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Effect of different agromining treatments on soil quality

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STATUTORY DECLARATION

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ABSTRACT

Serpentine soils are naturally enriched in nickel, chromium and cobalt, but deficient in essential nutrients. Furthermore, they show a low Ca:Mg ratio, organic matter content and water holding capacity. These soil properties make them unfavourable for conventional agriculture but open opportunities for new technologies. Agromining comprises the use of hyperaccumulators in combination with agronomic practices on metalliferous soils, to increase metal extraction while improving soil quality. The harvested biomass is further processed for metal recovery. A field experiment was set up on a serpentine site in Austria, to evaluate the effect of agromining on Ni-availability and soil quality, under different amendments. The Ni-hyperaccumulator *Odontarrhena chalcidica* (syn. *Alyssum murale*) was planted in six different treatments: i) control, ii) mineral fertilizer (NPK), iii) cow manure, iv) pig manure, v) compost and vi) low distance plantation (30 cm, others 50 cm). Soil samples were taken before and directly after fertilization, as well as after harvest. To assess Ni-availability, labile $Sr(NO_3)^2$-Ni and extractable DTPA-Ni pools were analyzed. Soil quality was evaluated by using physicochemical (e.g. nutrient availability and -contents) and biological parameters, including mesofauna abundance and a biological quality index (QBS-index). The DTPA-extractable Ni pool significantly declined within one vegetation period. The application of mineral fertilizer decreased enzyme activity, while organic amendments improved soil physicochemical properties and biological activity. Manure treatments increased abundance of Collembola and Acari, while biological activity was not increased in organic matter despite containing highest physicochemical soil quality. Pig manure significantly increased to a QBS-index of high quality soils. We observed no negative impact of Ni-agromining on soil organisms and suggest that application of animal manure potentially improves soil quality.

Key words: serpentine, hyperaccumulation, Ni-availability, soil mesofauna, *Odontarrhena chalcidica*, soil quality indicators

ZUSAMMENFASSUNG

Serpentinböden kennzeichnet ein hoher Anteil an Magnesium und Schwermetallen (Nickel, Chrom und Kobalt), während essenzielle Nährstoffe, organischer Kohlenstoff und Wasser unzureichend vorhanden sind. Diese Böden eignen sich für den Anbau von Hyperakkumulatoren, Pflanzen, welche hohe Mengen an Metallen in ihren oberirdischen Organen anreichern können. Agromining, der Anbau von Hyperakkumulatoren unterstützt durch ausgewählte landwirtschaftliche Praktiken, kann die Metallaufnahme der Pflanze erhöhen, um schließlich Metalle aus der geernteten und verbrannten Biomasse rückzugewinnen. Um den Einfluss von Agromining auf die Nickelverfügbarkeit und Bodenqualität in Österreich zu testen, wurde ein Feldversuch auf einem Serpentinstandort durchgeführt. Die Nickel-hyperakkumulierende Pflanze *Odontarrhena chalcidica* (syn. *Alyssum murale*) wurde in Behandlungen mit 50 cm Pflanzenabstand: i) Kontrolle, ii) mineralischer Dünger, iii) Rindermist, iv) Schweinemist, v) Kompost und einer ungedüngten Behandlung mit höherer Bepflanzungsdichte (30 cm), untersucht. Die Bodenproben wurden vor und nach der Düngung, sowie nach der Ernte entnommen. Die Nickelverfügbarkeit wurde mittels im Boden elektrostatisch gebundenen $Sr(NO_3)^2$-Nickel und komplexierten DTPA-Nickel Fraktionen untersucht. Für die Evaluierung der Bodenqualität wurde ein Set aus physikalisch-chemischen (Nährstoffkonzentrationen und -verfügbarkeiten) und biologischen Indikatoren (FDA-Enzymaktivität, biologischer Bodenqualitätsindex (QBS-index), Mesofauna-Abundanz), analysiert. Die Ergebnisse deuten auf einen signifikanten Rückgang von DTPA-Nickel innerhalb einer Vegetationsperiode hin, vermutlich aufgrund einer Aufnahme durch die Pflanze. Die Aufbringung von mineralischem Dünger verringerte die Enzymaktivität, während Rinder- und Schweinemist die Abundanz der Mesofauna signifikant erhöhten. Zudem führte die Behandlung mit Rindermist zu einem signifikanten Anstieg des Bodenqualitätsindexes.

Schlüsselwörter: Serpentinböden, Hyperakkumulation, Nickelverfügbarkeit, Bodenmesofauna, *Odontarrhena chalcidica*, Bodenqualitätsindikatoren

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ABBREVIATIONS

ANOVA	Analysis of variance
Bd	Bulk density
C:A	Ratio of Collembola to Acari
CEC	Cation Exchange Capacity
dw	Dry weight
excl. S	Exclusive sulphur plots
EMI	Ecological-Morphological Index
h	Hours
ICP-MS	Inductively Coupled Plasma – Mass Spectrometry
ICP-OES	Inductively Coupled Plasma – Optical Emission Spectrometry
IEK	Isotope exchange kinetics techniques
incl. S	Inclusive sulphur plots
indiv.	Individuals
min	Minutes
MO	Microorganism
Nmin	Mineral nitrogen
rpm	Revolutions per minute
sign.	Significant(-ly)
SOC	Soil organic carbon
TC	Total Carbon
TN	Total Nitrogen
WC	Water content
wt (%)	Weight per cent
DTPA	Diethylene triamine pentaacetic acid
QBS	Qualità Biologica del Suolo (Soil biological quality)

1. INTRODUCTION

Ultramafic soils are naturally enriched in heavy metals and derive from the weathering of ultramafic bedrock. Ultramafic rocks are a common constituent of the Earth's upper mantle (Alexander and DuShey, 2011), but are only covering a very small part of the terrestrial surface worldwide (< 1%) (Brooks, 1987). The world's largest ultramafic outcrops occur in temperate (e.g. Balkans, Turkey, California) and in tropical climates (e.g. New Caledonia, Cuba, Brazil, Malaysia, Indonesia) (Ent et al., 2018), where they often coincide with ophiolite belts along tectonic plate margins (Echevarria, 2018).

Ultramafic rocks (peridotites) contain > 70% mafic minerals and have a particularly high concentration of magnesium (Mg) and iron (Fe) (= ferromagnesian), but a low silica (Si) content (Coleman, 1971; Alexander and DuShey, 2011). The main minerals in ultramafic rocks are olivine, clinopyroxene, orthopyroxene, amphibole, biotite and serpentine. Most ultramafic rocks contain a mix of mafic minerals, such as peridotite which consists mainly of olivine and pyroxene with a small proportion of chromite (Brooks, 1987; Alexaner and DuShey, 2011). Peridotites are igneous ultramafic rocks that derive from ophiolites, "out-of-place" fragments of the oceanic crust. The ratio of olivine to clino- and ortho-pyroxene determines the type of peridotite. The most common peridotites are harzburgite, lherzolite, dunite and pyroxenite (Echevarria, 2018). The last two are monomineralic ultramafic rocks. Thus, they are pure in one ultramafic mineral, such as dunite (olivine), pyroxenite (pyroxene), hornblendite (amphibole) and biotite (biotite) (Brooks, 1987). Peridotites can alter to serpentinites, due to a metamorphic process, called serpentinization (Alexander and DuShey, 2011). Serpentinization usually occurs at the sea floor along tectonic boundaries (e.g. near mid-ocean ridges) or during continental emplacement (Ent et al., 2018). During serpentinization olivines and pyroxenes, the primary minerals in peridotites, are hydratised (addition of 13-14 % of water) under pressure and low temperature (< 500°C) to serpentine clay minerals and magnetite. Besides that, also chlorite, talc and brucite are formed, as it is described in Eq. 1.1 (Alexander and DuShey, 2011; Echevarria, 2018; Pędziwiatr et al., 2018).

$$\underset{\text{Olivine (Forsterite)}}{Mg_{1.82}Fe_{0.18}SiO_4} + wH_2O \rightarrow \underset{\text{Serpentine}}{0.5(Mg,Fe^{II},Fe^{III})_3(Si,Fe^{III})_2O_5(OH)_4} + \underset{\text{Brucite}}{x(Mg,Fe)(OH)_2} + \underset{\text{Magnetite}}{yFe_3O_4} + zH_2$$

Eq. 1.1: *Weathering of olivine to serpentine, brucite and magnetite (Echevarria, 2018 cited in McCollom et al., 2016).*

Hence, rocks deriving from this process are rich in serpentine and magnetite and are called serpentinites. Serpentinite refers to rocks consisting > 50% out of serpentine minerals, obscuring the original and not metamorphosed mineralogy of the primary minerals (Alexander and DuShey, 2011). Serpentine is 1:1 clay mineral and occurs in three different types, namely chrysotile (form of asbestos), lizardite and antigorite (Echevarria, 2018).

