006; Keeping, 2017). Crops are often Si accumulators and by harvesting them Si is permanently exported out of the system, resulting in a depletion of plant-available Si. The combination of chemical and physical degradation due to soil perturbation and crop removal in long term agricultural systems results in increasing acidification and desilication. Plant-available Si is lost through leaching processes which come along with a decline of the cation exchange capacity, hence the ability to retain essential plant nutrients declines (Berthelsen et al., 2003; Datnoff et al., 1997). Tropical soils that are Si poor in their native state include oxisols and ultisols, whose profile is characteristically highly weathered, leached, acidic and low in base saturation. Histosols, more common in the temperate and boreal zone, hold large amounts of organic matter but have low mineral contents in the upper horizons. They frequently reveal a scarcity of Si as well (Korndörfer & Lepsch, 2001; Tubaña & Heckman, 2015).

1.6 Essentiality of silicon in plants

The essentiality of nutrients for plants was defined by Arnon and Stout (1939) by the following criteria:
"An element is not considered essential unless

> (a) a deficiency of it makes it impossible for the plant to complete the vegetative or reproductive stage of its life cycle;
> (b) such deficiency is specific to the element in question, and can be prevented or corrected only by supplying this element; and
> (c) the element is directly involved in the nutrition of the plant quite apart from its possible effects in correcting some unfavorable microbiological or chemical condition of the soil or other culture medium."

The essentiality of Si for higher plants is much debated (Coskun et al. 2018). However, with the exception of certain groups of plants it could not be proven to be essential in the sense of the definition by Arnon and Stout (Epstein, 1999). Those plants Si is considered essential for are certain algae including prominently the diatoms, and plants of the family Equisetaceae (Epstein, 1994). Epstein (1994) argues that this definition is conceptually simple, however hard to apply in practice. In the case of Si, the element is known to be an omnipresent contaminant. It is present in water, even if it is distilled or demineralized, in tools, glass containers and dust, which makes it very hard to maintain an experimental environment free from it. Epstein (1994) also stresses the conspicuous feature of Si, not being considered essential but being consistently present at concentration levels as high as those of macronutrients.

According to Epstein and Bloom (2005), essentiality is given through the fulfilment of either one or both of the following points: (a) The element is part of a molecule which is a specific component of the metabolism or structure of the plant and (b) if the plant is severely deficient of the element it exhibits abnormalities in growth, development or reproduction compared to plants with lower deficiency. Silicon may fit into this criterion of essentiality (Sapre & Vakharina, 2016). Based on the definition by Arnon and Stout (1939) a favorable response by adding an element does not make it essential. However, their experiments were suggesting a perfect stress-free environment that will never occur in nature (Coskun et al., 2018). In agricultural science the beneficial role of Si for plant growth and health and for soil productivity is receiving ever more attention (Conley, 2002). The advantages of Si for plants, especially in stressed conditions, are increasingly well documented, which led the International Plant Nutrition Institute (IPNI) to nominate it a beneficial substance in 2015 (Coskun et al., 2018).

1.7 Silicon in plants

The plant-available form of Si in which it can be taken up by plant roots from the soil solution is the undissociated orthosilicic acid H_4SiO_4. Plants grown in soil contain 1 to 100 g kg^{-1} Si (on a dry weight basis) in their tissue, depending on plant species, soil properties and Si source (Epstein, 1994). Uptake mechanisms vary between plant species and can be either active, passive or strongly restricted (excluder plants) (Mitani & Ma, 2005). Passive uptake along the transpiration stream has been observed for most dicotyledonous species. Active uptake by transporters is prevailing in many crop species like rice (*Oryza sativa* L.), maize (*Zea mays* L.), sugarcane (*Saccharum officinarum* L.) and wheat (*Triticum aestivum* L.) (Adrees et al., 2015). After being taken up by the plant, Si moves with the water flow in the xylem. Most of it is translocated to the shoots and ultimately deposited as hard amorphous silica gel, $SiO_2 \cdot nH_2O$. The deposition happens mainly at transpiration sites, where water is lost. Once deposited the silica is immobile and will not be transported any further. These immobile depositions of amorphous silica are called phytoliths (Ma & Yamaji, 2006; Adrees et al., 2015; Tubaña & Heckman, 2015).

With more than 10 g kg^{-1} Si in dry leaf matter plants are considered as Si accumulators (Epstein, 1994). High Si accumulation of more than 40 g kg^{-1} was shown for members of the families Poaceae, Equisetaceae and Cyperaceae. It was also observed that Si accumulation varies within different plant parts (Currie & Perry, 2007). The beneficial effects of Si under stressed conditions emerge more clearly in Si-accumulators (Ma, 2004), which is important, as many among the worldwide most important crops as wheat, rice, barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.), sugarcane and maize are ranked among the Si accumulators (Epstein, 2001).

The precise role of Si in plants is still largely subject to research. Many studies report that in the absence of stress additional Si has little to no effect, but there is mounting evidence on the beneficial effect of Si under stress, be it biotic or abiotic (Coskun et al., 2018). Its beneficial effects are suggested to be derived from a mechanical protective layer it lends the plant through deposition, reactions in the soil solution and plant-internal mechanisms (Tubaña & Heckman, 2015). The phytholith deposition in the shoots creates a hard layer and improves mechanical strength. It has been shown to be effective against damage caused by insects and grazing animals (Tubaña & Heckman, 2015) and to improve drought stress tolerance (Janislampi, 2012). External mechanisms of Si are characteristically the inhibition or reduction of metal-ion absorption by plants. Inside of the plant Si can be involved in uptake processes (eg. water, metals, nutrients), the antioxidant systems, complexation, co-precipitation and compartmentalization of metal ions (Liang et al., 2007; Tubaña & Heckman, 2015).

1.8 Silicon in agriculture

As Coskun et al. (2018) state, a more applicable and realistically more important question than the essentiality of Si for plants is whether plants will benefit from additional Si fertilizer. From a practical perspective Si-containing fertilizer was used since the Middle Ages. In Europe phosphate-, silicate- and calcium-rich slag-based fertilizer, a by-product of iron-making industry, was applied, taking advantage of its good performance as fertilizer and liming material to rectify soil pH and increase crop yield. In China the recycling of Si-containing materials such as organic manure and the returning of ash and cinder to cropland was performed, as beneficial effects were observed from doing so. Beginning over 150 years ago a multitude of beneficial effects regarding resistance against biotic or abiotic stresses were attributed to Si. In many countries there are early records on agriculturally important effects of Si (Liang et al., 2015). However, it was not until a few decades ago, that Si as a fertilizer became subject of increased attention. Nowadays, it is globally accepted as an important addition in agriculture as with its beneficial effects it contributes to food safety and allows for higher production at lower costs and less negative environmental impacts (Epstein, 1999; Liang et al., 2015).

Research is ongoing on the mechanisms involved in the roles of Si in plant biology, emphasizing on questions relevant for agriculture, such as the effects of Si on crop productivity and quality, stress alleviation, availability and uptake of the element and the manufacturing and management of the fertilizer (Liang et al., 2015). It is mostly monocotyledonous crops that show a positive response to additional Si. Rice, wheat, maize, barley, millet (*Setaria italica* (L.) P.BEAUV), sorghum (*Sorghum bicolor* (L.) MOENCH) and sugarcane can be counted among them. Among dicotyledonous crops there are also some widely used representatives that are able to accumulate Si, such as cotton (*Gossypium hirsutum* L.), soybean (*Glycine max* (L.) MERR) and some vegetable and fruit crops, including some of the European main crops as rapeseed (*Brassica napus* L.), sugar beet (*Beta vulgaris* L.) and potato (*Solanum tuberosum* L.) (Artyszak, 2018; Ma et al., 2001; Liang et al., 2015). On a large scale however, it is mostly rice and sugarcane where Si-based fertilizers are included in conventional agricultural practice (Coskun et al., 2018; Liang et al., 2015), owing to their economic value and because they are often grown on (sub-) tropical soils, which are known for being highly weathered and low in plant-available Si. There is much less research and knowledge about the topic in agroecosystems of temperate zones (Haynes, 2014). However, it could become more relevant in Europe, as organic farming is getting ever more importance and application of Si to the soil or leaves has, based on the current state of knowledge, no detrimental environmental effects (Artyszak, 2018). Only recently soil Si status of several soils in Lower Austria was invesigated. Examined soils include important arable and grassland soils. Schiefer (2019) found that 51% of 81 examined Cambisols had Si concentrations lower than 20 mg kg^{-1}, and Reiter (2019) found that 50% of 99 examined Chernozems and Phaeozems had Si concentrations lower than 44 mg kg^{-1} and 5% had Si concentrations lower than 20 mg kg^{-1}. Also ever more soils show concentrations of plant-available Si lower than 20 mg kg^{-1} due to human land use (47% of 95 sampled sites; Cocuzza, 2017).

Worth consideration is also the increasing use of crop residues for bioenergy or biorefinery. What is meant to be a strategy for replacing fossil fuels with renewable energy sources to mitigate climate change-causing greenhouse gases can induce negative effects on soil functioning and ecosystem services soil systems provide. The harvest of crop residues was shown to lead to increased compaction and risk of erosion, further it was shown to negatively effect soil physical and hydraulic processes, and structure and diversity of microorganisms. Further, crop residues constitute an important source of nutrients for subsequent crops. If taken out from the system there might be an increased need to apply mineral fertilizers. (Cherubin et al., 2018)

1.9 Beneficial effects of silicon

In literature a large number of reports exists that document the various beneficial qualities of Si (Coskun et al., 2018). Beneficial effects of Si include a fortified plant structure, resistance to lodging, resistance to biotic stresses (herbivory by various arthropods and vertebrates and infection by plant pathogens), resistance to abiotic stresses like drought, salt, heavy metal, extreme temperatures (heat, cold), increased yield and better quality and the contribution in the uptake of other nutrients (Epstein, 1999; Janislampi, 2012; Keeping, 2017; Ma, 2004; Berthelsen et al., 2003; Coskun et al., 2018). Liang et al. (2015) further extend the list with an improvement in light interception and hence facilitation of photosynthesis, resistance to shading, inhibition of transpiration and hence promoting drought tolerance and water use efficiency, resistance to UV radiation, effects on enzyme activities and promotion of N_2 fixation in legumes. Most studies have been conducted with plants known to be susceptible to Si fertilization, like rice and sugarcane. The documented effects listed above can therefore not be generalized for all plants.

1.9.1 Silicon alleviation of copper stress

In small doses copper is known to be essential for plant growth as it participates in several physiological processes. Copper takes part in photosynthetic electron transport, oxidative stress response, mitochondrial respiration and cell wall metabolism. It also plays a role in different proteins, enzymes and hormones (Yruela, 2009). However, plants exposed to high levels of copper show signs of toxification. Toxic levels of copper in soil can occur naturally (Yruela, 2009), but many sources of copper are anthropogenic, e.g. mining, smelting, electronics and pharmaceutical industry, coal burning, agriculture and waste disposal technologies (Yruela, 2009; Nowakowski & Nowakowsaka, 1997; Wu et al., 2013). It is mostly some regions in Asia that face the problem of high heavy metal concentrations in agricultural soils, but also regions of the USA and European countries like Spain and Slovakia are affected (Su et al., 2014). In Europe soil contamination with copper is mainly attributed to long used vinyards, followed by olive groves and orchards (Ballabio et al., 2018).

Plants from contaminated areas often have a considerably higher copper content than those from non-polluted sites. Toxic effects of Cu include reduced biomass, poor root development and chlorotic symptoms. An excessive amount of copper leads to inhibition of photosynthetic electron transport, alteration of chlorophyll amount and structure of chloroplasts in leaves, decreased DNA synthesis, oxidative stress (Nowakowski & Nowakowsaka, 1997; Yruela, 2009; Liang et al., 2015). As heavy metals may be transported through the food chain polluted soils not only state a danger for plants, but also for animal and human health (Adrees et al., 2015; Wu et al., 2013; Podlešáková et al., 2011).

The beneficial effect of Si for plants under heavy metal toxicity is well documented. External and internal mechanisms have been proposed as explanation for how Si alleviates metal toxicity (Liang et al., 2015 and references therein). External mechanisms include immobilization of the metal and elevating the pH value, especially when it is applied in the form of a basic silicate. Besides the increased mechanical strength which Si provides for plants, suggestions for internal mechanisms include the inhibition of metal transport from root to shoot, the compartmentalization of metals, a shift of metals from the symplast to the cell wall and the reduction of oxidative stress by enhancing the activities of antioxidant enzymes. Also, it is debated whether Si might be involved in regulating the expression of genes responsible for plant metabolic processes under heavy metal stress conditions (Liang et al., 2015; Adrees et al., 2015).

1.9.2 Silicon alleviation of drought stress

Drought stress is a worldwide limiting factor for plant growth and productivity. Based on current projections of climate models, the severity and frequency of droughts may increase in several regions of the world (IPCC, 2013). Therefore, it seems appropriate to enhance the drought tolerance of crops to keep up food supplies. Water deficiency in plants negatively affects plant growth and physiological processes, related to photosynthetic activity and the antioxidant defense capacity (Liang et al., 2015). Effects can be observed on cellular and whole-organism levels and usually include reduced leaf, stem and root size and reduced water use efficiency. Responses to drought stress can be stomatal closure, thereby reducing CO_2 assimilation, membrane damage, malfunctioning of various enzymes, increased oxidative stress and damage to macromolecules due to the increased number of reactive oxygen species (Farooq et al., 2009).

Several studies report that Si application results in increased tolerance towards drought stress. Mechanisms that play a role in this protection include physical, biochemical and physiological aspects. Silicon's role at the molecular level still remains unclear (Liang et al., 2015 and references therein). Liang et al. (2015) give a summary on possible Si-mediated mechanisms of stress alleviation. It was found that Si increases photosynthesis and growth by increasing the activity of photosynthetic enzymes and the photochemical efficiency and by facilitating nutrient uptake (Liang et al., 2015). Furthermore, there are indications, that Si can stabilize the structure, integrity and functions of plasma membrane, as it strengthens antioxidant defense mechanisms and thus reduces oxidative damage (Vangeesh et al., 2011; Liang et al., 2015; Sapre & Vakharia, 2016). Addition of Si can improve the water status of plants under drought conditions through improved root growth and better root water uptake and by decreased transpiration and osmotic adjustment, as well as by Si deposits in cell walls of xylem vessels preventing the compression of the vessels under conditions of drought stress (Ma et al., 2001; Hattori et al., 2009; Liang et al., 2015; Sapre & Vakharia, 2016). However, no general predications should be made, as the exact effects of Si might vary with different crops and different stress and site parameters.

Sapre and Vakharia (2016) provide an extensive review on studies documenting the effects of Si on different crops under drought stress.