1.1 Serpentine vs. Ultramafic

The term serpentine is often used interchangeably for all ultramafic rocks, such as peridotite and serpentinite, which is not correct. Similarly, also soils deriving from these rocks are mostly called serpentine soils, due to the unique plant community occurring on both substrates (Brooks, 1987; Alexander and DuShey, 2011). While this generalization might be appropriate for botanists, in geology a clear distinction between the terms: peridotite, serpentinite, serpentine and ultramafic, is needed (Alexander and DuShey, 2011; Ent et al., 2018).

Even though serpentinite and peridotite are chemically very similar (except for lower concentrations of Ca and Si in serpentinite due to leaching during serpentinization), they differ substantially in their mineralogical and structural composition (Fig. 1.1). First, during serpentinization olivine and pyroxene are altered to serpentine and magnetite, while chromite remains unaltered (Alexander and DuShey, 2011). Thus, also the optical appearance changes. Peridotites are dark black with oxidised weathering sheath (due to high amount of Fe-oxides), while the mix of green serpentine, chlorite, white talc and other clay minerals resembles kind of a snake skin and gives serpentinites ("serpens" latin for snake) their name (Echevarria, 2018). Second, serpentinization is accompanied by a vol. expansion (33 %). The bulk density (Bd) decreases from (3.2-3.3 Mg m^{-3}) in peridotite, to (2.4-2.6 Mg m^{-3}) in serpentinite. Besides that, the extension also triggers structural differences. First, expansion causes fracturing, which results in serpentinite commonly breaking into smaller blocks than peridotite. Second, serpentinite is more sensitive to mass failure, due to a reduced shear strength caused by smoother and polished surfaces (Alexander and DuShey, 2011).

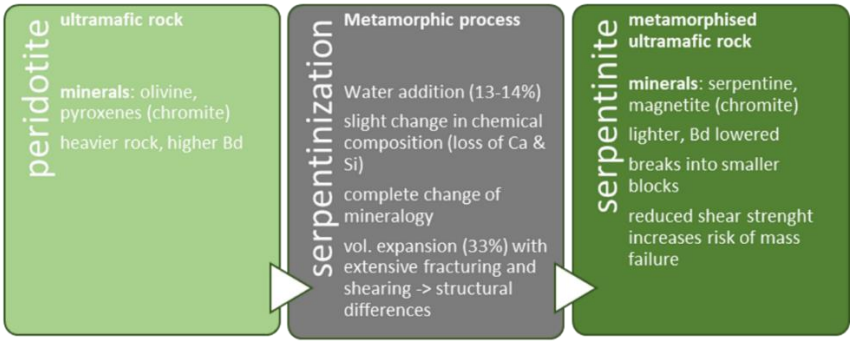

Fig. 1.1: *Differences between peridotite and serpentinite due to serpentinization.*

The term "ultramafic" refers to rocks, which are rich in ferromagnesian (mafic) minerals and have a low Si content (Brooks, 1987). However, Brooks (1987) outlines that the term "serpentine" should only be used for minerals with the general formula $Mg_3Si_2O_5(OH)_4$ and for minerals as well as soils deriving from serpentinized ultramafic rocks. Thus, all serpentine soils derive from ultramafic substrate, but not all ultramafic soils contain serpentine minerals. This clarification is crucial, as the degree of serpentinization controls soil chemistry (Echevarria, 2018). Table 1.1 gives again a short overview of the different terms.

Table 1.1*: Overview of different terms after Brooks (1987, p. 8) and modified after Alexander and DuShey (2011), Pędziwiatr et al., (2018).*

Term	Rock and mineral description
Ultramafic rocks	Rocks containing > 70% ferromagnesian (mafic) minerals, particularly high concentrations of magnesium (Mg) and iron (Fe)
Peridotite	Ultramafic rock, rich in minerals olivine and pyroxene, but without feldspar (low Si content). Common types: harzburgite, Iherzolite, dunite and pyroxenite
Serpentinite	Rocks deriving from serpentinization of peridodite, rich in serpentine minerals.
Serpentine	1:1 clay mineral, occurs in 3 different mineral forms: antigorite, chrysotile or lizardite, which derive from the hydratisation of olivines and pyroxenes

1.1.1 Pedogenesis

Due to the mineralogical and structural differences of ultramafic and serpentinite bedrock, the soils deriving from weathering of these substrates possess different properties (Brooks, 1987). Also, Alexander and DuShey (2011) postulated that the nature of the parent ultramafic rocks substantially influences pedogenesis and soil characteristics, besides climate, topography and vegetation (Echevarria, 2018). Thus, soils on peridotite or serpentinite usually reach a limited development stage, independent of latitude or elevation. The weathering state is primarily

determined by the minerology of the bedrock (Echevarria, 2018). The dissolution of serpentine minerals and talc in serpentinite, is a rather slow process compared to the dissolution of olivines or pyroxenes dominating peridotite (van der Ent et al. 2018). This can be partially explained by the Goldich scheme. Goldich (1938, cited in Brooks, 1987) proposed that minerals precipitating first during magma differentiation (e.g. olivine), are the least resistant to weathering. Primary minerals of igneous rocks weather in following order from least to most resistant: olivine → augite → hornblende → biotite → potash feldspar → muscovite → quartz (Brooks, 1987). Thus, peridotite (rich in olivine) weathers faster than serpentinite. During weathering, these primary minerals are formed into secondary minerals, which is also shown in fig. 1.2. Olivines (e.g. in peridotite) first transform to amorphous Fe-oxyhydroxides under a loss of Si and Mg, but also weather to clay minerals (e.g. kaolinite). In contrast, serpentine minerals transform into secondary 2:1 phyllosilicates. This neo-formation of 2:1 clay minerals during weathering, is a crucial difference between serpentinized and non serpentinized peridotites (Echevarria, 2018).

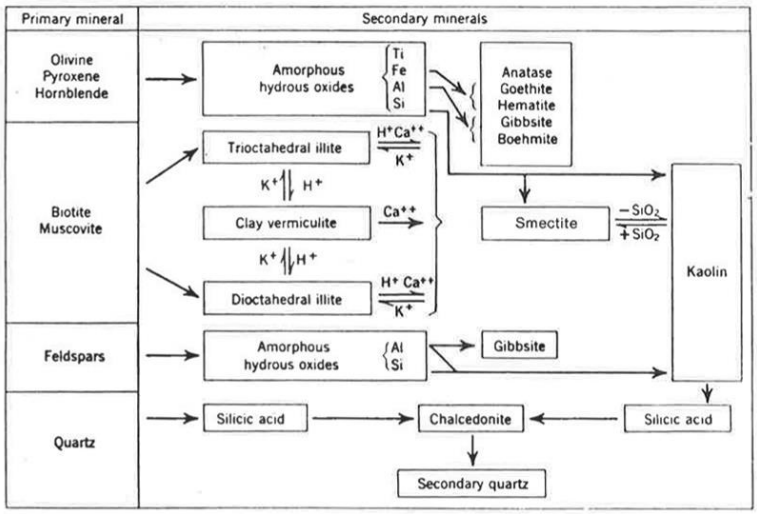

Fig. 1.2: *Weathering of primary to secondary minerals (Brooks, 1987, p. 19).*

Azonal serpentine soils in temperate climates contain primary "serpentine" minerals (chrysotile, antigorite, lizardite) and secondary phyllosilicates (smectite, magnetite, chlorite, talc) (Chardot et al., 2007). During soil formation, magnesium is partially leached due to incomplete hydrolysis, which results in formation of 2:1 clay minerals, such as vermiculite (e.g. from Mg-rich chlorite) and Fe-rich smectite (e.g. from serpentine minerals). Furthermore, Fe is released during weathering. The free Fe causes a usually higher chroma of serpentine soils in comparison to surrounding soils on non-ultramafic bedrock, but lower than soils on non-serpentinized peridotite. Intense weathering in the tropics causes total loss of Mg and Si and results in an

accumulation of free Fe oxides (Chardot et al., 2007; Echevarria, 2018). Furthermore, soils on serpentinite contain less Ca than on peridotite, as Ca is partially lost during serpentinization (Alexander and DuShey, 2011). Thus, common soil types on serpentinite in temperate climates are high pH Regosols or Leptosols with cambic properties and a cation exchange complex (CEC) dominated by Mg over Ca, as well as neutral to slightly acidic Cambisols (Chardot et al., 2007; Echevarria, 2018).