1.10 Silicon fertilization

Being so prevalent in its occurrence (Struyf et al., 2009), finding Si sources is an easy task. However, to perform as a fertilizer, certain characteristics have to be met that will be decisive for the fertilizer's attractivity. Plants can only take up Si from the soil solution in the form of monomeric silicic acid. A high concentration of soluble Si is therefore a crucial quality for a fertilizer – and one that is hard to find. Silicon is usually combined with other elements and most natural sources result insoluble (Gascho, 2001). Also, a potential source should be in an area not too far away from the point of application, as transport costs (economic and environmental) might be higher than the revenues from potentially higher yields and benefits for the soil (Gascho, 2001). Another challenge lies in the application technology. The material must be in a suitable form for fertilizer spreader, to allow for a uniform application. Usually Si sources are finely ground before being spread on the ground. A smaller particle size enables rapid dissolution, however accurate and uniform spreading becomes more difficult the finer the material gets (Gascho, 2001). Another point worth consideration are possible contaminations of fertilizers. Silicon sources often contain heavy metals, linked to their origin and manufacturing, as by-products of the steel and iron industries are often considered as Si fertilizers. Silicon needs to be supplied in relatively high concentrations, which could result in heavy metal levels in soils not safe anymore for food supply and eventually in a degradation of farmland and waterways (Gascho, 2001; Prentice & Crooks, 2011). Finally, costs to acquire Si fertilizer have to be feasible for farmers/farm managers (Gascho, 2001).

It is notable that fertilizers with a pH-corrective capacity, as wollastonite, slag-based fertilizer (Babu et al., 2016) or manufactured calcium silicate (Keeping et al., 2017) in studies often have resulted in greater Si uptake by plants than fertilizers that do not have such properties, (Keeping, 2017; Silva de Oliveira & Ferreira Canuto, 2016). This may be based on the pH-dependent solubility and availability of silicic acid, which is also related to adsorption/desorption processes on soil colloids. Solubility of Si is highest at low pH at which it can easily get washed out. With increasing pH the adsorption of Si to solid soil surfaces increases, reaching a maximum at pH = 9 and providing more reserves of plant-available Si (Keeping et al., 2017; Phonde 2014; Oliveira et al., 2007).

The use of Si fertilizers is already established in some countries, mainly for rice and sugarcane production. For these two crops a large number of studies can be found that document the exceptional potential for increasing farm revenue, that might be applicable to other crops, especially to Si-accumulators (Ma et al., 2001). Some authors stress that Si should be applied on all agriculturally used land, not only to obtain higher yield but also to counteract soil degradation (Matichencov & Bocharnikova, 2001). Silicon fertilizers could also become an important component in sustainable and organic agriculture (Prentice & Crooks, 2011).

Many Si sources have been considered and evaluated for use in agriculture, reaching from plant residues and natural minerals to chemical products and by-products of the steel and iron industries (Gascho, 2001; Liang et al., 2015). Plant residues from harvested crops are used as Si source intentionally or incidentally. However, since crops are continually removed from agricultural systems, the Si demand cannot be covered with plant residues only (Gascho, 2001). Liang et al. (2015) provide a detailed insight into currently available Si fertilizers. They name slag-based silicate fertilizers as calcium silicates, that are processed using slags, by-products or waste materials of the iron and steel-making industries. They have a long history, as they are appreciated also as liming or phosphorus-containing amendments (Liang et al., 2015). As some of the slags contain heavy metals strict maximum allowable limits exist to prevent toxicity and environmental harm (Gascho, 2001; Ma and Takahashi, 2002). Further fertilizer options are silicates fused with other nutrients (potassium and magnesium), porous hydrate calcium silicate, a waste product of building industry (Ma and Takahashi, 2002), soluble silicate fertilizer (sodium silicate and potassium silicate can be water soluble), which

demonstrate to be quite effective in increasing productivity (Elawad et al., 1982; Liang et al., 2015). As they are too expensive for field incorporation they are mostly used for foliar applications (Gascho, 2001). In some cases, the rather poorly soluble Silica gel is used (Ma and Takahashi, 2002). Natural occurring Si-containing materials (besides plant residues) that are used as fertilizer in agriculture are minerals such as wollastonite ($CaSiO_3$), olivine ($MgSiO_3$) and diatomaceous earth (Gascho, 2001; Prentice & Crooks, 2011; Berthelsen & Korndörfer, 2011; Liang et al., 2015).
Out of the different types of Si fertilizers, calcium silicate (wollastonite or slag-based calcium silicates) have emerged as the most widely used and effective sources of Si in agriculture, as they meet best above described requirements (Gascho, 2001; Nagabovanalli et al., 2017; Babu, 2015). Slag-based calcium silicate can be a cost-effective Si source, provided it is free from heavy metals and locally available. In its manufacturing process the high temperatures release Si from its original crystalline form to more reactive and soluble forms. Other nutrients present in the slag account for more advantages of the fertilizer (Ma & Takahashi, 2002; Nagabovanalli et al., 2017).
Wollastonite is a mineral of metamorphic origin. It is rarely found by itself but mixed with other minerals. Natural wollastonite may contain minor amounts of various metal ions such as aluminum, iron, magnesium, manganese, potassium and sodium, substituting for calcium (Purle, 2018). Wollastonite is also associated with models for CO_2 capture and storage (CCS) (Ruiz-Agudo, 2018; Haque et al., 2019).

1.11 Critical level of plant-available silicon

In crop production it is important to make efficient and rational decisions on the use and amount of nutrient inputs to apply the least amount of fertilizer necessary to obtain an economically optimum yield. A critical level has been established for most plant nutrients, as it represents the point until which a yield response to the added nutrient can be expected. The critical level varies among nutrients, plants and soils. For Si however, as it is not counted among the essential plant nutrients, little is known about its critical level. Deficiency of Si under stressed conditions can be problematic for the plant. Applied in excess Si is not becoming toxic for plants, however it may lead to increased Si polymerization and thus become unavailable to plants. It is therefore desirable to determine a critical/optimal level for Si that makes a well-directed fertilization with the element possible allowing for stress resilience and optimal growth. (Sahrawat, 2006; Kanamugire et al., 2006; Babu et al., 2016; Korndörfer et al., 2001, Tubaña & Heckman, 2015).

Silicon is nowadays applied regularly only in rice and sugarcane cultivars, therefore most research had been conducted for these plants and information is lacking for most other crops (Coskun et al., 2018; Liang et al., 2015). For the assessment of a critical level of Si several challenges arise: The critical limit is not a universally defined number, but dependent on the soil type and its properties, such as pH, texture and organic matter, as well as on the water regime and may lead to wide variations among different soil types (Kanamugire et al., 2006; Babu et al., 2016). Further it is important to identify the most appropriate soil test procedure that is simple to use and reflects the amount of plant-available Si (Kanamugire et al., 2006; Berthelsen et al., 2003; Babu et al., 2016). The choice of extractant might be based on its ease of adoption for a particular laboratory and its suitability for specific soil characteristics (Berthelsen & Korndörfer, 2011). Consequently, there might not be a universal extractant that is suitable for determining available Si that will cover all soil types and soil conditions (Gascho, 2001). To allow for routine testing of soil Si status it is necessary to conduct more calibration studies, over a longer period and across different soil types for establishing soil type-specific critical levels (Babu et al., 2016).

As extractant 0.01M calcium chloride ($CaCl_2$) has proven to be utile for indicating the amount of readily available Si in soil. Other extractants that have shown to be useful for extracting slightly different pools of Si in soil are for example distilled water, diluted acetic acid, sulfuric acid or ammonium acetate (Korndörfer et al., 1999; Berthelsen et al., 2003; Ma & Takahashi, 2002; Liang et al., 2015; Berthelsen & Korndörfer, 2011). Few studies on the critical levels of Si in soil have been conducted and several different critical levels have been proposed, which are summarized in Table 1.

Table 1: Summary of studies on the critical level of Si in soil
** USDA soil taxonomy*
*** USDA soil taxonomy and WRB for soil resources*
*** Latosols are usually classified as oxisols in the USDA soil taxonomy or as ferralsols in the WRB for soil resources.

Extractant	Region	Soil	Plant	Suggested critical level (mg kg^{-1})	Reference
Deionized water	Louisiana	Fine-silty, mixed, superactive, thermic Fluventic Eutrudepts*	Rice	71	Babu (2015)
Distilled water 1 h shaking	South India	Ultisols*, acid (Typic Kandiustult, Paleustult, Ustic Palehumults)	Rice	14	Narayanaswamy & Prakash (2009)

Distilled water 4 h shaking	South India	Ultisols*, acid (Typic Kandiustult, Paleustult, Ustic Palehumults)	Rice	30	Narayanaswamy & Prakash (2009)
H_2O	-	Gibbsihumox*	Sugarcane	2	Fox & Silva (1978)
H_2O	Florida	euic, hyperthermic Lithic Medisaprist*	Sugarcane	2 - 8	Elawad et al. (1982)
0.01 M $CaCl_2$	North Queensland, Australia	acidic	Sugarcane	20	Haysom and Chapman (1975)
0.01 M $CaCl_2$	Louisiana	Fine, smectitic, thermic Typic Albaqualfs*	Rice	37	Babu (2015)
0.01 M $CaCl_2$	Louisiana	Fine-silty, mixed, superactive, nonacid, thermic Fluvaquentic Endoaquepts *	Rice	43	Babu (2015)
0.01 M $CaCl_2$	Louisiana	Very-fine, smectitic, thermic Chromic Epiaquerts*	Rice	110	Babu (2015)
0.01 M $Ca\ Cl_2$	South India	Ultisols*, acid (Typic Kandiustult, Paleustult, Ustic Palehumults)	Rice	43	Narayanaswamy & Prakash (2009)
0.05 M $CaCl_2$	North Queensland, Australia	Acid, low base status	Sugarcane	10	Berthelsen et al. (2003)
0.5 M acetic acid	Northern India	-	Rice	20 - 25	Singh et al. (2006)
0.5M acetic acid	Florida	Histosol**	Rice	15	Snyder (1991
0.5M acetic acid	Louisiana	Very-fine, smectitic, thermic Chromic Epiaquerts*	Rice	272	Babu (2015)
0.5M acetic acid	Louisiana	Fine, smectitic, thermic Typic Glossaqualfs*	Rice	221	Babu (2015)
0.5M acetic acid	Florida, Everglades	Histosol**	rice	19	Korndörfer et al. (2001)
0.5M acetic acid	Florida, Everglades	-	Rice	24	Barbosa et al. (2001)
0.5 M acetic acid 1h shaking	South India	Ultisols*, acid (Typic Kandiustult, Paleustult, Ustic Palehumults)	Rice	54	Narayanaswamy & Prakash (2009)

0.5M acetic acid 2h shaking	South India	Ultisols*, acid (Typic Kandiustult, Paleustult, Ustic Palehumults)	Rice	87	Narayanaswamy & Prakash (2009)
Acetate – buffer	Malaysia, Thailand	Tropical paddy soils	Rice	33	Kawaguchi 1966
Na acetate – acetic acid	Sri Lanka	Tropical soils	Rice	38	Takijima et al. 1970
Na acetate – acetic acid	China	calcareous	Rice, Wheat	71 - 181	Liang et al. (1994)
Na acetate – acetic acid	Japan	Acid and neutral	Rice	49 - 60	Imaizumi & Yoshidai (1958), Lian (1976)
Na acetate – acetic acid	China	Acid and neutral	Rice	38 - 60	He (1980), Zhang et al. (2003)
Na acetate – acetic acid	-	Inceptisol*, calcareous	Wheat	80	Xu et al. (2001)
0.5 M NH4O Acid	South India	Ultisols*, acid (Typic Kandiustult, Paleustult, Ustic Palehumults)	Rice	32	Narayanaswamy & Prakash (2009)
0.5 M NH4O Acid	-	Latosol***	Sugarcane	20 - 40 (marginal - adequate)	Fox et al. (1967) Wong You Cheong & Halais, (1970)
N NaO Acid 1h continuous shaking	South India	Ultisols*, acid (Typic Kandiustult, Paleustult, Ustic Palehumults)	Rice	75	Narayanaswamy & Prakash (2009)
N NaO Acid 5h occasional shaking	South India	Ultisols*, acid (Typic Kandiustult, Paleustult, Ustic Palehumults)	Rice	85	Narayanaswamy & Prakash (2009)
0.1 M Citric acid	South India	Ultisols*, acid (Typic Kandiustult, Paleustult, Ustic Palehumults)	Rice	185	Narayanaswamy & Prakash (2009)
0.005 M H_2SO_4	Queensland, Australia	-	Sugarcane	100	Hurney (1973)
0.005 M H_2SO_4	South India	Ultisols*, acid (Typic Kandiustult, Paleustult, Ustic Palehumults)	Rice	207	Narayanaswamy & Prakash (2009)
Modified Truog: 0.01 M H_2SO_4 containing 3 g $(NH4)_2SO_4$ L^{-1}	-	Latosol***	Sugarcane	40 - 100 (marginal - adequate)	Fox et al. (1967)
Phosphate acetate	-	Latosol***	Sugarcane	50 - 150 (marginal - adequate)	Fox et al. (1967)
-	Thailand	-	Rice	31	Ullah et al. (2018)

-	tropical and subtropical areas of south China	low-pH oxisols and ultisols *	Rice	49 - 56	Liang et al. (2015)
-	Korea	Paddy soils	Rice	60	Park (2001)

1.12 Experimental plant – rye, *Secale cereale* L.

As experimental plant of this study rye (*Secale cereale* L.) was selected. It belongs to the family of Poaceae. The plant can reach a height of 2 m. Rye has its origin in the Caucasian area from where it was brought to Europe. Its beginning of cultivation lies about 3000 years behind. Nowadays it is an important winter cereal in Central and Eastern Europe and is grown mainly as cereal for bread, but also as fodder plant for cattle and as a renewable resource for bioethanol and biogas production and the chemical industry. Winter rye is planted in the end of September, with low temperatures in winter stimulating the formation of the florescence. The pollination then happens by wind (Minol, 2008).

Rye is known for some extraordinary features that allows it to be grown even in the farther north and in mountainous regions and to endure the intermittent droughts in spring and early summer common in European sub-continental temperate climate. The cereal shows modest needs in terms of temperature and soil and it is remarkably firm against the wind and tolerant against water deficit stress (Minol, 2008; Hattori et al., 2009; Czyczyło-Mysza & Myśków, 2017). Rye has a larger root biomass and length and higher density in deeper soil layers than most cereals. This well-developed root system enables rye to exploit an extensive area of soil and acquire more water and nutrients from it. However, if shortage of precipitation happens during vegetative growth, after winter rest, the root system development is limited, which is recognized as a main factor hindering rye production (Hattori et al., 2009).
Most graminaceous plants, including rye, are known to be Si-accumulators, although roots capacity to take up Si is lower than in rice and sugarcane (Liang et al., 2015), which makes a critical level above that of rice (43 mg kg^{-1}) and sugarcane (10-20 mg kg^{-1}) possible.

2 Research question

Research on the critical level of Si in soil has been going on mostly in tropical and subtropical climate, and for very few plants only (mainly rice and sugarcane). Although Si has been reported to be beneficial for plants especially under diverse stresses information is lacking on critical levels under stress conditions. In times of climate change however, this aspect becomes ever more important, as plants could be exposed to more intense, more frequent or elongated drought stress, than what they are used to. Therefore, it is necessary to establish critical levels for different agro-climatic zones, including the temperate zone, soils that are commonly used in these agricultural systems and plants, that are cultivated there and also show promising features with respect to changing climatic conditions, to be able to give fertilizer recommendations and food security in the future.

Based on literature values (Table 1) of a critical Si level in soil for rice (43 mg kg^{-1} $CaCl_2$-extractable Si) and sugarcane (20 mg kg^{-1} $CaCl_2$-extractable Si) a greenhouse pot experiment was set up using rye (*Secale cereale* L.) as experimental plant and one Si deficient soil (Dystic Relictistagnic Regosol). The study was conducted with the objective to establish a critical Si level for rye under drought and copper stress (EC50 level), considering also the aspect of ageing of added Si.

Hypothesis H1a: Stressed plants have a positive growth response to increasing soil Si until a certain (critical) level.

Hypothesis H1b: Si concentration in the plant tissue (roots and shoots) increases with increasing soil Si in the control and under stress conditions.

Hypothesis H1c: Root biomass is expected to increase with higher levels of soil Si under stress conditions and lower the shoot:root ratio.

Hypothesis H1d: Increasing soil Si level increases leaf water potential under drought stress.

3 Material and Methods

3.1 Preliminary experiment on the solubility of two potential fertilizers (SiO_2 and $CaSiO_3$)

To assess the appropriateness of the fertilizer for the experiment a pre-trial was set to assess the solubility of $CaSiO_3$ (~ 200 mesh $CaSiO_3$ 99%. ALDRICH Chemistry; CAS: 10101-39-0) and SiO_2 (Produced by drying [60°C, 24 h] and milling a 50% SiO_2 suspension; LUDOX® TM-50 colloidal silica, SIGMA-ALDRICH) in soil. Soil was incubated with different levels of Si (0, 50, 100, 150, 200, 250, 300 mg kg^{-1}) over a period of one week. The two fertilizers were added in amounts as depicted in Table 2. Afterwards, Si extraction and measurement were performed as described in 3.4.4.

Table 2: Fertilizer amendments of $CaSiO_3$ and SiO_2 in pre-trial to assess fertilizer solubility.

Si level in soil (mg kg^{-1})	Added $CaSiO_3$ (mg kg^{-1})	Added SiO_2 (mg kg^{-1})
0	0	0
50	208.7	109.2
100	417.5	218.3
150	626.2	327.5
200	835.0	436.6
250	1043.7	545.8
300	1252.5	654.9

3.2 Establishing stress levels

For the stressed groups Dr and Cu it was attempted to determine the EC50 level, which is the effective added concentration that causes 50% growth inhibition, i.e. a reduction in dry matter yield of 50% compared to the control.

The EC50 level of drought stress was set after a literature review. A drought level of 25-30% FC (field capacity) was considered appropriate, while the control group was held at 70% FC. (Czyczyło-Mysza & Myśków, 2017; Pereira de Melo et al., 2003; Hattori et al., 2009).

To determine the EC50 level of Cu stress, literature was reviewed and also a short pre-trial was conducted. In the pre-trial the same soil and plant (rye) as in the main trial were used for an assessment of the toxicity of different concentrations of copper. Different amounts of Cu (Table 3) were added to 1 kg of soil and homogenized by mixing it by hand with additional 100 mL of water. After one week of incubation 25 rye seeds, var. "Amilo", were planted per pot and held at well-watered conditions (70% of FC) for two weeks. After harvesting and oven drying (65°C for 48 h) plant dry weight suggested an EC50 level at 400 mg Cu per kg soil (Figure 3).

Table 3: Copper amendments in pre-trial to establish stress level for main trial.

Cu level in soil (mg kg^{-1})	Added $CuCl_2$ (mg)
0	0
50	108
100	216
150	324
200	431
300	647
400	863
500	1079

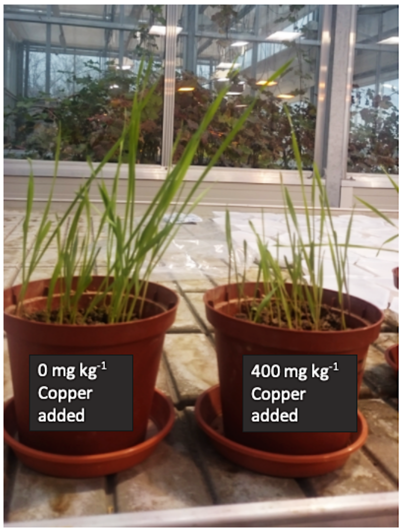


Figure 3: Pre-trial suggesting an EC50 level for copper of 400 mg copper per kg soil.

Literature values for an EC50 level of copper ranged from 36 to 536 mg kg^{-1} (for barley root elongation) (Rooney et al., 2006) and from 109 to 1039 mg kg^{-1} (tomato and barley growth) (Ruyters et al., 2013). Rooney et al. (2006) examined a number of different soil types of which the majority had EC50 levels below 500 mg kg^{-1}. For the main trial the value of 400 mg copper per kg soil was adopted.

3.3 Experimental soil

The soil used in the trial originated from an agricultural site and was collected in September 2017 from a field close to Sigmundsherberg, Lower Austria (48.7094°N and 15.74445°E; 450 m a.s.l.) It was classified as Dystic Relictistagnic Regosol (according to Cocuzza, 2017; WRB for soil resources, FAO, 2015) which corresponds to carbonate-free Reliktpseudogley in Austrian soil taxonomy (Nestroy et al., 2011). The soil was classified as low-value farmland

(BFW-Bodenkarte, 2019). Physical and chemical characteristics that had been investigated by Cocuzza (2015) are summarized in Table 4 and completed by data retrieved from the digital soil map eBod (BFW-Bodenkarte, 2019). Soil was collected from the upper 20 cm of the soil profile, which were recognized as part of an Ap horizon (p for plowed, Table 4) (Nestroy et al., 2011; BFW-Bodenkarte, 2019). Soil samples were then air dried, sieved to a grain size of 0-4 mm and homogenized before soil was filled in pots (1 kg per pot).

Table 4: Physical and chemical characteristics of the experimental soil (Cocuzza, 2017; BFW-Bodenkarte, 2019)

Soil group according to WRB	Average yearly precipitation (mm)	Average yearly temperature (°C)	pH	Sand ($g\ kg^{-1}$)	Silt ($g\ kg^{-1}$)	Clay ($g\ kg^{-1}$)	Organic Matter ($g\ kg^{-1}$)	Lime ($g\ kg^{-1}$)
Dystic Relictistagnic Regosol	557	9.5	6.72	450	450	100	9	0
Profile description	Ap	E	Cvrel	Cv				
	0-20 cm	20-40 cm	40-65cm	65-200cm				

3.4 Main trial

3.4.1 Experimental setup

To assess the critical level of plant-available Si in soil a pot trial was conducted under greenhouse conditions. Humidity was held at 60 %, different temperatures were induced at day and night, which were 25°C and 15°C respectively, and the day/night rhythm was 16/8 h. Sodium vapor lamps were used to artificially supply daylight and allow minimum photosynthetically active radiation (PAR) of 300 to 400 µmol $m^{-2}\ s^{-1}$. The experiment was conducted in the greenhouses of the UFT (Universitäts- und Forschungszentrum Tulln) in Tulln, Lower Austria in the period of September 2017 to February 2018.

The experimental setup was as following: Si was added at seven different levels (Table 5), that were based on literature values of critical Si values, in the form of $CaSiO_3$, to a control group (C) and two groups of plants that were submitted to stress. Drought stress (Dr) and copper stress (Cu) were selected as stress types. For each group and each Si level four repetitions were made. There was one extra repetition in the control group and the drought group to use them later in order to determine the leaf water potential without affecting plant biomass of the four repetitions.

Additionally to the Si fertilizer $Ca(NO_3)_2$ (as $Ca(NO_3)_2 \cdot 4H_2O$, amount added was adjusted accordingly) and NH_4NO_3 were added in amounts according to Table 5, to level out influences of Ca added with the fertilizer. $CaSiO_3$, $Ca(NO_3)_2$ and NH_4NO_3 were added to the soil only once, homogenized well by mixing by hand and watered one week before the seeds were planted, to allow the fertilizer to dissolve.

Copper was added to the Cu group only. An amount of 400 mg kg^{-1} copper was added in the form of $CuCl_2$ (863 mg kg^{-1}) only once, one week before the seeds were planted and distributed evenly by mixing by hand.

Table 5: Amendments of $CaSiO_3$, $Ca(NO_3)_2$, NH_4NO_3 to soil (1 kg) in the main trial.

Soil Si level (mg kg^{-1})	Added $CaSiO_3$ (mg kg^{-1})	Added $Ca(NO_3)_2$ (mg kg^{-1})	Added NH_4NO_3 (mg kg^{-1})
0	0	1019	0
10	42	934	29
20	84	849	58
40	167	679	115
60	251	509	173
80	334	340	230
120	501	0	345

Pots were filled with 1 kg of < 4 mm sieved soil. Rye seeds were sown in the pots (25 seeds per pot) on December 20th, 2017 and covered with a small layer of soil that had been sieved to grain sizes less than 2 mm. Water was added to facilitate germination.

To allow the soil to maintain constant soil moisture each pot was weighed, and its target weight determined. The weight of the pots was then controlled continually during growth phase to determine the water volume needed for watering. The C group and the Cu group were held at 70% of the FC (field capacity). To induce drought stress Dr group was held at 30% of the FC. Field capacity of the soil was calculated to be 174 g kg^{-1} of the < 4 mm grained soil (Duboc, personal communication, 2017). The differentiation of water regime in the soil started in the phase of tillering. Watering was conducted with high purity water (laboratory water type I; 18 MΩ cm^{-1}) to avoid contamination with Si through tap water.

In order to not have any other stress affecting the plants 50 mL of a nutrient solution were added weekly, starting one week after planting. Refer to Table 6 for the nutrient solutions composition (modified from Middleton & Toxopeus 1973).

Table 6: Composition of the nutrient solution. 50 mL of the nutrient solution were applied per pot once a week during the period of the main trial (7 weeks).

	N	P	K	S	Mg	Ca	Na
$g\ L^{-1}$	1.401	0.297	0.659	0.328	0.044	0.208	0.147
	B	Cu	Mn	Mo	Zn	Fe	
$g\ L^{-1}$	0.105	0.075	1.528	0.047	0.144	0.137	

Rye plants were kept in a green house and grown for seven weeks, until harvest. Pots were distributed randomly within blocks and moved once a week on the table to avoid effects of possible site-specific differences of light or humidity (Figure 4).

An extra set of pots in two repetitions was filled with soil and the different levels of Si (1-7) were applied. The pots were left without plants to monitor the Si concentration in the soil. Amendments with $CaSiO_3$, $Ca(NO_3)_2$ and NH_4NO_3 were as in groups C, Dr and Cu, and moisture in these pots was maintained at 70% FC by regularly weighing the pots and adjusting them to target weight.

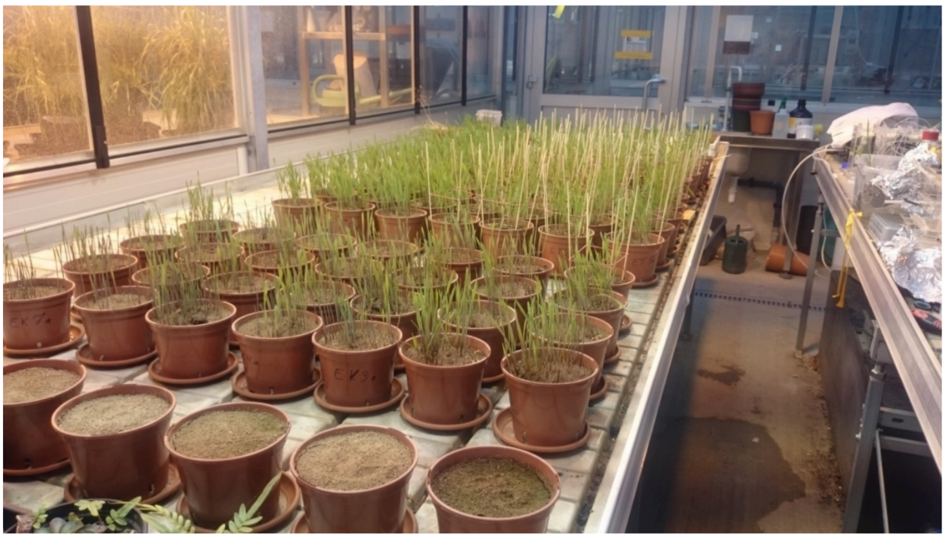


Figure 4: Main trial: Pots in the greenhouse, one week after sowing.

3.4.2 Leaf water potential (LWP)

The leaf water potential in groups C and Dr was measured with a Scholander pressure chamber (3000 Series Plant Water Status Consoles, Soilmoisture Equipment Corp.) Three measurements were made during two weeks (week five to seven) before harvesting. At each measurement two to three replications were made, and the average value was established. Measurements were conducted at 7:00h am, 12-15 hours after watering to target weight (according to water regime in each group).

3.4.3 Shoot and root biomass

The rye shoots and leaves were harvested on 1st and 2nd of February 2018, after seven weeks of growth by cutting off shoots with scissors at the level of the pot's edge. They were rinsed with high purity water, put in paper bags and oven-dried at 65°C for 48h. The dry plant tissue was then weighed. In group Cu some of the plants had died off already. Plant tissue was still available, but brown and dry when harvested.

Roots of Si levels 1 and 7 were harvested from groups C, Dr and Cu from all four repetitions. It was notable that roots of group Cu were not well developed. They stayed within the upper 2 cm of the soil and due to very little biomass could not be used for digestion and their Si concentration could not be measured.

The soil was washed off from the roots manually and they were cleaned with tap water, as for this procedure large amounts of water were needed, and then rinsed with high purity water before continuing with further procedures.

The fresh weight was measured. As roots had to be used for establishing root dry weight, but also for evaluating root parameters, fresh roots were split in two parts and both weighed to later be able to calculate dry weight and root parameter values for the whole root sample. One share of the roots was put in paper bags and oven-dried for 48 h at 65°C weighed and the total dry mass calculated. The other share was put in 30% - 50% Ethanol and stored at 4°C for further measurements (see root parameters chapter 3.5).

3.4.4 Silicon analysis

3.4.4.1 Soil sample preparation

Soil Si concentration was measured in all samples of the pre-trial one week after Si amendment had taken place. In the plantless pots soil Si concentration was measured after seven weeks (at the same time as the plants were harvested). Simultaneously to sample preparation Si calibration standards for soil were prepared in the same matrix (0, 0.5, 3, 6, 9, 12,16 mg L^{-1}) which were also measured with the photometer.

Extraction of Si from soil was conducted as described in Haysom and Chapman (1975). For the Si extraction 2 g of soil, sieved to grain sizes less than 2 mm, were amended with 20 mL of a 0.01 M $CaCl_2$ solution and put on an overhead shaker (GFL Overhead shaker 3040) for 16 hours, with a speed of five turns per minute. Further procedures to assess the Si concentration were filtration of the Si extract with paper-filters, staining (described in 3.4.4.3), and measurement in the photometer (described in 3.4.4.3).

3.4.4.2 Plant sample preparation

Shoot dry matter, as well as root dry matter was ground after being dried, at a speed of 8500 turns per minute, for 40 seconds (Retsch GM 200 Grindomix). The digestion of plant biomass was conducted for aboveground biomass of all groups and all Si-levels, as well as for root biomass of groups C and Dr, levels 1 and 7.
Simultaneously to sample preparation Si calibration standards for plants were prepared in the same matrix (0, 20, 40, 60, 80, 100 mg L^{-1}) which were also measured with the photometer.