1.1.2 Properties of serpentine soils

Soils on serpentinite are often shallow with a loamy (skeletal) structure. The coarse skeleton content, a shallow soil profile and low content of organic matter cause a low water holding capacity (WHC) and restriction of root development (Brooks, 1987). Hence, plants growing on these soils often suffer from drought. Besides that, the low water content results in high temperature fluctuations, which are typical for serpentine soils (Michalek et al., 2015). Therefore, the low soil moisture content might be another reason for the scarce vegetation on serpentine soils, besides their chemical peculiarities (Brooks, 1987). Serpentine soils show elevated concentrations of Mg (18-24%) and Fe (6-9%) and a low Ca:Mg ratio (< 1) (Nkrumah et al., 2016). Usually, a high Mg content in the substrate antagonizes Ca uptake by plants and causes together with high concentrations of Ni and Cr, toxicity to most terrestrial plants (Adriano, 2001). Furthermore, serpentine soils are naturally deficient in macronutrients (nitrogen (N), phosphorous (P) and potassium (K)) and micronutrients (molybdenum (Mo), bor (B)). In contrast, they contain high levels of the siderophile elements nickel (Ni), chromium (Cr) and cobalt (Co) (Brooks, 1987; Alexander, 2004; Nkrumah et al., 2016; Kidd et al., 2018). The elevated concentrations of Ni, Cr and Co in ultramafic bedrock result from ionic substitution during weathering. Thus, the ionic radii of the divalent states Ni^{2+} (0.072 nm) and Co^{2+} (0.069 nm), are similar to that of Mg^{2+} (0.072 nm). Similarly, Cr^{3+} (0.064 nm) substitutes Fe^{3+} (0.067 nm) during serpentinization (Brooks, 1987). Thus, Ni concentration can rise to 1400 - 2000 mg kg^{-1} in ultramafic bedrock, while mean Ni abundance in the Earth`s crust is only around 20 mg kg^{-1} (Kabata-Pendias, 2010). Especially in humid tropical climates, ultramafic bedrock can weather to nickel laterite soils, which are a major target for the nickel and cobalt mining industry (Echevarria, 2018). Thus, ultramafic and serpentine soils are an important resource for minerals of Ni, Cr and Co, while they are unfavourable for traditional agriculture due to their infertility and low productivity (Echevarria, 2018; Kidd et al., 2018). Summarized

as the “serpentine factor”, serpentine soil properties represent a hostile environment for most plants. Hence, high rates of endemic plant species that adapted morphologically and physiologically to the soil conditions are usually found on serpentine substrate, which makes them also clearly distinguishable from the flora of surrounding areas (Brooks, 1987; Alexander and DuShey, 2011).

1.2 Plants on serpentine soils

Trace elements are essential nutrients (e.g. Mn, Ni, Zn), but become toxic at high concentrations for most plants. Hence, plant species possess mechanism to control metal homeostasis, determined by their individual nutrient demand as well as metal availability in soils (Merlot et al., 2018). Plants growing on metal rich substrate can exhibit three strategies to cope with high metal concentrations: i) exclusion, ii) indication and iii) accumulation (Baker, 1981). Excluders inhibit metal accumulation in photosynthetically active shoot tissues by: i) limitation of metal absorption by roots, ii) increased metal efflux from root tissues and iii) higher storage of metals in non-active root cell walls and vacuoles (Merlot et al., 2018). Second, indicators increase uptake and accumulation of elements to aerial plant parts as a response to elevated soil contents. Third, (hyper)- accumulators concentrate enormous amounts of metals in their aboveground biomass, independent of soil metal levels (Baker, 1981). In contrast to excluders, the metal homeostasis network in (hyper)- accumulators is altered to enable translocation of metals to shoot tissues for sequestration (Merlot et al. 2018). Furthermore, Merlot et al. (2018) suggested that hyperaccumulation requires:

i) increased mobilization, uptake and radial transport of metals in roots, towards root vascular tissues (with reduced sequestration in root vacuoles),
ii) enhanced metal translocation from roots to shoots through xylem, to enable metal storage in shoot tissues, and
iii) unloading of xylem to store metals in shoot vacuoles with high storage capacity.

The summary of these processes in roots and shoots enables detoxification via storage of metals in non-photosynthetic active parts (e.g. vacuoles) (Merlot et al., 2018).

1.2.1 Ni-hyperaccumulation

Plants growing on Ni-rich substrate evolved strategies to regulate homeostasis and hyperaccumulation of Ni. To transport Ni from roots to shoots, metal transporters and chelators are needed to bind Ni at different stages of transport and pH environments. Regarding the later, Ni can be complexed to organic acids, such as the carboxylic acids citrate and malate, to ensure binding in acidic environments (e.g. vacuoles and xylem). While, NA (nicotianamine) complexes Ni (NA - Ni) in compartments with neutral pH (e.g cytoplasm and phloem) (Merlot et al., 2018). Up to now it is not clear, which transporters are specifically involved in Ni-hyperaccumulation. Merlot et al. (2018) claimed that generally two types of transporter can be distinguished: i) divalent metal importers (e.g. ZIP/IRT, NRAMP) or transporters for complexed Ni phases (e.g. Yellow Stripe-Like (YSL) family). Hence, Merlot et al. (2018) proposed following strategy for Ni transport in hyperaccumulators, also visualized in fig. 1.3.

i) First, Ni is taken up from soil solution by metal transporters (ZIP/IRT, NRAMP), which are located at the plasma membrane of root epidermal cells.
ii) Then Ni is chelated (e.g. by nicotianamine to form [NA-Ni]-complex) to reduce its reactivity for the transport through cortex and endodermis and to inhibit vacuolar sequestration.
iii) Next, Ni and chelator molecules (e.g. NA, Citrate) are loaded in xylem, where the complexes (e.g. NA-Ni, Ni-Citrate) are translocated to the shoot by YSL transporters.
iv) Finally, Ni is unloaded in xylem and transported to the leaf epidermal cell, where Ni is eventually stored in the vacuole by iron transporters (IREG).

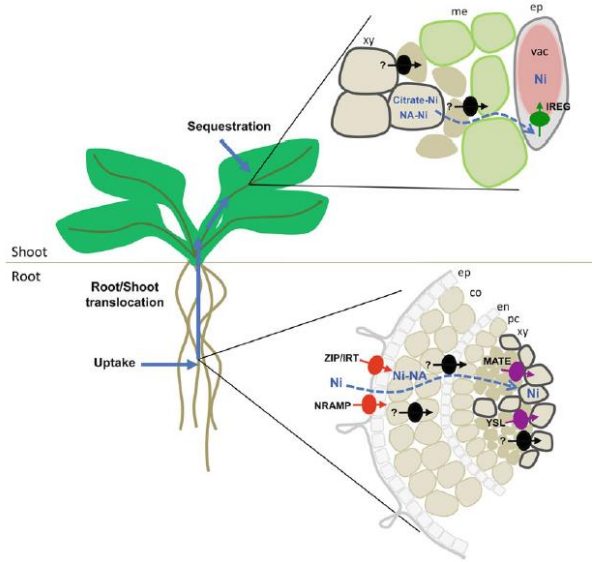

***Fig. 1.3**: Transport of nickel (Ni) in hyperaccumulators, proposed by Merlot et al. (2018, p. 102).*

1.2.2 Hyperaccumulators

Hyperaccumulation was first observed by Minguzzi and Vergnano (1948, cited in Brooks, 1987), who determined unusually high concentrations of Ni (> 1%) in dried leaves of the crucifer *Alyssum bertolonii*. The concentration of Ni in the biomass of this serpentine-endemic plant was about a factor 100 higher than ever been reported. In the following years, Ni-hyperaccumulation was found in various species of the genera *Odontarrhena* (family *Brassicaceae*), *Hybanthus* (family *Violaceae*) and *Homalium* (family *Salicaceae*). This motivated Brooks et al. (1977, cited in Brooks, 1987) to investigate species of the genera *Homalium* (240 taxa) and *Hybanthus* (150 taxa) from soils in tropical to warm-temperate climates around the world. Interestingly, the collection sites of the metalliferous plants corresponded to several of the world`s major serpentine reservoirs (Brooks, 1987). Thus, metalliferous plants are considered being a valuable geobotanical indicator for mineral deposits as well as anthropogenic contaminations, due to the fact that their occurrence is mostly linked to a specific type of soil or bedrock (Baker and Brooks, 1989). Furthermore, Ni concentrations of plants growing on ultramafic bedrock mostly exceed 1000 ppm, while on normal soils Ni levels in plant aerial parts seldomly rise beyond 5 ppm (Adriano, 2001).

In their study Brooks et al. (1977, cited in Brooks, 1987) used the terms “hyperaccumulators” and “nickel plants” to describe higher plants that are able to concentrate > 1000 µg Ni g^{-1} DW (0.1%) in their aerial tissues, and thus introduced the still valid definition for hyperaccumulators. However, this threshold value for Ni-hyperaccumulators, is not universal for all trace elements. For instance, accumulations of > 1000 µg Zn g^{-1} DW are not uncommon in “normal” plants, since it is an essential plant nutrient, which is needed in higher quantities than Ni. Thus, Zn-hyperaccumulation begins with values above 1% (Baker and Brooks, 1989). Therefore, after Baker and Brooks (1989) hyperaccumulators are plants, which have the ability to accumulate:

i) > 100 mg kg^{-1} DW of Se or Cd,
ii) > 1,000 mg kg^{-1} DW of Cu, Co, Cr, Ni, As, Pb; or
iii) > 10,000 mg kg^{-1} DW of Mn or Zn, in their vegetative tissues.