The original method of OID (Oven-induced digestion method) was developed by Kraska and Breitenbeck (2010) as a "simple, robust method for quantifying Si in plant tissue". This method was applied here. Adaptations of the method were made according to an update of the method made in October 2017 by Olivier Duboc, Anja Robbe and Paul Schabl. The following modifications were made: Five drops of octyl alcohol of the published method are equal to 80µL. A transfer pipette was used instead of counting the drops. Vials vere capped loosely instead of tight to prevent pressure development inside the vials. The 50% NaOH were understood as *w/v.* In the second phase of digestion heating was for five hours, in contrast to four hours in the published method, to increase Si extraction from plant biomass.

The digestion was conducted as following: 100 mg of ground plant tissue were brought to a 50 mL vial. 80 µl octyl alcohol were added to reduce foaming. 2 mL of 30% H_2O_2 were added, and with the cap put loosely the vial was kept in the oven for 30 minutes at 95°C. Then 4 mL of 50% w/v NaOH were added to the hot sample, which was again capped loosely, and gently vortexed. The vial was then put in the oven again for five hours, at 95°C. Afterwards 1 mL of 5 mM NH_4F was added to the sample to facilitate the formation of monosilicic acid. The sample was then brought to a final weight of 51.921 g with high purity water to reach a final volume of 50 mL.

3.4.4.3 Photometric analysis

For measuring the Si concentration, a colorimetric method was used. The same staining method was used for plant digests and soil extracts, using only different concentrations depending on the type of sample, and following the instructions given by Morrison & Wilson (1963) and Webber & Wilson (1964): To 1 mL of the soil-extract sample 7.75 mL of water were added (0.2 mL sample and 8.55 mL of water for plant digests). 0.5 mL of acidified molybdate solution were added, mixed immediately by hand and left for ten minutes. 0.5 mL tartaric acid solution 28% w/v (28 g tartaric aid powder in 100 mL high purity water) was then added, again vortexed immediately and left for five minutes. 0.25 mL reducing agent solution was added, and again mixed by hand. Plant and soil calibration standards were stained in the same way. A photometer (Varian UV visible spectrophotometer DMS 200) and the calibration standards were used to determine the amount of Si in soil and plant samples. For measuring Si, a wavelength of 810 nm was used. The measurement with the photometer was done within one to three hours after adding the reducing agent solution

3.5 Root parameters

To investigate certain root features a root scanner (Epson perfection V700 PHOTO) and corresponding software (Win RHIZO 2013e, Regent Instruments 2013) were used. The part of the root that had been set aside and stored in ethanol was used for the scanner. Roots were made suitable for the scanner by staining. The roots were rinsed with distilled water at least two times and put in the Giemsa working solution, a staining mixture of methylene blue, eosin, and Azure B, diluted 1 to 25 with distilled water according to Himmelbauer et al. (2004), heated up to 40°C, for ten minutes. The stained roots were washed under running, deionized water for at least three minutes and stored in 30% - 50% ethanol until scanning.

The scanning software identified root volume, diameter, length, surface area, projected area, tips, forks, crossings and fractions (per diameter class) of several of the listed parameters. As only a share of the whole root mass was used for the scanner the identified values were extrapolated for the entire root mass, according to the share of root mass that was withdrawn after rinsing the fresh roots for the purpose of root parameter identification. (e.g. if 50% of the root sample was scanned, the results were multiplied by 2 to derive a result for the whole root sample).

3.6 Statistics

For descriptive statistics Microsoft® Excel (Version 16.37) was used. To estimate the critical level of Si in soil different methods are possible (Babu, 2015). The parameters shoot dry weight, shoot Si concentration and Si uptake (plant biomass times Si conc.) were used as responses to Si amendments and considered in establishing a critical level or optimum soil Si plant availability. In the quadratic model the minimum Si concentration in soil that corresponds to the maximum yield can be determined as an optimal Si level. The critical level for Si response is the value associated with highest yield level, which corresponds to the peak of the projected quadratic function (Waugh et al., 1973). The linear-plateau model identifies the point, at which further soil Si amendments are unlikely to result in higher yield, by depicting a linearly increasing response (yield) that levels out to a plateau (Kuzyakov et al., 1997). With the graphical Cate Nelson model data can be divided into two groups to establish a critical x and y level, that divide the plot into four quadrants, where most data points should remain in the second and fourth quadrant. The critical x-level is found by calculating the Sum of Squares for each potential critical x-value (Mangiafico, 2013; Cate & Nelson, 1971).

If a strong linear relation is obtained, it is unlikely, that the critical/optimal level has been reached yet.

For some of the data, none of the models gave a good fit. Regression models were statistically evaluated using the coefficient of determination (R^2) and p-value (significance level = 0.05). Best model fit was established for response variables shoot dry weight, shoot Si concentration and Si uptake. Linear and quadratic models were performed with Microsoft® Excel, whereas the linear-plateau model and the Cate-Nelson model were performed with RStudio (RStudio, Inc., Version 1.2.5033).

A Shapiro-Wilk test, which tests the null-hypothesis of normal distribution and confirms it with values of significance > 0.05, was performed for root data (dry weight, Si concentration and root parameters). A Shapiro Wilk test was also performed for shoot Si concentration data at Si levels 1 and 7 of groups C, Dr for further comparisons between roots and shoots. Levene’s test was used to test for homoscedasticity. If normal distribution and homoscedasticity were given a parametric t-test was performed for assessing significant differences ($p < 0.5$) between two groups of values. This was the case for most data. If normal distribution and/or homoscedasticity were not given a non-parametric Mann-Whitney u-test was performed for assessing significant differences ($p < 0.5$) between two groups of values. P-values derived with the Mann-Whitney u-test will be marked with superscript "u" in data tables. These tests were performed with XLSTAT (Version 22.3.1; Addinsoft; an add-in for Microsoft® Excel).

Significant differences in sample values ($p < 0.5$) were assessed between root and shoot Si concentration in groups C and Dr at level 1 and 7. Also, significance of differences in sample values were evaluated in root data at level 1 and 7 within each group and within each level, for root biomass (groups C, Dr, Cu) and root Si concentration (groups C, Dr). Significance was also tested for root parameters between and within groups and for shoot:root ratio of biomass. A one-way ANOVA was performed to test for difference in means of C, Dr, Cu of Si levels 1 to 7 of shoot biomass and shoot Si concentration. Means are significantly different from each other if F > Fcrit. T-test and ANOVA were performed with Excel.

3.7 Quality assurance

All tools used during the trial were acid washed (5% HNO_3), soaked in a base bath (0.1 mol L^{-1} NaOH) for at least five hours and afterwards rinsed with deionized water three times to avoid contamination with Si.

4 Results

4.1 Pre-trial

To decide which fertilizer was more suitable for the main trial, in a pre-trial two fertilizers, $CaSiO_3$ and SiO_2, were compared. After a week of incubation $CaSiO_3$ showed a strong positive correlation between the amount of fertilizer that was added and amount of $CaCl_2$-extractable Si (Figure 5; $R^2 = 0.98$, $p < 0.01$). On average 26.5 % of the added Si via $CaSiO_3$ were found to be extractable and therefore possibly plant-available. This corresponds to 6.3 % of the total mass of added fertilizer.

When SiO_2 was used as a fertilizer, no significant ($p = 0.6$) correlation between fertilizer added and extracted Si was found (Figure 6; $R^2 = 0.04$). $CaCl_2$-extractable Si after fertilizer treatment (13.9-17.0 mg kg^{-1}) had similar concentrations to $CaCl_2$-extractable Si in soil without any fertilizer addition (14 mg kg^{-1}). $CaSiO_3$ was determined as fertilizer for the main trial.

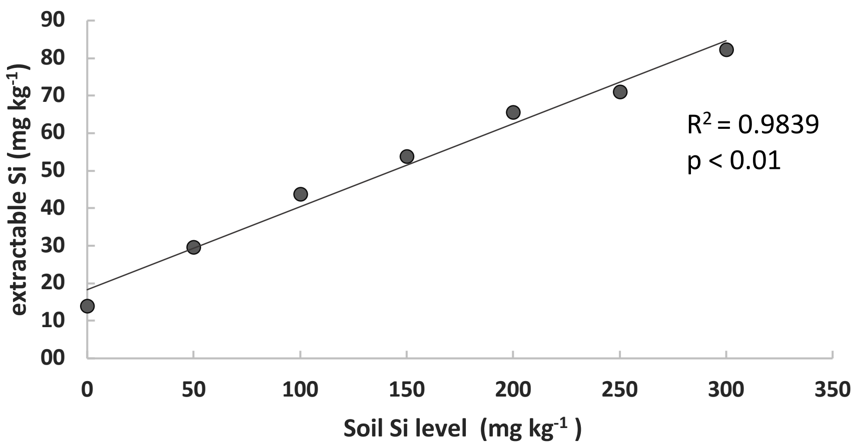


Figure 5: $CaCl_2$-extractable Si in soil from $CaSiO_3$ amendments after one week of incubation (1 repetition) in pre-trial.

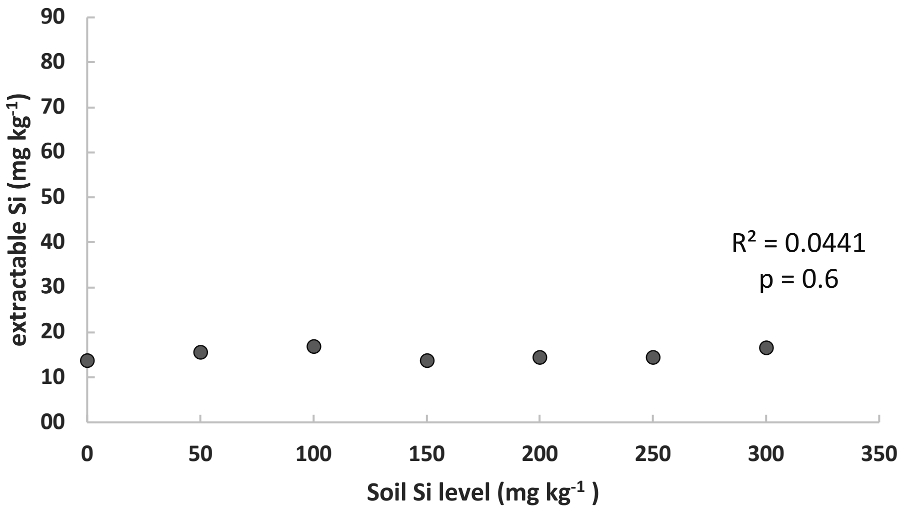


Figure 6: $CaCl_2$-extractable Si from SiO_2 amendments after one week of incubation (1 repetition) in pre-trial.

4.2 Main trial

4.2.1 $CaCl_2$-extractable silicon in soil over time

Figure 7 illustrates the availability of Si in soil added in the form of $CaSiO_3$, in week one and week seven of the experiment. Week seven had lower amounts of $CaCl_2$-extractable Si. Measurements of both weeks however correlated with the amount of Si that had been added (week 1: $R^2 = 0.98$, week 7: $R^2 = 0.956$).

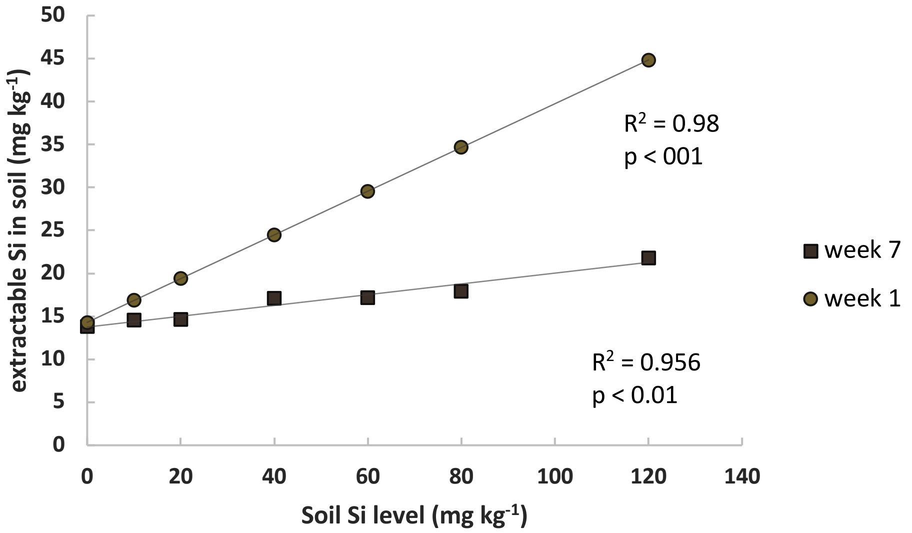


Figure 7: $CaCl_2$-extractable Si in soil after one week (1 repetition) and after seven weeks (mean of 2 repetitions) with trendline. SD for week 7 is 0.38, 0.29, 0, 0.29, 0.19, 0.28, 0.19 according to levels 1 to 7.

4.2.2 Leaf water potential and its correlation with soil silicon level

The leaf water potential was higher in group C than in group Dr (Figure 8). Regression analysis shows a significant positive correlation between LWP and Si levels, with $R^2 = 0.752$, $p < 0.05$ for the C group and $R^2 = 0.819$, $p < 0.01$ for the Dr group. The rate at which LWP of both groups increased as Si in soil increased was constant (Table 7), which means that Si raised the LWP in both groups evenly.

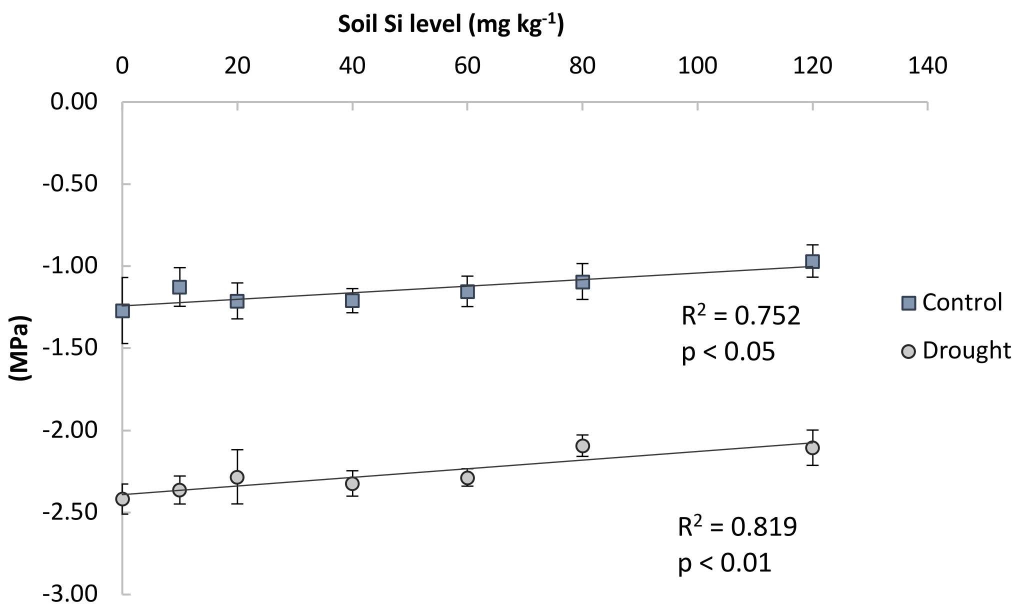


Figure 8: Mean leaf water potential of C and Dr group with trendline. Error bars indicate standard deviation, n=6-7

Table 7: Rate of Dr:C LWP

Soil Si level (mg kg^{-1})	Rate Dr:C
0	0.525
10	0.477
20	0.531
40	0.521
60	0.504
80	0.522
120	0.460

4.2.3 Plant biomass

4.2.3.1 Shoot dry weight

The highest amount of shoot biomass was found in the C group, followed by the Dr group and then the Cu group (Figure 9).

Analysis of variance informs that the average dry weight did not significantly change in C group as Si levels changed. For Dr and Cu group however, mean values of dry weight were significantly different at each Si level (Table 8).

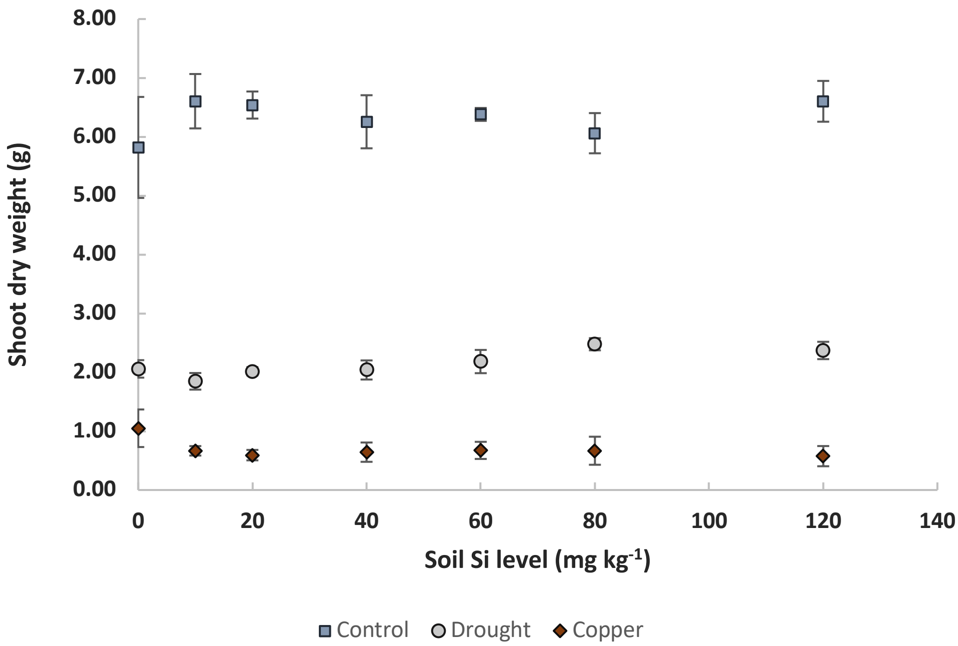


Figure 9: Mean shoot dry weight of groups C, Dr, Cu at different soil Si levels. Error bars indicate standard deviation, n=4

Table 8: ANOVA for C, Dr, Cu shoot dry weight. Means are significantly different from each other if F > Fcrit.

ANOVA	F value	p-value	F crit.
C	1.72	0.17	2.57
Dr	9.26	0.00005	2.57
Cu	2.9	0.03	2.57

4.2.3.2 EC 50 level

The proposed EC50 level at which stress is introduced at a level that will reduce plant biomass by 50% compared to the control was not reached in the trial. The EC level was 35.5 in the Dr group and 18.1 in the Cu group. The effects of Si fertilization might be different at this level than at the EC50 level.

4.2.3.3 Root dry weight

Root parameters were only determined for Si levels 1 and 7 (no Si added and 120 mg kg^{-1} added, resp.). Figure 10 shows, that highest root biomass was produced by the C group, followed by the Dr group, and lowest root biomass production was observed in the Cu group.

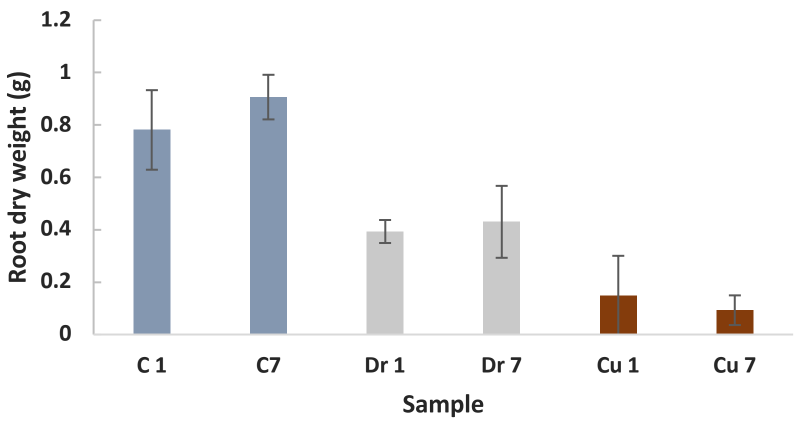


Figure 10: Mean root dry weight of levels 1 and 7 of groups C, Dr, Cu. Error bars indicate standard deviation, n=4

4.2.3.4 Shoot:root ratio of biomass and significance of differences in the ratio

Shoot:root ratio for plant biomass is given in Table 9, significances of differences in the ratio are given in Table 10. In the Cu group the shoot:root ratio decreased from 13.94 at level 1 to

10.48 at level 7, however not significantly ($p = 0.71$). The ratio in the Dr group at both levels was lower than in group C, however only the difference at level 1 was significant ($p < 0.05$).

Table 9: Shoot:root ratio of plants biomass.

Sample	Shoot:root ratio
C1	7.55
C7	7.31
Dr1	5.31
Dr7	5.90
Cu1	13.94
Cu7	10.48

Table 10: Significance of differences in shoot:root ratios. significant differences ($p < 0.05$) in red. [u]: Mann-Whitney u-test, all other: t-test

Sample	p	Sample	p
C1-C7	0.886[u]	C1-Dr1	0.029[u]
Dr1-Dr7	0.886[u]	C7-Dr7	0.343[u]
Cu1-Cu7	0.712	C1-Cu1	0.383
		C7-Cu7	0.343[u]

4.2.4 Shoot silicon concentration

Figure 11 shows increasing Si concentrations in tissue of plant shoots of all three groups, as the soil Si level rises. The shoot Si concentration in the Cu group was lower than that of groups C and Dr at all 7 levels. At level 1 the Dr group had a lower Si concentration in its shoots tissue than the C group, however, at level 7 it was higher than in the C group. The means at all Si levels were significantly different in all groups (Table 11).

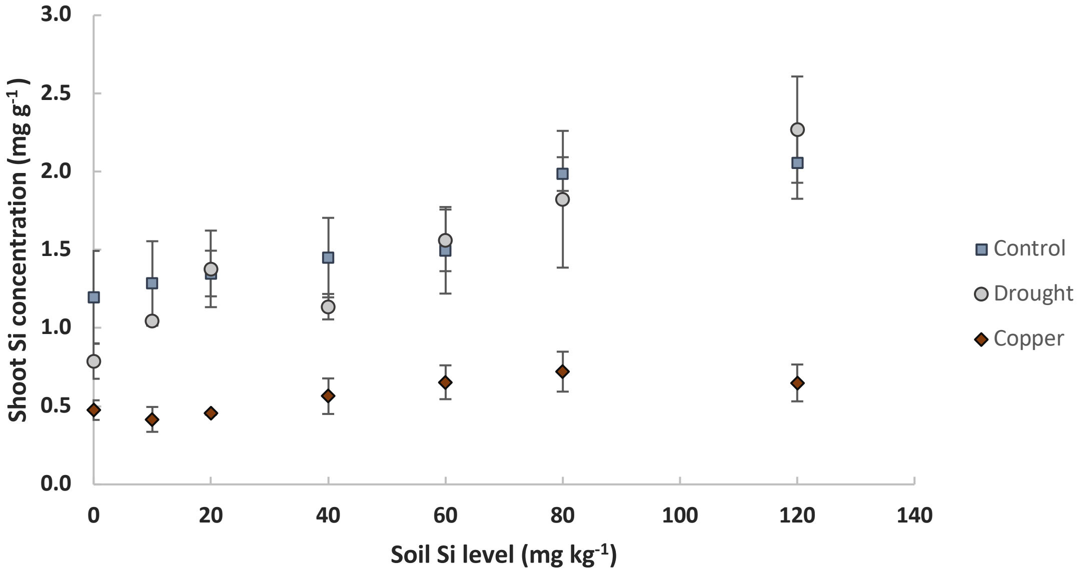


Figure 11: Mean Si concentration in shoots of groups C, Dr, Cu at different soil Si levels. Error bars indicate standard deviation, n=4

Table 11: ANOVA for Si concentration of C, Dr, and Cu. Means are significantly different from each other if F > Fcrit.

ANOVA	F-value	p-value	F crit
C	8.35	0.0001	2.57
Dr	16.72	0.00005	2.57
Cu	5.86	0.001	2.57

4.2.5 Comparing root and shoot dry weight and silicon concentration

Dry weight and Si concentration of shoots and roots are compared in Table 12. As root samples were only taken from levels 1 and 7 only comparisons with shoot Si concentration at the respective levels could be conducted. Table 13 shows that the mean Si concentration was significantly ($p < 0.05$) higher in roots compared to shoots in groups Dr and C at levels 1 and 7. Considering the root data in Table 14, a significant difference for root biomass could be found for the two stressed groups in comparison with the control group at level 1 and 7 ($p < 0.05$). The root dry weight at level 7 was not significantly different to root dry weight at level 1 ($p > 0.05$) for no group. The root Si concentrations at level 7 was significantly different to root Si concentrations at level 1 ($p < 0.05$) in groups C and Dr (no data for group Cu).

Table 12: Shoot and root mean dry weight, mean Si concentration and SD of samples C1, C7, Dr1, Dr7

Shoot sample	Mean dry weight (g)	Mean Si concentration (mg g^{-1})	SD dry weight (g)	SD Si concentration (mg g^{-1})
C1	5.819	1.196	0.86	0.30
C7	6.603	2.057	0.35	0.23
Dr1	2.057	0.789	0.15	0.11
Dr7	2.370	2.269	0.15	0.34

Root sample	Mean dry weight (g)	Mean Si concentration (mg g^{-1})	SD dry weight (g)	SD Si concentration (mg g^{-1})
C1	0.78	2.650	0.15	0.57
C7	0.91	4.546	0.09	0.16
Dr1	0.39	2.349	0.04	0.18
Dr7	0.43	4.635	0.14	0.56

Table 13: Significance of differences ($p < 0.05$) in Si concentration in shoots and roots. Significant differences in red. [u]: Mann-Whitney u-test, all other: t-test

Sample	p
C1 shoots-C1 roots	0.004
C7 shoots-C7 roots	0.000002
Dr1 shoots-Dr1 roots	0.029[u]
Dr7 shoots-Dr7 roots	0.00035

Table 14: T-test to investigate significant differences between root biomass and Si concentration at Si levels 1 and 7 within a group and among groups. P-values in red indicate significance $p < 0.05$.

Sample	Root biomass	p	Root Si concentration	p
C1-C7		0.2		0.0007
Dr1-Dr7		0.627		0.00024
C1-Dr1		0.0027		0.356
C7-Dr7		0.0011		0.769
Cu1-Cu7		0.519		
C1-Cu1		0.0011		
C7-Cu7		0.0000039		

4.2.6 Silicon uptake

The Si uptake is the product of shoot dry weight and shoot Si concentration. It increases in C and Dr group as soil Si increases, but not in group Cu (Figure 12).

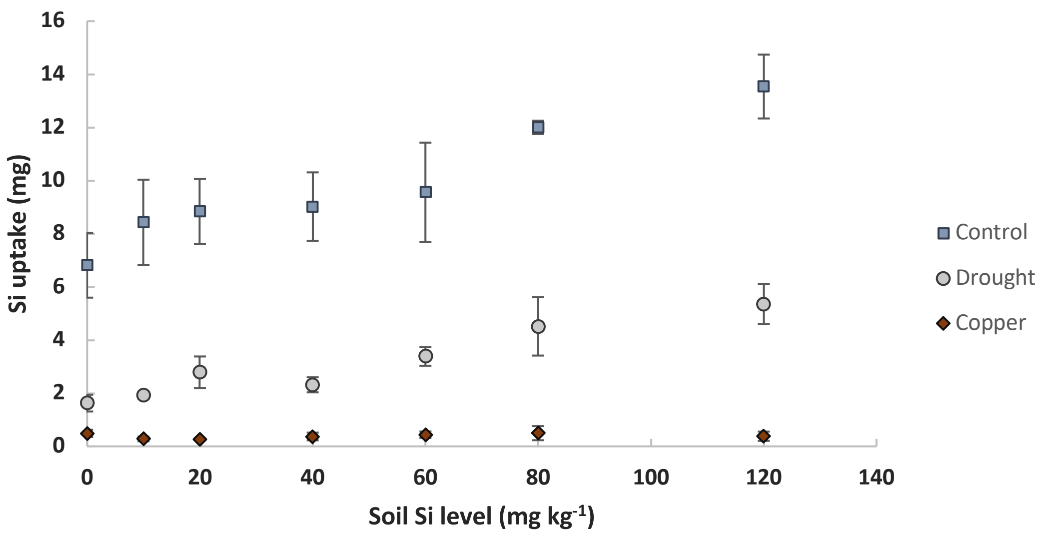


Figure 12: Mean uptake of Si in rye plant of groups C, Dr, Cu at different soil Si levels. Error bars indicate standard deviation, n=4

4.2.7 Root parameters and significance of differences in root parameter values

Values in Table 15 show clear differences between the groups. Root length, surface area and number of tips generally decreased between the treatments, in the order C > Dr > Cu. As seen in Table 16 most differences between groups are significant ($p < 0.05$), while differences between level 1 and 7 within the groups are not ($p > 0.05$).

Table 15: Mean root parameters values, n=4

Sample	Length (cm)	Projected area (cm^2)	Surface area (cm^2)	Average diameter (mm)	Length per volume ($cm\ cm^{-3}$)	Root volume (cm^3)	Tips
C1 mean	11304	277	872	1.04	2150	5	28825
C7 mean	13541	363	1140	1.32	1803	8	27530
Cu1 mean	694	33	105	1.00	481	1	1837
Cu7 mean	534	27	86	0.93	484	1	1429
Dr1 mean	5923	146	458	0.55	2139	3	12395
Dr7 mean	5179	138	432	0.47	1808	3	9255

Table 16: Significance of difference in root parameter values. Significant differences in red ($p < 0.05$). u: Mann-Whitney u-test, all other: t-test

T-test between groups	Length (cm)	Projected area (cm^2)	Surface area (cm^2)	Average diameter (mm)	Length per volume (cm cm^{-3})	Root volume (cm^3)	Tips
C1-Dr1	0.006	0.013	0.013	0.08	0.962	0.027	0.003
C7-Dr7	0.0002	0.0006	0.0006	0.009	0.982	0.029[u]	0.0004
C1-Cu1	0.000046	0.0003	0.0003	0.85	0.00003	0.0018	0.029[u]
C7-Cu7	0.029[u]	0.000006	0.000006	0.2[u]	0.029[u]	0.029[u]	0.00005

T-test within groups	Length (cm)	Projected area (cm^2)	Surface area (cm^2)	Average diameter (mm)	Length per volume (cm cm^{-3})	Root volume (cm^3)	Tips
C1-C7	0.16	0.11	0.11	0.34	0.11	0.01	0.75
Dr1-Dr7	0.43	0.73	0.73	0.886[u]	0.18	0.93	0.17
Cu1-Cu7	0.58	0.59	0.59	0.886[u]	0.97	0.56	0.61

In both stressed groups most values at Si level 7 were lower: Absolutely, compared with Si level 1 (Table 15), and relatively, in comparison with the C group (Table 17). An exception were naturally the average root diameter and length per volume. As can be seen, the length per volume was more or less the same in C and Dr group which can be attributed to their very similar growth patterns.