However, hyperaccumulation is uncommon and known in only 0.2% of vascular plants worldwide. The majority (450 out of 500 taxa) hyperaccumulate Ni and are generally found on ultramafic soils (Pollard et al., 2014).

1.2.2.1 Distribution of Ni-hyperaccumulators

All Ni-hyperaccumulators discovered worldwide so far, can be grouped into seven distinct regions, which have never been glaciated (fig. 1.4): 1) New Caledonia, 2) Western Australia, 3) Southern Europe and Asia Minor, 4) The Malay Archipelago, 5) Cuba, 6) Western United States, and 7) Zimbabwe (Great Dyke).

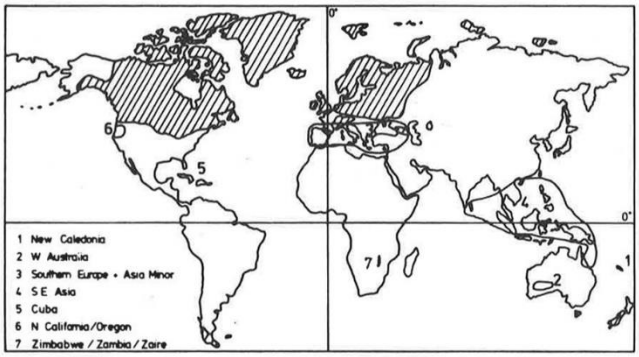

Fig. 1.4: Distribution of Ni-hyperaccumulators (Brooks, 1987, p. 93).

In Europe, most endemic Ni-hyperaccumulators are found in the Mediterranean region (fig. 1.5), extending from Portugal through Italy and the Balkans to Turkey and neighbouring countries (Reeves et al., 2018). Especially the Balkan Peninsula is an outstanding hotspot of serpentine endemic flora (Brooks, 1987). Due to a large distribution of ultramafic bedrock and no former glaciation, Albania and Greece possess many native Ni-hyperaccumulators.

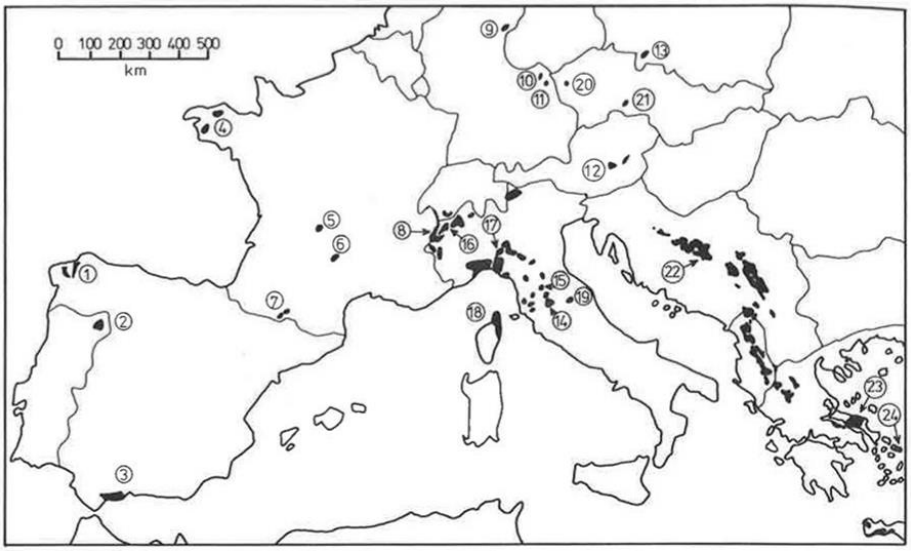

Fig. 1.5: *Distribution of ultramafic (including serpentinite) outcrops in Europe: 1) La Coruña, 2) Bragança, 3) Malaga, 4 Massif Armoricain, 5) Aveyron, 6) Haute-Vienne, 7) Ste Béat,8) Val d'Ayas, 9) Harz, 10) Grötschenreuth, 11) Wurlitz, 12) Kraubath, 13) Zabkowicz, 14) Impruneta, 15) Monte Ferrato, 16) Val d'Aosta, 17) Bobbio, 18) Corsica, 19) San Stefano, 20) Marianske Lazne, 21) Mohelno, 22) Gostovic, 23) Mantoudi (EuboaJ, 24) Tinos (Brooks, 1987, p. 210)*

The taxonomic and phylogenetic distribution of hyperaccumulators is however uneven, since nearly all species recorded until now, belong to the family *Brassicaceae* (crucifers) (Brooks, 1987). Within the crucifers, all species hyperaccumulating Ni are concentrated in 2 out of 23

tribes, namely *Coluteocarpeae* and *Alysseae*. *Alysseae* represents with 21 species, belonging to the three genera *Odontarrhena*, *Alyssoides* and *Bornmuellera*, the biggest and most promising tribe for Ni-hyperaccumulation (Kidd et al., 2018). Within the taxonomic classification of hyperaccumulators, various changes happened in the past years. The most prominent reclassifications comprise that of *Thlaspi* to *Noccaea* (Al-Shehbaz, 2014) and *Alyssum* to *Odontarrhena* (Španiel et al., 2015). Until the publication of Španiel et al. (2015), it was assumed that the genus *Odontarrhena* was one section of the genus *Alyssum* (Kidd et al., 2018). Hence, most publications refer to *Alyssum* species (e.g. *A. murale*), while nowadays being classified as *Odontarrhena*. Within the "true" *Alyssums* there is no species hyperaccumulating Ni. However, around 90 taxa belonging to *Odontarrhena* and growing on ultramafic soils in Southern Europe, Mediterranean Area and Western Asia are Ni-hyperaccumulators (Španiel et al., 2015). Thus, within this paper it will only be referred to *Odontarrhena*, even though original publications reported *Allyssum*.

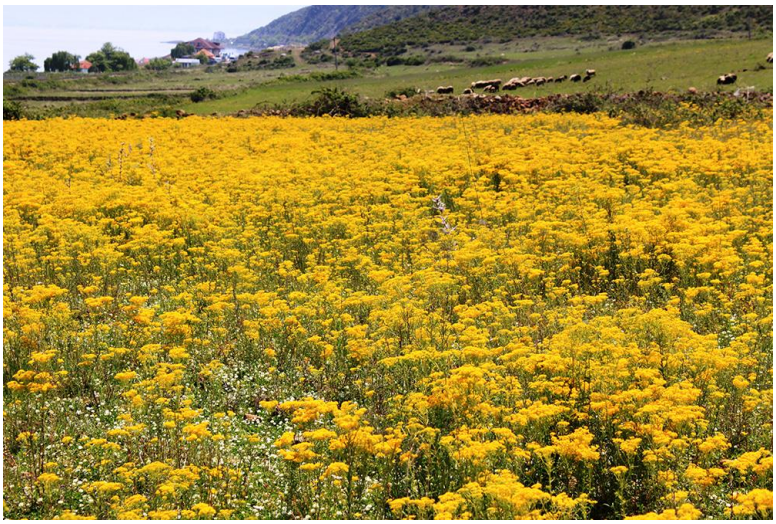

***Fig. 1.6**: Field of O. chalcidica in June, in Albania, Pojskë (http://www.life-agromine.eu).*

Hyperaccumulators are not restricted to serpentine bedrock. For example, two of the most promising taxa, *O. muralis s.l.* and *O. chalcidica*, are both facultative hyperaccumulators and thus not restricted to ultramafic habitats. The taxa *O. chalcidica* (fig. 1.6) is native to Greece, Albania, former Jugoslavia and Kosovo, while *O. muralis* is distributed in Bulgaria, Greece, Romania and Serbia (Španiel et al., 2015). Even though they are facultative hyperaccumulators, species of *Odontarrhena* are restrictively found on ultramafic and serpentine bedrock in the Mediterranean region (Brooks, 1987; Baker and Brooks, 1989). Brooks (1987) postulated, that

there is an inverse relationship between the extent of the distribution of those species and the amount of Ni taken up. For instance, the species *O. constellatu* (syn. *A. constellatu*) can concentrate over 10,000 µg Ni g^{-1} DW in its leaves but is only found in Eastern Turkey and Northern Iraq. In contrast, *Odontarrhena* species containing 1,000 - 5,000 µg Ni g^{-1} DW show a broader distribution (e.g. *O. alpestere* and *O. obovatum*). Hence, Brooks (1987) suggested a linkage between diversity, endemism and enormous high Ni levels within *Odontarrhena* species. This can be as well linked to an evolutionary adaption, which is typical for ancient floras. The outstanding ability to concentrate normally phytotoxic levels of Ni, enabled genera like *Odontarrhena* to survive on very hostile edaphic conditions, which might be a survival or defence strategy against competition from other species and animals feeding on plants. Especially taxa of *Odontarrhena* seem quite successful, since on serpentine outcrops in the Balkans extensive or nearly pure populations of *O. chalcidica*, in absence of any other competing species, occur (Brooks, 1987). Their advantage of not being restricted to serpentine habitats and their ability to hyperaccumulate trace elements in aboveground plant parts, qualifies taxa of the genera *Odontarrhena* as valuable candidates for phytoextraction (Bani et al. 2015a,b; Kidd et al. 2018).