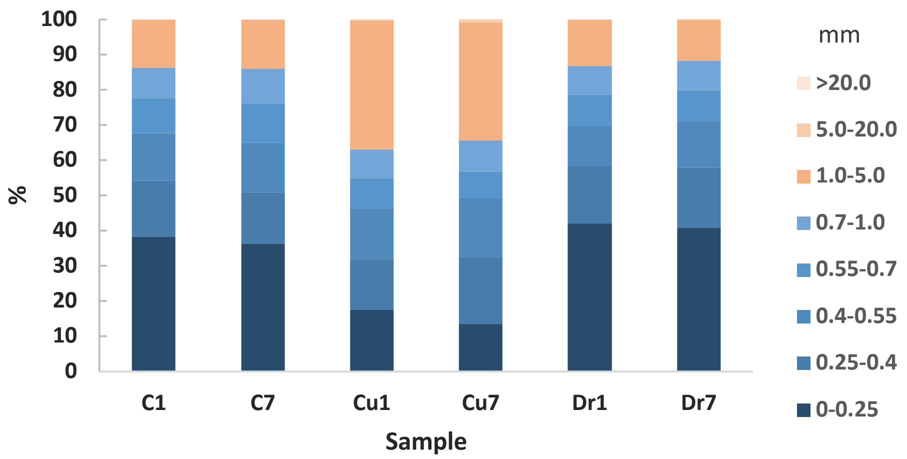
Table 17: Percental comparison of root parameter values of stressed groups Dr and Cu with Control group C

	Length (cm)	Projected area (cm^2)	Surface area (cm^2)	Average diameter (mm)	Length per volume (cm cm^{-3})	Root volume (cm^3)	Tips
% Dr1 from C1	52	53	53	53	99	53	43
% Dr7 from C7	38	38	38	36	100	37	34
% Cu1 from C1	6	12	12	96	22	24	6
% Cu7 from C7	4	8	8	70	27	14	5

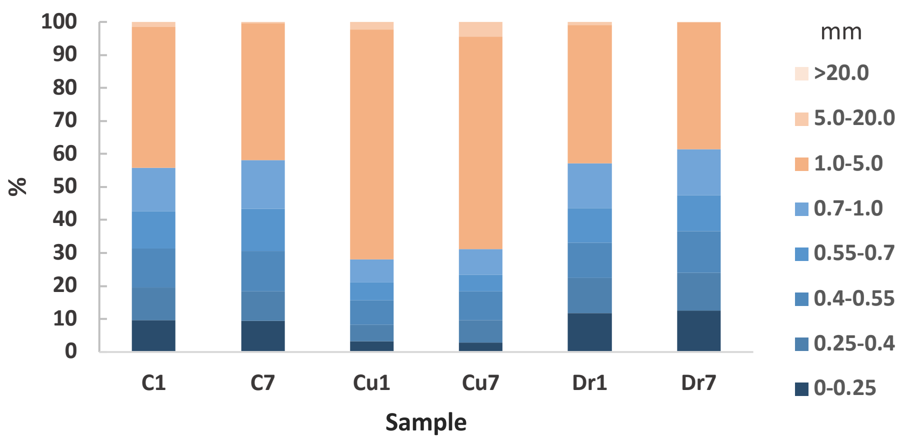
4.2.7.1 Parameter distribution as a function of root diameter

To illustrate to what degree roots of a certain diameter contribute to the total of root parameters, root diameter was split in 8 classes: 0-0.25; 0.25-0.4; 0.4-0.55; 0.55-0.7; 0.7-1.0; 1.0-5.0; 5.0-20; >20,0 mm. Results of parameter distribution are shown in Figure 13. In C and Dr group very fine roots (0-0.25 mm) accounted for more than 70% of root length. Naturally, fine roots also accommodated root tips. The major part of the surface area (36-42%) was made up by the smallest root diameter fraction. The rest was rather evenly distributed between classes 2-6 (0.25 to 5 mm). Roots of the larger diameter fraction (> 1.0 mm) accounted for most of the root volume (41-43%). In the Cu group, roots with a larger diameter often accounted for a major share of the root parameter.

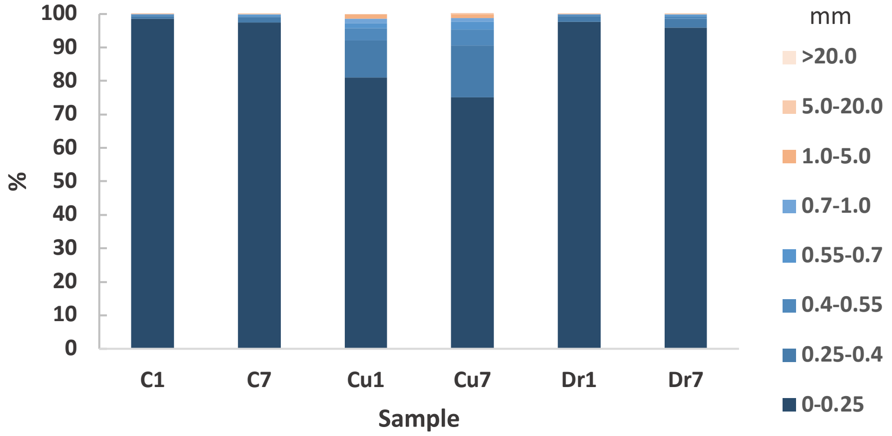
a) Projected Area



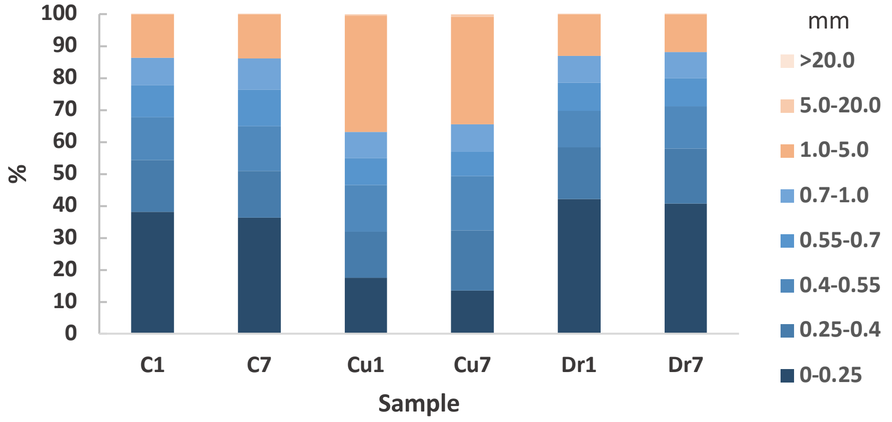
b) Volume



c) Tips



d) Surface Area



e) Length

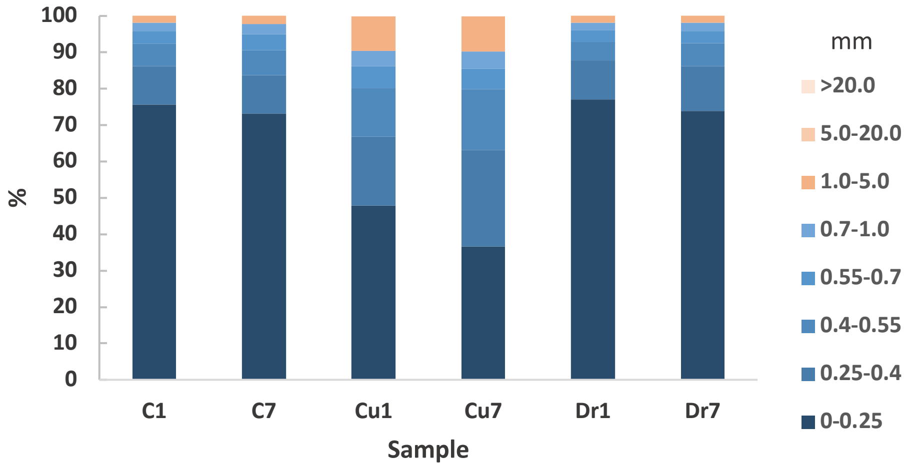


Figure 13: Parameter distribution in function of root diameter. a) Projected area, b) Volume, c) Tips, d) Surface area, e) Length; in blue: root diameter < 1.0 mm; in orange: root diameter >= 1.0 mm.

5 Discussion

5.1 Ageing of silicon

The availability of Si was not constantly as high as directly after amendment. After seven weeks the $CaCl_2$-extractable Si in soil had decreased in comparison to extractable Si directly after fertilization. Processes in soil (e.g. complexing, adsorption, washing out) as well as the time of fertilizer application and its dissolution rate should be considered when using Si fertilizers. In agriculture the period between the germination of rye seeds and the harvest of the cereal is 280-320 days (Düll & Kutzelnigg, 2011), which clearly exceeds the period of the experiment. Gascho (2001) points out the lack of studies addressing the potential of providing a longer-term release of Si. Including Si components with a lower dissolution rate or mixing different particle sizes in the fertilizer might provide Si over a longer time.

5.2 Establishing a critical level of silicon in soil

The critical level of an element is usually related to plant biomass, as it is desirable to optimize fertilizer amendment and crop yield. In this study shoot Si concentration and Si uptake were too considered as response parameters to Si amendments that might reach an optimal level, even if not directly linked to a higher yield. Response parameters are listed with best fitting model in Table 18 and are depicted in Figures 15-21.

*Table 18: Best model fit for response parameters (dry weight, Si concentration, Si uptake) and possible critical/optimal level. Amounts of Si added (0, 10, 20, 40, 60, 80, 120 mg kg^{-1}) correspond to 14.0, 16.9, 19.4, 24.5, 29.6, 34.7, 44.8 mg kg^{-1} $CaCl_2$ extractable Si respectively. Response parameters are derived from shoots only. *: possible critical/optimal level derived from 2nd best model fit.*

Model	Group, response parameter	2nd best model fit, if relevant	R^2	p	Possible critical /optimal level Si added (mg kg^{-1})
No model fit	C, dry weight		0.098	0.49	
	Cu, Si uptake		0.044	0.65	

Linear regression	C, Si concentration	Cate-Nelson	0.910	< 0.01	40-60*
	C, Si uptake	Cate-Nelson	0.936	< 0.01	40-60*
	Dr, Si concentration	Cate-Nelson	0.918	< 0.01	40-60*
	Dr, Si uptake	Cate-Nelson	0.942	< 0.01	40-60*
Quadratic regression	Cu, dry weight		-0.403	0.44	
Linear-plateau	Cu, Si concentration	Quadratic (R^2 = 0.846, p = 0.13), Linear (R^2 =0.710, $p < 0.05$)	0.892	< 0.05	60-80 100*
Cate-Nelson	Dr, dry weight	Quadratic (R^2 = 0.720, $p < 0.05$) Linear (R^2 = 0.712, $p < 0.05$)			40-60 > 100*

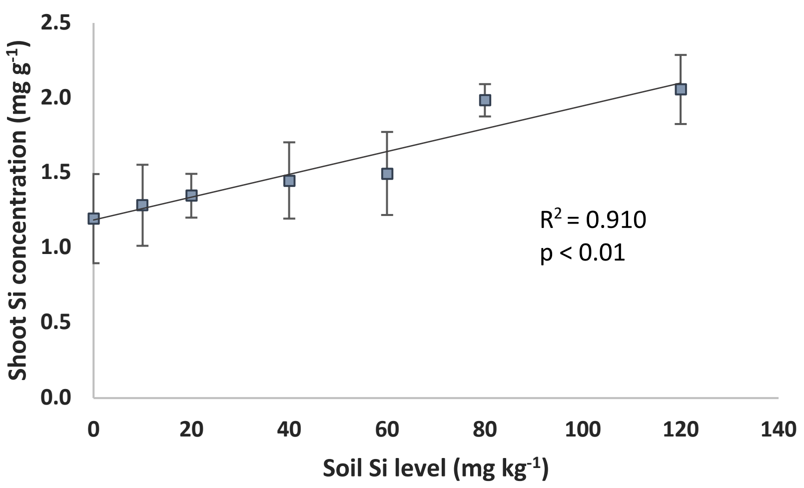


Figure 14: Linear regression model for C group, Shoot Si concentration; Mean values with trendline. Error bars indicate standard deviation, n=4

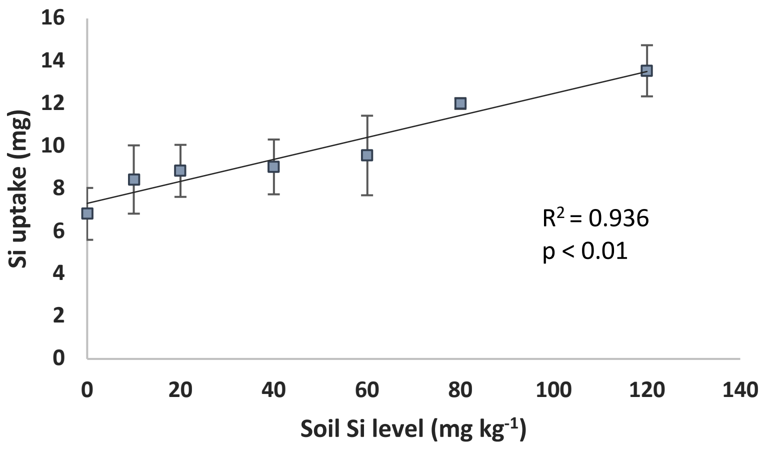


Figure 15: Linear regression model for C group, Si uptake; Mean values with trendline. Error bars indicate standard deviation, n=4

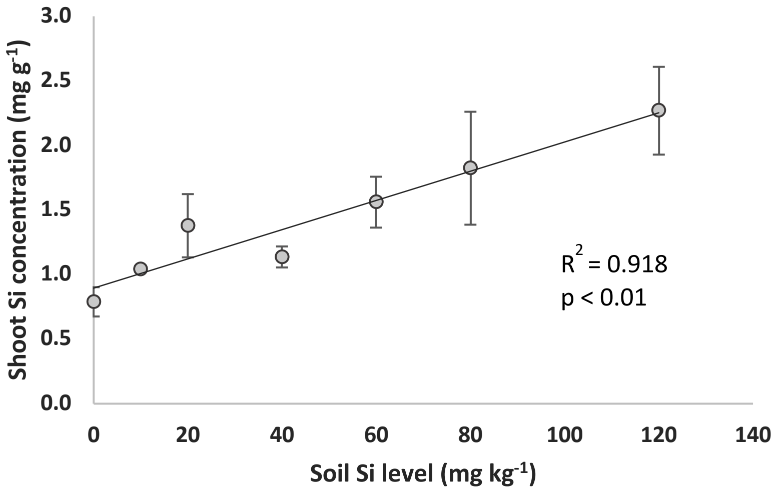


Figure 16: Linear regression model for Dr group, Shoot Si concentration; Mean values with trendline. Error bars indicate standard deviation, n=4

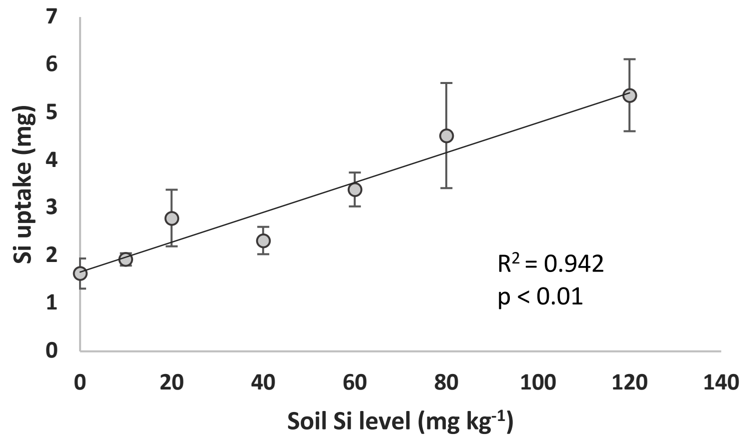


Figure 17: Linear regression model for Dr group, Shoot Si uptake; Mean values with trendline. Error bars indicate standard deviation, n=4

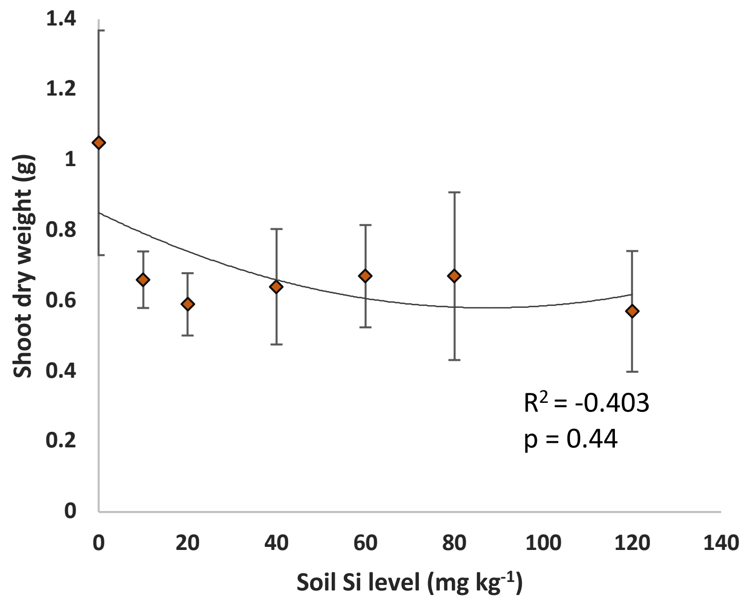


Figure 18: Quadratic regression model for Cu group, Shoot dry weight; Mean values with trendline. Error bars indicate standard deviation, n=4

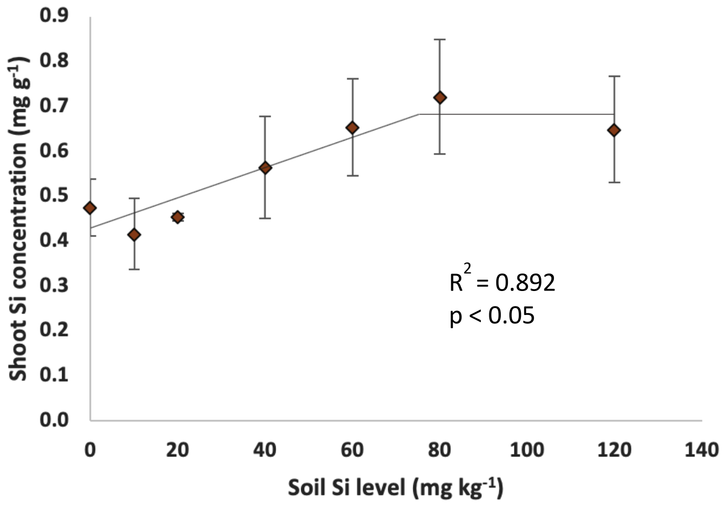


Figure 19: Linear-plateau model for Cu group, Shoot Si concentration; Mean values with trendline. Error bars indicate standard deviation, n=4. suggesting an optimal level between soil Si level 60 and 80 mg kg^{-1}.

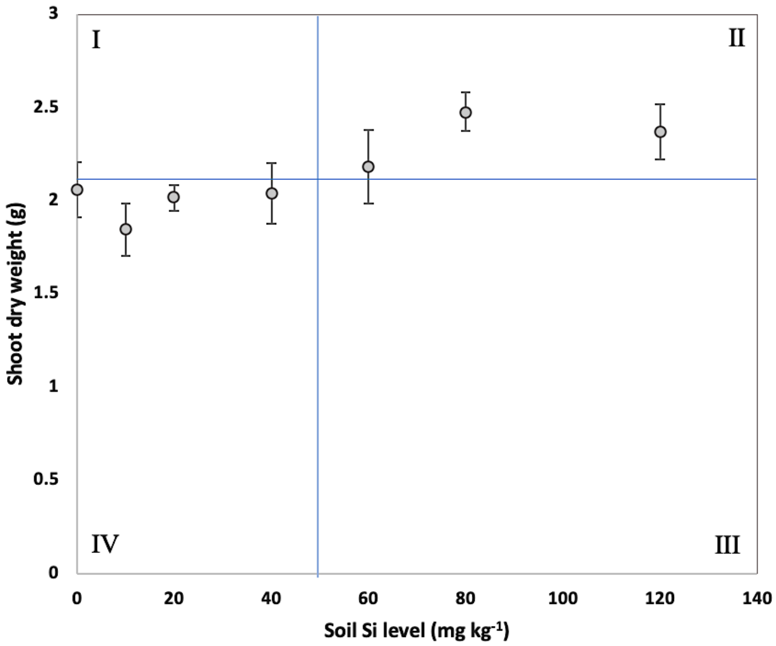


Figure 20: Cate-Nelson model for Dr group, shoot dry weight; Mean values and x-critical level and y-critical level. Error bars indicate standard deviation, n=4, Suggesting a critical level between soil Si level 40 and 60 mg kg^{-1}.

5.2.1 Critical level under stress-free conditions

Shoot biomass did not seem to be affected by different levels of Si (Table 18), which contrasts papers reporting effects of Si even under no-stress conditions: In a pot experiment with wheat Gong et al. (2003) observed greater plant height, leaf area, and dry matter in pots with Si compared to those without Si under well-watered conditions. Hattori et al. (2009) observed a difference between Si-supplied and non-Si-supplied rye plants at well-watered and dry conditions. The total dry matter of shoot and root was higher in the wet treatment than in the dry treatment, but there was also a significant ($p < 0.05$) increase due to Si application in both water regimes. Other authors stress the point that the effects of Si are becoming noticeable only, or especially under stressed conditions (Coskun et al. 2018; Ma, 2004), which would be conform with data from this experiment.

The strong correlation between soil Si and shoot Si concentration and uptake, as well as root Si concentration (Table 12, 14, 18) agrees with Hypothesis H1b and patterns observed by Pereira de Melo et al. (2003), who found that Si application to Brachiaria grasses (*Brachiaria sp.*, Poaceae) increased the Si concentration in the plants but did not affect dry matter yield in a water regime of 60% FC. Faria (2000) observed for rice, that effects of Si upon grain yield were greater under a higher water stress, but that even under well-watered conditions Si concentration in rice increased with Si application.

5.2.2 Drought stress

In the drought group there was a clear positive correlation between shoot biomass and soil Si level, in accordance with Hypothesis H1a and several studies. Gong et al. (2003) report from a pot experiment with wheat that several plant features were ameliorated by Si amendments under drought stress. Among them are higher relative water content in the plants, water potential and leaf area, and slightly higher root weight. Moreover, the plant dry matter of Si-supplied plants under drought stress was not significantly different to the control, while in plants without Si amendment growth of the shoots was strongly inhibited. Water deficit stress was applied by refraining already 26-day old seedlings from watering for 12 days. Janislampi (2012) too observed a Si-induced increase in biomass under drought conditions for corn, wheat, soybean and rice.

The Cate-Nelson model suggests a critical level between 24.5 and 29.6 mg kg^{-1} $CaCl_2$-extractable Si. However, biomass was still increasing at higher Si levels and also the quadratic regression model of shoot biomass had a fit with $R^2 = 0.720$, insinuating that the critical level might be higher. Shoot Si uptake and Si concentration showed similar patterns to the C group, with strong linear correlations ($R^2 = 0.942$, $R^2 = 0.918$; $p < 0.01$; Table 18), supporting Hypothesis H1b. This is valid for an EC level of 35.5 which was applied in this study. The effects of Si fertilization might be different at the EC50 level.

Si significantly ($p < 0.05$) raised the LWP in the Dr group and at the same rate as in the C group (Table 7), supporting hypothesis H1d. Similar patterns were found by Hattori et al. (2009), who compared rye growth at two different water regimes, and held a set of samples without Si amendment against a set with Si amendment. Leaf water potential was significantly ($p < 0.05$) lower in the dry setup and Si amendment significantly ($p < 0.05$) increased leaf water potential in both water regimes. Furthermore, Hattori et al. (2009) observed, that Si increased water use in both water regimes, and did not affect water use efficiency. These parameters however, were not investigated in this thesis. It is suggested that Si amendment helps in water uptake and to maintain leaf water potential. With this mechanism stomatal closure can be delayed, resulting in a higher dry matter yield (Hattori et al., 2009).

The shoot:root ratio in the Dr group at level 1 and 7 was lower than in group C, however only the difference at level 1 was significant ($p < 0.05$), hypothesis H1c cannot be supported. Hattori et al. (2009) observed, that Si amendments did not affect the shoot:root ratio under the well-watered condition, while they lowered the ratio under drought stress. This stands in contrast to results given in Table 9. Again, however it has to be stressed, that the values were not significantly different ($p > 0.05$).

Drought stress was apparent in root growth of Dr groups. Root parameters showed lower values compared to the control in root length, surface area, root volume and average diameter.

Drought caused inhibition in root development, however the aspect of the whole root systems was rather similar to the control, and mainly only smaller.

In Figure 13 it becomes visible that C and Dr groups showed similar patterns in the diameter class distribution, while Cu group showed a very different distribution compared to Dr and C groups for most parameters.

5.2.3 Copper stress

Hypothesis H1a cannot be supported by results from the Cu group and a critical level for copper stress of EC level 18.1 cannot be established. Biomass of the Cu group showed a weak negative ($R^2 = -0.403$) and not significant ($p = 0.44$) correlation with increasing levels of Si (Figure 18). This contradicts findings by Nowakowski & Nowakowsaka (1997), who report that Si amendments to seven-day old seedlings of wheat reduced toxic effects of copper (217 mg kg^{-1}) on biomass production and led to a higher water content in shoots and roots. Moreover, Si was found to reduce absorption of Cu from the soil solution.

The difference in the findings could be a consequence of the high stress level added Cu induced. It is possible, that freshly added Cu led to a larger fraction of plant-available and mobile Cu compared to contaminated soils, where the total Cu concentration might be the same, but, over time, was subjected to soil processes, e.g. complexing, immobilization, and therefore results in a lower stress level for plants. Such was found by Pump et al. (2019). Biochar-based amendments to Cu contaminated soils were able to immobilize Cu and reduce the fraction of extractable Cu. This effect increased, the more time passed.

The shoot:root ratio in the Cu group is higher than in C and Dr group, owing to a very poor root development, which matches with Nowakowski & Nowakowsakas (1997) findings that Cu toxicity was evident especially in the plant roots rather than in shoots. The ratio decreased from 13.94 at level 1 to 10.48 at level 7, however not significantly ($p = 0.712$; Table 9, 10), hypothesis H1c can therefore not be supported. Root parameters showed lower values compared to C and Dr group, in root length, surface area and root volume (Table 15). What might seem surprising at first is a rather large root diameter in the copper group which was similar to the control. However, looking at chapter 4.2.7 it is noticeable that the Cu group had a very different appearance of its root system compared to C and Dr group (see also Annex Figures 22, 23, 24). As a whole, the root development was very poor and limited to the upper soil layer and especially fine roots were poorly developed, therefore leading to a larger mean diameter. In the Cu group, roots with a larger diameter often accounted for a major share of the root parameter (Figure 13).

Shoot Si concentration showed a good fit for a linear plateau model suggesting an optimal level between soil Si level 60 and 80 mg kg^{-1} (Figure 19; $R^2 = 0.892$, $p < 0.05$), supporting Hypothesis H1b.

6 Conclusion

Extractable Si decreased over time, indicating, that more Si was in a plant-available/labile state shortly after Si amendments with wollastonite, suggesting that sowing should not happen too long after fertilization. However, the greenhouse study does not represent a farming situation and further studies are necessary.

Silicon amendments did not result in a higher biomass in the control group. In the drought group, however, a good positive correlation between biomass and soil Si level was found, indicating, that Si fertilization states a promising tool also in Austrian agricultural systems, to obtain better yields in case of drought during growth period. As the critical level is linked with soil properties and also differs for each plant species, further research could examine other Austrian and European soils, as Cambisols, Chernozems and Phaeozems, and crops that are typically used in agriculture.

Drought stress in this study was quite severe. The critical level suggested should be tested also at the EC50 level. An interesting aspect would also be to vary the beginning of the drought stress. In the control group Si uptake was higher than in stressed groups. Given drought started, e.g. four weeks after sowing, the higher Si concentration already present in plants might account for better resistance and higher yields (Gong et al., 2003).

The amount of copper added to induce heavy metal stress was above the targeted EC50 level. Stress became visible in root and shoot appearance and in growth inhibition. It is probable that Cu-toxicity outweighed effects of Si. In further experiments a lower level should be chosen in order to get more precise information about the critical level. Also, it can be considered to extend the incubation time of the metal or use historically contaminated soil.

Due to restricted disposable time the experiment was conducted in the short period of seven weeks, which is much shorter than rye would need to build up grains. As this is an essential factor for food production, studies over a longer period would be desirable.

The north-eastern part of Austria is influenced by pannonic climate and therefore already subject to drought periods, which could increase in number, intensity or length with proceeding climate change. As recent studies by Cocuzza (2017), Schiefer (2019) and Reiter (2019) show, many arable soils in Austria are possibly Si deficient. According to this thesis' findings soils and crop yields can, especially under drought conditions, benefit from Si amendments.