1.2.3 Nickel

Nickel (Ni) is besides palladium (Pd) and platinum (Pt), the third element of the group 10 of the periodic table. Furthermore, it belongs together with iron (Fe) and Cobalt (Co) to the iron family. (Kabata-Pendias, 2010). Ni shows calcophilic and siderophilic affinities. Because of its siderophility, Ni readily combines with metallic Fe, which is also reason for the frequent occurrence of Ni-Fe compounds in the Earth's core. Furthermore, associations of Ni with sulphur segregates are quite abundant, due to the affinity of Ni for sulphur (S). Thus, Ni metallic ores are often composed of pentlandite $(Ni,Fe)_9S_8$ and pyrrhotite (iron sulphide with variable Fe content). Moreover, Ni in rocks is principally segregated to sulphites (NiS), arsenides (NiAs) and antimonides (NiSbS). Furthermore, Ni also forms sulphides and sulphar-arsenides together with Fe and Co, associated with Fe minerals (Kabata-Pendias, 2010).

During weathering, Ni is released and free Ni coprecipitates with Fe and Mn oxides, gets included in Fe minerals (e.g. goethite, limonite, serpentinite), or associates with carbonates, phosphates and silicates. Furthermore, organic matter has a strong affinity to absorb Ni, which is also reason for high Ni levels in coal and oil. Hence, combustion of oil and gas contributes to

industrial pollution of Ni through the air (Kabata-Pendias, 2010). Besides that, agricultural soils are often polluted due to application of Ni-rich sludge and phosphate fertilizers. Thus, Ni concentrations of surface soils are not only a result of the weathering of the parent material, but might also derive from anthropogenic activities (Kabata-Pendias, 1993). Geogenic Ni can derive from various rock types. Ultramafic rocks (e.g peridotite, dunite, pyroxenite) show the highest Ni concentrations, followed by mafic- (e.g. gabbro, basalt) and intermediate rocks, or igneous rocks rich in ferromagnesian and sulphide minerals containing Ni (e.g. pyroxene, olivine, biotite, chlorite) (Massoura et al., 2006).

1.2.3.1 Ni-availability in serpentine soils

The availability of Ni in soils deriving from Ni rich bedrock (geogenic), is controlled by the Ni bearing phases present, as well as the speciation of Ni within these phases (Massoura et al., 2006; Chardot et al., 2007). Furthermore, in Ni-rich soils (e.g. serpentine soils), the plant-available fraction of Ni is determined by the weathering intensity, which is influenced by climatic conditions and moisture regimes (Massoura et al. 2006; Alexander, 2004).

The Ni bearing phases in serpentine soils comprise: i) primary phyllosilicates (e.g. serpentine, chlorite, talc), ii) secondary clay minerals (e.g. illite, smectite) and iii) Fe-Mn oxihydroxides (Adriano, 2001; Echevarria, et al., 2006; Massoura et al., 2006;). Thus, in the solid phase of serpentine soils, Ni is represented in various chemical forms (Adriano, 2001):

i) Bound to exchange or specific absorption sites (e.g. organic matter)
ii) Adsorbed or occluded within sesquioxides (e.g. Fe-Mn oxyhydroxides)
iii) Bound inside clay lattices (e.g. primary-, secondary clay minerals)
iv) Fixed in organic particles and microorganisms

During weathering Ni is released due to mineral dissolution of primary minerals containing Ni. The mobilized Ni is relatively stable in the aqueous soil phase (soil solution), which enables Ni to move between soil horizons. The mobility of Ni depends on the chemical form. Ionic species of Ni in soils are: Ni^{2+}, $(NiOH)^{+}$, $(HNiO_2)^{-}$ and $Ni(OH)_3$ (Kabata-Pendias, 2010). The most abundant chemical form of Ni in soils is Ni^{2+}, due to its stability over a broad range of pH and redox conditions (Adriano, 2001).

In soil solution Ni occurs free in its ionic form (Ni^{2+}) or complexed to inorganic and organic ligands. The solubility of Ni-complexes depends on the ligand. Thus, formed Ni-halides and salts of oxo-acids are mostly water soluble, while Ni-carbonates are hardly soluble (Adriano, 2001). Furthermore, the surface charge and hence the sorption of Ni onto the mineral surface is pH dependent (Massoura et al., 2006). The intensity of the Ni concentration in soil solution, is usually negatively correlated to soil pH (Massoura et al., 2006; Echevarria, 2018). The mobility of free Ni during weathering is mostly limited, as it is easily i) incorporated or exchangeably bound to newly formed 2:1 clay minerals, ii) complexed to organic matter, or iii) reversibly absorbed to amorphous Fe-oxyhydroxides at slightly alkaline pH (Massoura et al., 2006; Chardot et al., 2007; Kabata-Pendias, 2010; Echevarria, 2018). Thus, Ni is mostly bound to the soil solid phase. However, Ni-availability in the topsoil can be increased by compounds with a high sorption capacity (e.g. organic ligands, clays), which have the potential to remobilize Ni from the solid phase and thus increase its availability. For example, Cambisols and Calcisols are soil types usually containing the highest amounts of Ni worldwide (Kabata-Pendias, 2010). To summarize, the Ni concentration in soil solution is controlled by various soil properties, comprising: i) total metal levels, ii) pH, iii) CEC, iv) contents of organic matter and hydrous oxides, and v) texture and mineralogy of soil (Echevarria, 2018).

In temperate and Mediterranean climates, Ni-availability in slightly weathered serpentine soils, is usually controlled by the association of Ni with amorphous Fe-oxides (Cambisols) or with secondary clay minerals with high exchange capacity (smectite in Vertisols or Saprolites), which exchange Ni^{2+} between the soil solid phase and soil solution (Massoura et al., 2006; Chardot et al., 2007). Chlorite and talc can be present as primary- or secondary minerals in ultramafic soils (Echevarria, 2018). Chlorite is a 2:1 clay mineral with a high CEC, while Talc is extremely resistant to weathering and shows a CEC close to zero (Echevarria, 2018). The secondary clay mineral smectite (from primary minerals chlorite or serpentine), shows a high CEC and high specific surface charge, making it an essential source of Ni in serpentine soils (Bani et al., 2007). In tropical climates, Ni is mostly associated with goethite. Goethite, that forms after intense weathering, has a high retention capacity for trace elements and incorporates metals (e.g. Ni) into its crystal lattice. Thus, Ni in crystallized Fe, Al or Mn oxides is completely immobile, while Ni availability in soils is higher when Ni is associated with phyllosilicates and amorphous Fe oxides (Echevarria et al., 2006; Massoura et al., 2006; Chardot et al., 2007).

Ni is considered being an essential micronutrient for plants due to its role in the metabolism of urease (Merlot et al., 2018). However, if plants are exposed to elevated concentrations of Ni (> 10 mg kg^{-1}), nutrient imbalance, disruption of cell membrane functions, chlorosis and necrosis can occur (Pędziwiatr et al., 2018). Besides the metal level, the toxicity to plants and other biological effects also depend on the Ni species present. Cationic Ni (Ni^{2+}) is easier adsorbed and thus more toxic than complexed forms. Furthermore, the amount of Ni taken up by plants is influenced by i) soil properties, ii) the origin of Ni, and iii) the ability of a plant to accumulate Ni in its biomass (Kabata-Pendias, 2010).

1.3 Potential applications of hyperaccumulators

The ability of hyperaccumulators to tolerate and store high concentrations of heavy metals in their aboveground biomass (phytoextraction), opened research into their use to remediate contaminated soils (phytoremediation). Thus, hyperaccumulators were cropped to remove toxic metals from soils contaminated with heavy metals, low-grade ores, or on naturally metal enriched soils, which were not profitable for traditional mining activities (Li et al., 2003a,b).

The harvested biomass was considered toxic waste and put to landfills. With the idea of phytomining, first proposed by Chaney (1983, cited in Nkrumah et al., 2018), the metal rich biomass of *O. murale* (syn. *Alyssum murale*) developed from a waste product, towards a profitable alternative ore for Ni (Simonnot et al., 2018; Morel et al., 2018). In addition, phytomining was extended to a whole soil-plant-ore agrosystem by Morel (2013, 2015), who introduced the term "agromining" (Nkrumah et al., 2018). Agromining and phytomining are both based on the principle of phytoextraction. Van der Ent et al. (2015) distinguished these strategies based on the initial use of the site (e.g. mining or

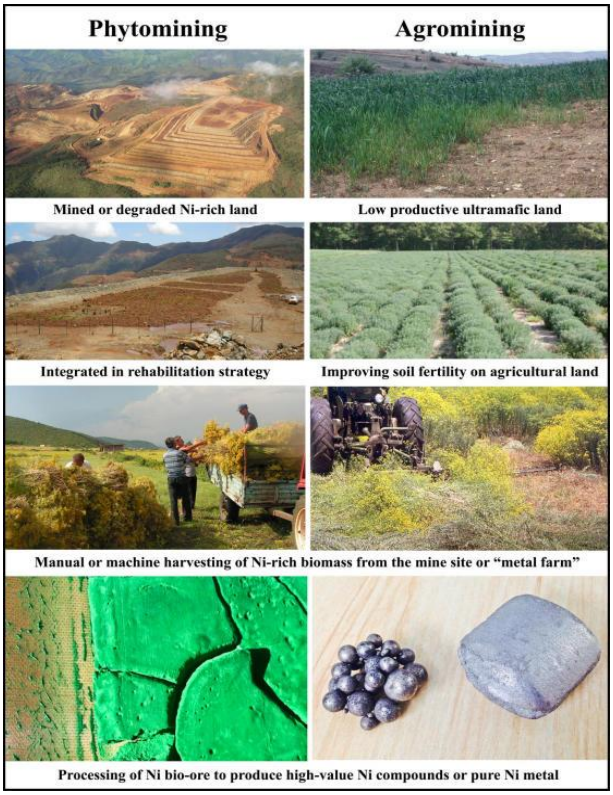

Fig. 1.7: *Differences between phytomining and agromining (van der Ent et al. (2015).*

agriculture), which is also well illustrated in fig. 1.7. They proposed, that phytomining is a form of rehabilitation strategy on degraded metal-rich land, such as Ni laterite mines or due to smelter activities, where metals are extracted for metal recovery. In contrast, agromining is an alternative to conventional agriculture on low productive ultramafic substrate, with the benefit of improving soil quality and ecosystem services, while gaining higher revenues for the local farmers (Van der Ent et al., 2015; Kidd et al., 2018). Both techniques have been proven to be especially appropriate for Ni. First, most hyperaccumulators are known for Ni. Second, over >1% of the Earth`s surface is covered by ultramafic soils enriched in Ni. Besides that, Ni is quite plant-available, compared to other elements present in ultramafic soils (Fe, Co, Cr). In addition, species, such as *Odontarrhena chalcidica*, produce high biomass and accumulate great amounts of Ni, which are both crucial factors to make phyto- or agromining commercially attractive (van der Ent et al., 2015).

1.3.1 Concept of Agromining

Besides extracting metals such as Ni from soil and thus decreasing the phytotoxicity of the substrate, agromining may have positive environmental effects (Kidd et al., 2018). Echevarria et al. (2015) defined various ecosystems services provided by agromining:

i) Carbon sequestration
ii) Enhanced soil biodiversity
iii) Renewable biomass production
iv) Improved agricultural productivity (safe edible and non-edible crops), and
v) Land restoration

Besides positive effects on the environment, there are also social benefits, such as the stimulation of rural development, where lately ultramafic soils have been abandoned by farmers (Kidd et al. 2018). Moreover, agromining can be an economically viable approach for elements with a high value, such as Ni, Co or Au (gold). Ni is a raw material with high commercial importance (2.7 million metric tons produced globally in 2019 (Garside, 2020). It is an essential component of magnets, electrical equipment, Ni alloys (e.g. for tools and vessels), dyes in ceramic and glass manufactures and batteries (Kabata-Pendias, 2010). The use of hyperaccumulators to recover Ni from ultramafic soils is a promising approach, due to the ability of hyperaccumulators to concentrate and purify the Ni taken up. The concept of Ni-agromining consists of i) cropping promising Ni-hyperaccumulators to gain maximal biomass with high Ni

concentrations, which can be improved by agronomic practices, and ii) conversion of the harvested biomass to valuable end products. After harvest, the dried hyperaccumulators are incinerated to remove organic matter, while increasing the Ni concentration in the obtained ash (e.g. approx. 12 times). In a further step, various Ni compounds can be produced from the ash or "bio-ore" (Kidd et al., 2018). The concentration of Ni in the bio-ore can vary between 10 - 25 wt %, while the Ni content in lateritic ores is usually < 1.5 wt % (Nkrumah et al., 2018). Possible end products comprise Ni metal, Ni-based catalysts, Ni salts or Ni oxides. Besides that, the energy produced during incineration of the Ni-rich biomass can be of value (Kidd et al., 2018; Simonnot et al. 2018). A Ni-based chemical that has already been successfully recovered from hyperaccumulators ash, is the mineral salt ANSH (Ammonium Nickel Sulfate Hydrated), with a purity of more than 99% and a value of $ 20,000 per ton (Echevarria et al., 2015). In Albania, phytomining has already been brought to field scale. Bani et al. (2015a,b) gained a yield of 105 kg Ni ha^{-1} from cropping *O. murale* (syn. *A. murale*) on ultramafic soil in Albania.

Generally agromining and phytomining require fast growing hyperaccumulators that achieve high biomass yield and high shoot Ni concentrations, since this determines the efficiency of the technology. Thus, not all hyperaccumulators are suitable for phytomining. Species that concentrate high levels of Ni in their tissues but show only low biomass production, are not a commercially viable option. To improve biomass production and Ni yield, crop- and agronomic management are needed (van der Ent et al., 2015).

1.3.2 Agronomic practices

The potential of agromining can be enhanced by improving the unfavourable soil conditions of serpentine soils through agronomic practices, such as (Kidd et al., 2018; Pędziwiatr et al., 2018):

i) Application of fertilizers (mineral or organic)
ii) Plant cropping patterns
iii) Irrigation management, and
iv) Weed control (e.g. herbicide application)

Mineral fertilizers (e.g. NPK) can increase biomass production and Ni yield of Ni-hyperaccumulators growing on ultramafic soils, as they potentially improve the nutrient status of the deficient substrate (Li et al., 2003a; Bani et al., 2015a; Kidd et al., 2015; Álvarez-López

et al., 2016). In contrast, organic amendments are suggested to increase biomass production by various effects. Besides supplying essential nutrients, organic fertilizers enhance soil quality and structure, reduce compaction and erosion, and indirectly stimulate biological activity (Nkrumah et al., 2016; Kidd et al., 2018).

Plant cropping patterns comprise co-cropping of hyperaccumulators with "normal" plants without hyperaccumulation ability. Co-cropping might enhance agromining efficiency, due to increased plant diversity. Because the presence of different plant species can change rhizosphere properties by releasing a mixture of different rhizodeposits, which affects abundance, functions and diversity of associated macro- and microorganism in the soil. Besides that, a multi-species vegetation offers a diverse mix of plant litter to the degrading organism, enhancing species richness (Kidd et al., 2018). Co-cropping with legumes is another option with the potential to enhance plant growth and metal phytoextraction, due to an improvement of soil nutrient status. First, symbiotic N_2-fixing rhizobium bacteria convert nitrogen (N) into a plant assimilated form and thus increase N content in deficient ultramafic soils. Second, decomposition of residues from legumes return N and C to the soil, increasing soil carbon stock. Besides that, legumes enhance soil physical properties, soil porosity and aggregate stability (Kidd et al., 2018). Furthermore, weed control is required to reduce competition for nutrients and water between weeds and hyperaccumulators (Bani et al., 2015a). Additionally, irrigation management can support plant growth, which is usually limited by draught on serpentine soils (Kidd et al., 2018).

Due to the beneficial effects that agronomic practices provide to infertile serpentine soils, van der Ent et al. (2015) claimed that agromining is a promising alternative to food crop production. They stressed that, agromining generates better economic return to the local community, does not compete with food crop production and might enhance soil quality and soil health. Moreover, continuous extraction of Ni from topsoil together with higher soil quality, might enable conventional agriculture after longer time periods (van der Ent et al., 2015).

1.4 Soil Quality

As mentioned before, a lot has already been published about the application of mineral or organic fertilizers, and their positive effect on Ni yield or biomass production. However, within the sustainable scope of agromining it is as well of interest to assess long-term impacts of cropping hyperaccumulators in combination with agronomic practices, on soil quality.