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9 Annex

9.1 Data tables

Table 19: Available silicon from two different fertilizers ($CaSiO_3$ and SiO_2) after one week of incubation. (n=1)

Si via $CaSiO_3$ (mg kg^{-1})	Added $CaSiO_3$ (mg kg^{-1})	Available Si (mg kg^{-1})
0	0	14.1
50	208.7	29.6
100	417.5	43.8
150	626.2	53.9
200	835.0	65.6
250	1043.7	71.0
300	1252.5	82.3

Si via SiO_2 (mg kg^{-1})	Added SiO_2 (mg kg^{-1})	Available Si (mg kg^{-1})
0	0	13.9
50	109.2	15.7
100	218.3	17.0
150	327.5	13.9
200	436.6	14.6
250	545.8	14.6
300	654.9	16.7

Table 20: $CaCl_2$-extractable Si after one week (n=1) and after seven weeks (mean value, n=2)

Si added to soil (mg kg^{-1})	$CaCl_2$-extractable Si in soil week 1 (mg kg^{-1})	$CaCl_2$-extractable Si in soil week 7 (mg kg^{-1})
0	14.30	13.81
10	16.85	14.55
20	19.39	14.62
40	24.48	17.12
60	29.57	17.19
80	34.66	17.93
120	44.83	21.79

Table 21: Leaf water potential of C and Dr group. Mean value and standard deviation, n=6, except **: n=7

Leaf water potential, Control group			
	Mean value		
Soil Si level (mg kg^{-1})	(bar)	(MPa)	SD (MPa)
0	12.7	-1.27	0.20
10	11.3	-1.13	0.12
20	12.1^{**}	-1.21^{**}	0.11
40	12.1	-1.21	0.07
60	11.5	-1.15	0.09
80	10.9	-1.09	0.11
120	9.7^{**}	-0.97^{**}	0.10

Leaf water potential, Drought group			
	Mean value		
Soil Si level (mg kg^{-1})	(bar)	(MPa)	SD (MPa)
0	24.2^{**}	-2.42^{**}	0.09
10	23.6	-2.36	0.09
20	22.8^{**}	-2.28^{**}	0.17
40	23.2	-2.32	0.08
60	22.9	-2.29	0.05
80	20.9	-2.09	0.07
120	21.1^{**}	-2.11^{**}	0.11

Table 22: Mean dry weight of shoot and standard deviation, n=4

Soil Si level (mg kg^{-1})	C mean dry weight (g)	Dr mean dry weight (g)	Cu mean dry weight (g)	C SD (g)	Dr SD (g)	Cu SD (g)
0	5.80	2.06	1.049	0.86	0.15	0.32
10	6.60	1.85	0.66	0.46	0.14	0.08
20	6.50	2.02	0.59	0.23	0.07	0.09
40	6.30	2.04	0.64	0.45	0.16	0.16
60	6.40	2.18	0.67	0.11	0.20	0.15
80	6.10	2.48	0.67	0.34	0.10	0.24
120	6.60	2.37	0.57	0.35	0.15	0.17

Table 23: Mean shoot Si concentration in all three groups and standard deviation. (n=4)

Soil Si level (mg kg^{-1})	C Si concentration (mg g^{-1})	Dr Si concentration (mg g^{-1})	Cu Si concentration (mg g^{-1})	C SD (mg g^{-1})	Dr SD (mg g^{-1})	Cu SD (mg g^{-1})
0	1.20	0.79	0.48	0.30	0.11	0.06
10	1.28	1.04	0.42	0.27	0.02	0.08
20	1.35	1.38	0.45	0.15	0.24	0.01
40	1.45	1.14	0.56	0.25	0.08	0.11
60	1.50	1.56	0.65	0.28	0.20	0.11
80	1.98	1.82	0.72	0.11	0.44	0.13
120	2.06	2.27	0.65	0.23	0.34	0.12

Table 24: Mean Si uptake (mg) in all three groups and standard deviation. (n=4)

Soil Si level (mg kg^{-1})	C uptake (mg)	Dr uptake (mg)	Cu uptake (mg)	C SD (mg)	Dr SD (mg)	Cu SD (mg)
0	6.82	1.63	0.49	1.22	0.32	0.13
10	8.43	1.92	0.28	1.60	0.13	0.09
20	8.84	2.79	0.27	1.22	0.59	0.04
40	9.02	2.32	0.37	1.29	0.29	0.15
60	9.56	3.39	0.44	1.87	0.35	0.12
80	12.00	4.52	0.50	0.25	1.10	0.27
120	13.54	5.36	0.38	1.20	0.75	0.17

9.2 Shoots and roots appearance

9.2.1 Root appearance

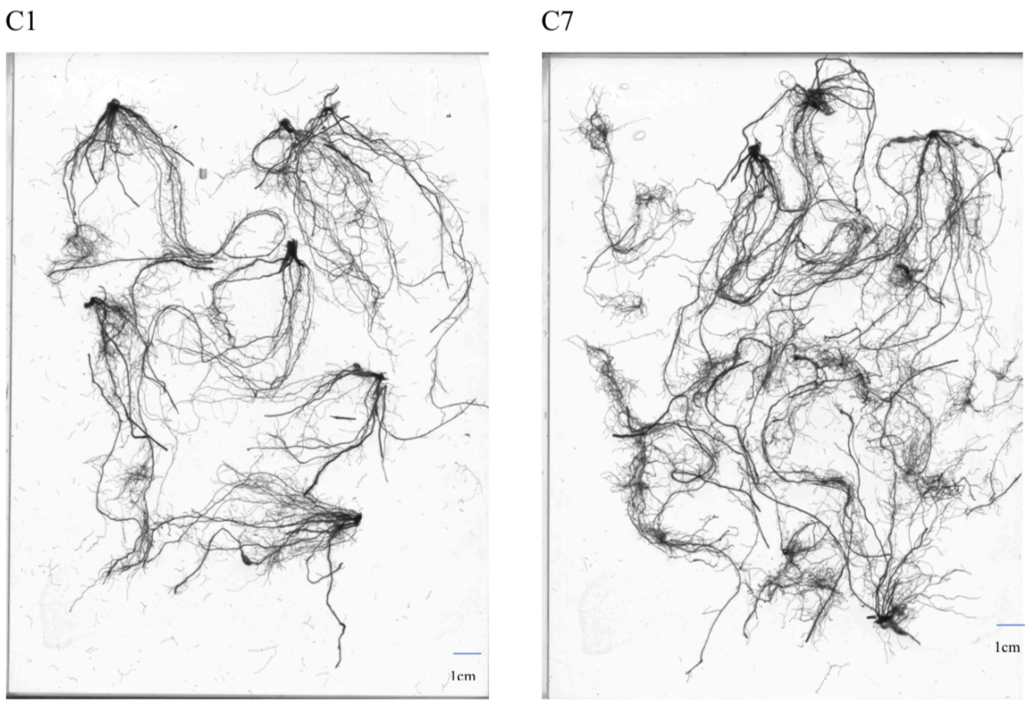

Figure 21: Control group root appearance after seven weeks. Pictures were obtained with a root scanner (Epson perfection V700 PHOTO) after staining. A visually representative part of the whole root mass is shown.

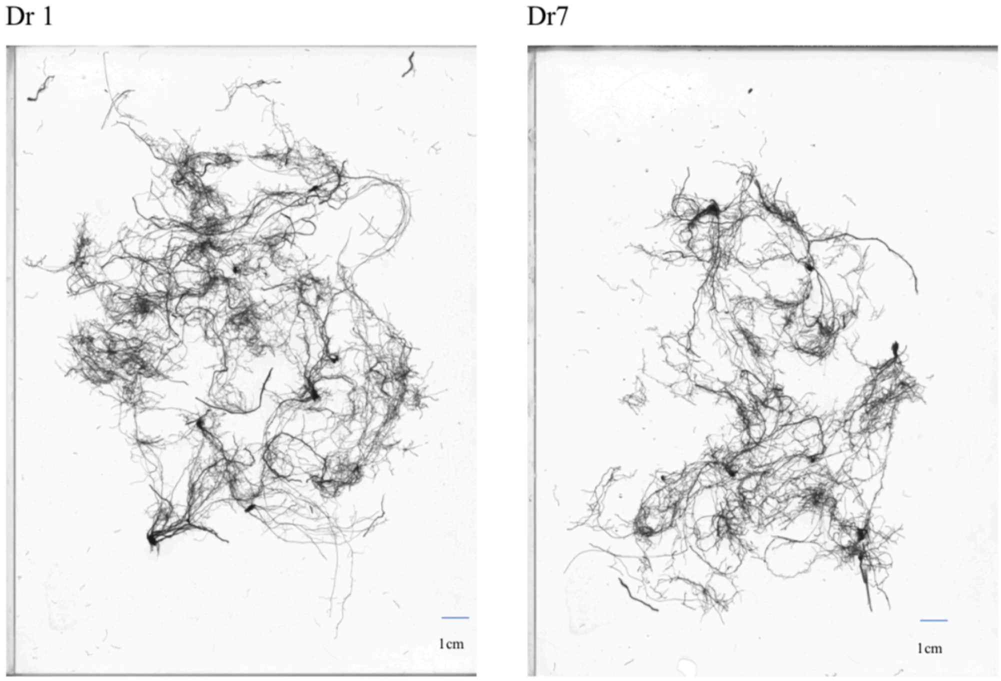

Figure 22: Drought group root appearance after seven weeks. Pictures were obtained with a root scanner (Epson perfection V700 PHOTO) after staining. A visually representative part of the whole root mass is shown.

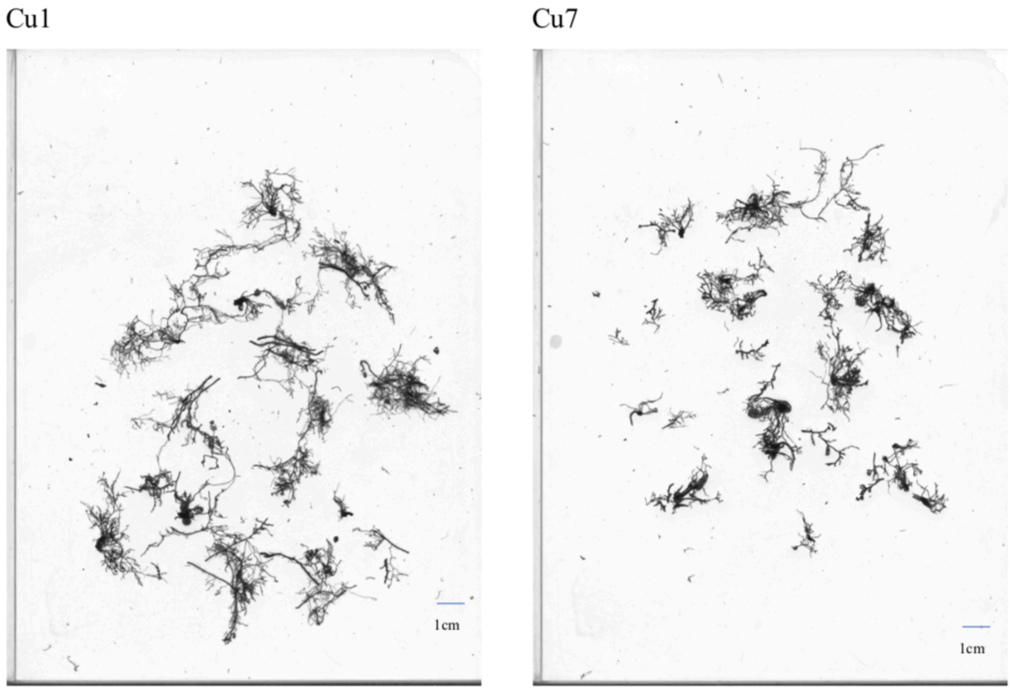

Figure 23: Copper group root appearance after seven weeks. Pictures were obtained with a root scanner (Epson perfection V700 PHOTO) after staining. A visually representative part of the whole root mass is shown.

9.2.2 Shoot appearance

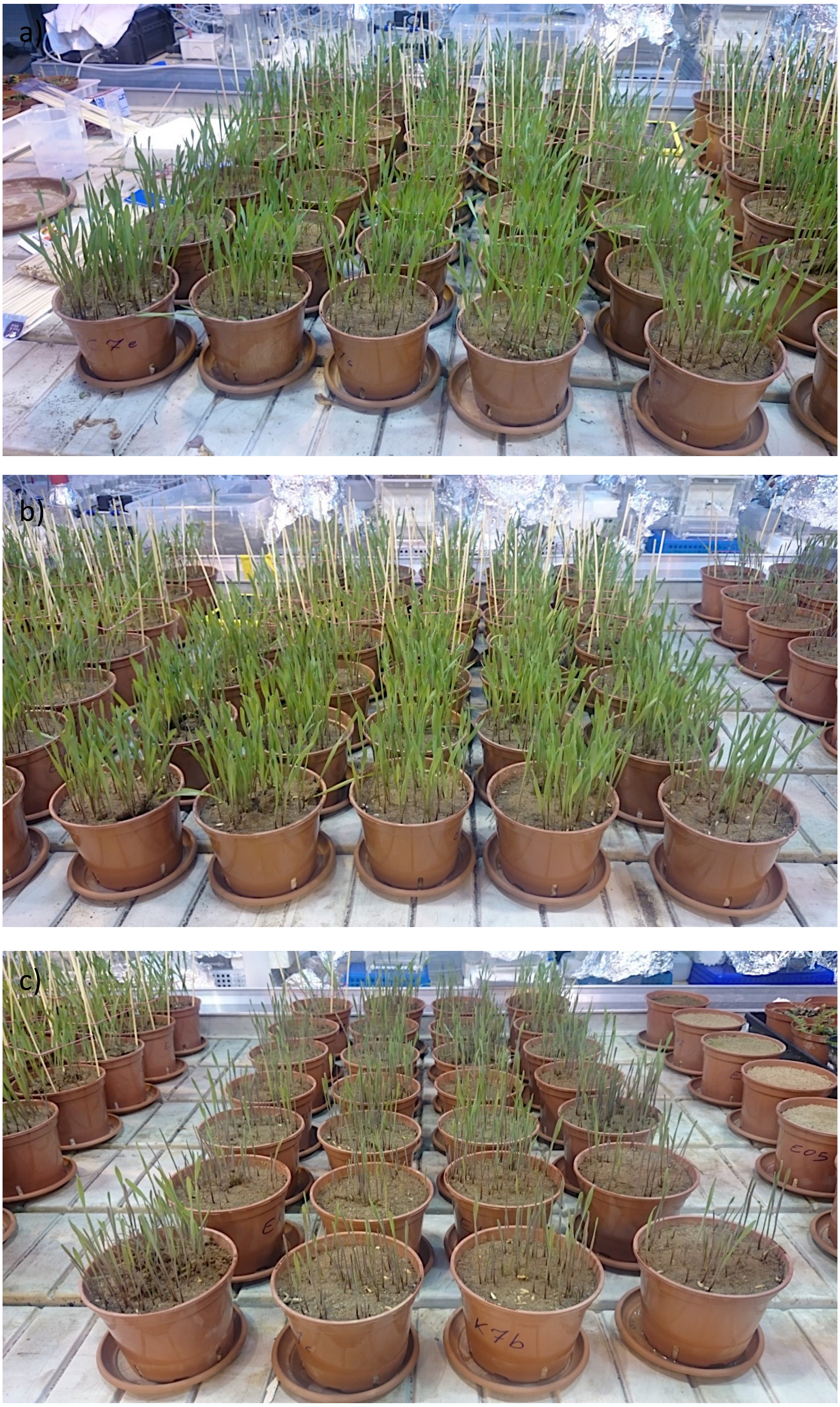

Figure 24: Plants in the greenhouse (one week after sowing) a) control group b) drought group c) copper group