Particularly, the investigation of possibly positive effects of the technology on the soil habitat, as a good soil quality is one component of a healthy environment (Acton and Gregorich, 1995). The importance of clean water and air is nowadays broadly understood, while the role of soil is often neglected. However, soil is a crucial component of the Earths biosphere, that needs protection too (Doran and Perkin 1996). It is often ignored, that soils provide many important ecosystem services (Acton and Gregorich, 1995), such as biomass production, biodiversity conservation, erosion control, pest and disease control, water quality and -supply as well as climate regulation (Bünemann et al., 2018). The ability of soils to continuously provide ecosystem services is endangered by soil threats, such as soil erosion, organic matter decline, contamination, sealing, compaction, soil biodiversity loss, salinization, desertification, flooding and landslides (Stolte et al., 2016).

1.4.1 What is soil quality?

Within the scientific community there are different terms that are often used to describe the state of a soil. The most popular terms are soil quality, soil health and soil fertility. These definitions are often used interchangeably, even though they do not have the exact meaning. In the past, humans used soil for mining and for agricultural production. The concept of soil fertility emphasises the ability of a soil to provide essential plant nutrients and water, to enable plant growth and reproduction, in the absence of toxic materials. Thus, the concept of soil fertility focuses only on the suitability of a soil for food production. Bünemann et al. (2018) criticised, that this approach comprises the chemical and physical conditions of soil but does not respect biological compounds. Another term often used is soil health. According to Acton and Gregorich (1995), soil quality or soil health is the fitness of a soil to enable crop growth, without risking soil degradation or harming the health of animals and humans. Moreover, the US Natural Resources Conservation Service (USDA, 2001) defined soil health or soil quality as:

> *"The capacity of a specific kind of soil to function within natural or managed ecosystem boundaries to: sustain plant and animal productivity, maintain or enhance water and air quality and support human health and habitation."*

Quite similarly, Doran and Perkin (1994, p. 7) defined soil quality as:

> *"The capacity of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health."*

Thus, both definitions emphasise the chemical, physical and biological components of soil and consider the contribution of soil to environmental quality, as well as health of plants, animals and humans (Bünemann et al., 2018). Within this study soil quality is understood according to the definition by Doran and Perkin (1994). In addition, we consider soil quality being equivalent to soil health, as it was recommended by Bünemann et al. (2018).

Soil quality can be split into inherent and dynamic quality (Bünemann et al., 2018; Roy et al., 2018). The inherent quality relates to the natural capacity of a soil due to its geology (parent material), climate, topography, vegetation and the duration of soil formation (USDA, 2001; Bünemann et al., 2018). These conditions are believed being more or less stable, without immediate changes after management practices (Bünemann et al., 2018). Loamy soils for instance, derive a higher inherent soil quality due to their better performance in retaining water, compared to sandy soils. Thus, soils naturally vary in their capacity to function, which is the reason for soil quality being specifically assessed for each soil type (USDA, 2001). In contrast, the dynamic soil quality emphasises changes of natural soil quality due to human activities (e.g. agricultural practices) and environmental factors (USDA, 2001; Roy et al., 2018). Agronomic measures can improve soil quality (e.g. use of cover crops to increase SOM), or negatively affect the ability of a soil to function (e.g. compaction due to tillage on wet soil) (USDA, 2001). In this study, soil quality reflects to the dynamic (manageable) properties of soil. Thus, soil quality assessment comprises the determination of the influence of different agricultural measures on soil quality on the same soil type, to monitor trends over time and evaluate the impact of management on soil quality.

Soil is a multiphase system (liquid, gaseous, solid) that is interacting with air and water. This makes it complex but is also reason for the many important functions it fulfils, such as (USDA, 2001; Bünemann et al., 2018):

i) Maintaining biological diversity, -activity and -productivity
ii) Regulating water flow
iii) Filtering, buffering and breakdown of organic and inorganic elements
iv) Storage and cycling of nutrients, water and organic matter
v) Sustaining and improving soil physical stability

As mentioned before, soil quality is defined as the ability of soil to perform its functions. Thus, monitoring is required, to ensure a continuous fulfilling of soil functions. However, measuring

functions directly is difficult. Hence, specific indicators (e.g. pH, nutrient availability) or soil conditions (fertility, structure, erodibility) can be used to assess soil quality indirectly (Acton and Gregorich, 1995).

1.4.2 Soil quality indicators

Doran and Perkin (1994) claimed that among scientists there is a general agreement that soil quality affects 3 domains: i) plant- and biological productivity, ii) environmental quality (quality of air, ground- and surface water), as well as iii) human and animal health (food quality). Therefore, a set of soil quality indicators should be chosen that i) enables a quantitative evaluation of soil functions, which relate to the performance of these 3 domains, and ii) provides a comprehensive understanding of the state of a soil. Besides that, indicators must fulfil certain criteria: i) they have to be relevant and related to a given soil threat, function or ecosystem service, ii) should integrate physical, chemical, and biological properties and processes, iii) are easy to sample and measure, iii) interpretable and iv) sensitive to detect changes in management and climate (Doran and Perkin, 1994; Bünemann et al., 2018). Following set of indicators is a symposium of parameters most mentioned in literature, after Bünemann et al. (2018), who reviewed 62 publications on soil quality indicators:

i) Biological: earthworm density, N mineralisation, microbial biomass, soil respiration, labile C and N.
ii) Chemical: Total organic carbon, pH, available K, N & P, total nitrogen, electrical conductivity, cation exchange capacity, heavy metals, other macronutrients (e.g. Ca, Mg, S), salinity, micronutrients.
iii) Physical: water storage, bulk density, texture, structural stability, soil depth, penetration resistance, hydraulic conductivity, porosity, aggregation, infiltration.

Physical indicators (e.g. texture, bulk density) can inform how well the soil retains and transports water and nutrients, or its compaction potential. Chemical properties should provide a better understanding of biological and chemical activity, availability of plant nutrients and potentials for loss of N and P. The biological assessment gives insight into the living component of the soil. Biological indicators are dynamic soil properties, that are sensitive to land management (e.g. cultivation) and disturbances (e.g. contamination) (Rusek, 1998; USDA, 2001; Bünemann et al., 2018). Various authors claimed that soil organisms are the most sensitive indicators in soil quality evaluation. First, they immediately respond to changes in soil

properties. Second, soil organisms contribute essentially to soil functions (Santorufo et al., 2012; Yan et al., 2012 Rieff et al., 2016; Bünemann et al., 2018).

1.4.2.1 Soil organisms

Generally, soil organisms can be classified according to their body size (Neher and Barbercheck, 1998):

i) Microflora (bacteria, fungi, algae, actinomycetes)
ii) Microfauna (protozoa), 0.005 - 0.2 mm length
iii) Mesofauna (nematodes, enchytraeids, mites, springtails), 0.2 - 10 mm length
iv) Macrofauna (insects), 10 - 20 mm length
v) Megafauna (earthworms), > 20 mm length

The majority of soil organisms, habitats the first 20 cm of soil. In contrast to soil macrofauna (earthworms, insects, ants), the organisms of the mesofaunal group do not have the ability to actively shape the soil, which is why they are restricted to existing pores within the soil profile. Hence, mesofauna activity is highly influenced by the water and air balance in soil pores (Neher and Barbercheck, 1998). Within the mesofaunal group, Collembola (springtails) and Acari (mites) are microarthropods playing an essential role in terrestrial ecosystems (Neher and Barbercheck, 1998; McIntyre et al., 2001; Moldenke, s.a.) and accounting for 95% of soil arthropods (Gbarakoro and Zabbey, 2013). Microarthropods together with microflora contribute directly to ecosystem processes, as they decompose organic material and release nutrients to the soil. Microorganism (e.g. bacteria, fungi, algae) primarily decompose organic matter and are responsible for humus production, nutrient cycling, energy release, fixation of elements, metabolic (enzyme) activity and the formation of chemical compounds to form soil aggregates. Soil mesofauna regulates the decay of organic material and nitrogen mineralization, by feeding on the microbes. Thus, mesofauna influences microbial growth and metabolic activities (Neher and Barbercheck, 1998). Especially the droppings of springtails and mites positively influence microbial activity, decomposition rate and transport processes in soil. This is because they break down bigger organic material, moisten it and enhance availability for microbial decay (Neher and Barbercheck, 1998; Moldenke, s.a.). Depending on the size, large Collembola taxa enhance N-mineralization by feeding selectively on fungi, while smaller species contribute to humus formation by mixing mineral and organic fragments in soil (Neher and Barbercheck, 1998). Elliot et al. 1988 (cited in Neher and Barbercheck, 1998)

claimed that soil mesofauna accounts for 30% of nitrogen mineralization in agricultural- and natural soils. Besides their role in ecosystem services, Collembola and Acari show the highest abundance and diversity among soil arthropods (McIntyre et al., 2001; Behan-Pelletier, 2003; Moldenke, s.a). Furthermore, they are relatively easy to sample, have short generation times and are highly dependent on their immediate environment, which is why the respond quickly to habitat changes (McIntyre et al., 2001; Parisi et al., 2005). Schloter et al. (2003) defined 3 requirements for soil organism used as soil quality indicators, namely i) being dominant in the regarding soil type, ii) high abundance and species richness, and iii) playing a crucial part in soil functioning. Hence, within the soil faunal community, the microarthropods (e.g. Acari and Collembola) are excellent candidates to assess the impact of land use (management practices) on soil quality (Rusek, 1998; Parisi et al., 2005; Bünemann et al., 2018).

1.4.2.1.1 Collembola

Collembola (springtails) are a group of wingless arthropods within the subphylum Hexapoda (Greek for six legs). They live in wet and dry ecosystems of different climatic zones worldwide (e.g. arctic, alpine tundra, desert, tropical rain forest). Depending on the ecosystem 1 - 3 to 50 60 species with several million individuals per m^2 can be present (Rusek, 1998). Rusek (1998) modified the life form system, first introduced by Gisin (1943), and suggested that Collembolans can be distinguished according to their ecomorphological life forms, which are linked to their main location within the soil profile. Thus, deeper horizons are dominated by small eu-edaphic life forms. Small individuals might also be present in upper horizons, whereas large eu-edaphic taxa never migrate deeper into the soil. Some life-forms follow a certain vertical distribution within the soil, which is influenced by i) pore size distribution, ii) humidity, and iii) food supply. Thus, the distribution of Collembolan life forms within a soil profile is an indicator for the state of soil development (Rusek, 1998).

Furthermore, the role Collembolans are playing depends on soil development. Especially in the earlier stages of pedogenesis and at climatic zones with weak soil development (e.g. arctic, alpine), the microstructure is substantially formed by springtails. Hence, in less developed soils, droppings of Collembolans with specific shape, size and inner structure, are sometimes forming the entire soil profile. This so called microarthropod moder is the simplest form of humus and mainly consists of microarthropod excrements. Besides that, also the excrements of other groups of mesofauna form soil microstructure (Rusek, 1998).

In more developed soils, Collembolans contribute primarily to the degradation of leaf litter and excrements of micro- and macrofauna. The morphology and ecological role are determined by feeding habits (Rusek, 1998). Most Collembolans feed on soil microbiota (fungi, bacteria, actinomycetes and algae), especially fungi (fungivores). Thus, they control the dynamics of those microbial populations. Furthermore, Collembolans degrade dead organic matter like excrements or leaf litter (detritivores), are facultative predators on nematodes and other Collembolans, or are omniphages (Crossley et al., 1992; Rusek, 1998).

Usually, collembolans follow a life cycle of several weeks to months. The abundance of mesofauna usually reaches its lowest value at the driest period of summer, which is different compared to other groups of soil fauna (Rusek, 1989). Collembolans are also a food source themselves. They are prey for beetles and their larvae, dipterans, ants, mites, other springtails and bigger animals (e.g. frogs, reptiles). Furthermore, they are host of various parasites and pathogenic bacteria, fungi and nematodes. A high number of parasited Collembolans can be a sign of soil pollution. Besides that, Collembolans are considered being a valuable indicator of soil quality for arable soils (Rusek, 1998).

1.4.2.1.2 Acari

Acari (mites) belong to the class of Arachnida, within the phylum Arthropoda. Mites can be found in all ecosystems globally, from soils in desert and arctic environments, to grasslands and tropical rainforests. They can survive in extremely acidic or alkaline, as well as nutrient poor or nutrient rich soils, at every latitude. Thus, they compete with Collembolans, as they show the same wide distribution in ecosystems worldwide. From all habitats, their greatest diversity and abundance happens to be in soils. Thus, 1 m^2 in the organic layer of forest soils, might be colonized by up to 250 different species and 800,000 individuals (Behan-Pelletier, 2003).

Like Collembolans, mites play a crucial role in decomposition and nutrient cycling (Seastedt, 1984). Generally, four predominant groups of mites can be distinguished in soils, namely Mesostigmata, Prostigmata, Oribatida and Astigmata, a highly derived group within Oribatida (Coleman et al., 2018). The suborder Oribatida fulfils similar functions as Collembolans in soil. They contribute to decomposition and nutrient cycling by feeding on fungi and dead organic matter. Their droppings contribute to soil structure (microarthropod moder). Belonging to the group of k-specialists, they show low fecundity and slow development. Due to that, their population declines rapidly, once their habitat is changed (Behan-Pelletier, 2003). In

contrast to Oribatida, Astigmata feed on plants, fungi, and algae and show higher production rates (r-specialists) and population densities in cultivated soils (Crossley et al., 1992; Behan-Pelletier, 2003). The second suborder, Prostigmata, include fungivores and predators and are among the most abundant soil mites in agricultural soils. As r-specialists they have short life spans and high fecundity (some taxa only live for one week). Thus, they usually respond quickly to changing soil properties after disturbances or nutrient pulses (e.g. tillage, fertilization). Mesostigmata are active and predaceous mites, which control populations of other microarthropods and nematodes in agricultural soils (Crossley et al., 1992). Besides that, some taxa also feed on fungi, bacteria or organic matter. Like Prostigmata, they have life cycles of one week. Compared to other soil arthropods, Mesostigmata are more sensitive to changes in the habitat within a shorter period. Application of fertilizers usually increases abundance of Astigmata and Prostigmata, while Oribatida populations decline (Behan-Pelletier, 2003).

1.4.2.2 Biological indices for soil quality evaluation

Biological soil quality assessment can comprise the evaluation of a single taxon (species richness and -diversity) or the general abundance of microarthropods. Even though biological indicators have a great potential to assess and prevent soil degradation, they are often neglected due to difficulties in classifying the taxa. In literature, biological indices are often based on a single taxon and their species diversity or density, such as for earthworms (Peres et al., 2006), nematodes (Biagini and Zullini, 2006) or oribatid mites (Gergocs and Hufnagel, 2009). However, grouping at species level is not always needed. A common way to assess impacts of land use on mesofauna without classification on species level, is the comparison of the abundance of Collembola in relation to Acari in a sample (C:A ratio), or vice versa (Bachelier, 1986 cited in Parisi et al., 2005). Another synthetic index was proposed by an Italian team (Parisi, 2001, Parisi et al., 2005), who invented a simple ecomorphological index, which doesn´t require complex taxonomic identification and thus enables a wider application, also for non-specialists. The QBS-index (Qualità Biologica del Suolo) stands for biological soil quality and emphasises the functional role of arthropods in the soil food web (eco-morphological form). This index is based on the concept, that a higher number of microarthropod groups well adapted to the soil (edaphic forms), indicates a higher soil quality (Parisi et al., 2005). They assumed that since soil is a peculiar environment without light and only limited space in soil pores, microarthropods have developed a certain morphology. This adaption to edaphic life usually

causes loss of pigmentation and visual structures, a streamlined body form, reduced appendages (hairs, antennae, legs) and reduction or loss of flying, jumping or running. For example, the furca of springtails (used to jump and giving them their name), is reduced or in high eu-edaphic life forms even absent due to edaphic adaption. As a result, eu-edaphic life forms are especially sensitive to changes in their environment (soil degradation). Thus, a soil of good quality has a high number of groups well adapted to the soil (eu-edaphic).

1.5 Agromine

Due to the growing interest in the concept of agromining, the EU project AGROMINE (LIFE15 ENV/FR/000512) has been established to create a network of agromining field studies on ultramafic soils, with different edaphic and climatic conditions across Albania, Austria, Greece and Spain. The aim is a full-cycle evaluation of i) effects of agromining cropping systems on ecosystems services, ii) recovery of Ni-products and iii) potentially environmental or socio-economical benefits (Kidd et al. 2018). Within the scope of AGROMINE, the objective of the Austrian field experiment was to identify the most effective agronomic management practice for improving the efficiency of Ni-agromining on a serpentine site in a temperate climatic zone, while all other field studies so far were conducted in the Mediterranean area (Kidd et al., 2018; Hipfinger et al., 2020*).* In the first year of the experiment (October 2016 to September 2017), the agromining potential of the endemic *Noccaea goesingensis* was compared to the Albanian hyperaccumulator *Odontarrhena chalcidica.* Additionally, plant cropping patterns (intercropping with *L. corniculatus*), planting densities (10 or 20 cm distance between plants) and pH adjustments (elemental sulphur application) were tested, to influence plant yields and Ni phytoextraction. As a result, the native *N. goesingensis* showed lower Ni-accumulation (7,900 mg Ni kg^{-1}) and biomass production (2.90 t ha^{-1}), compared to *O. chalcidica* with an average shoot Ni concentration of (12,400 mg kg^{-1}) and (3.77 t ha^{-1}) shoot dry weight (Rosenkranz et al., 2019). Further results and soil properties of the samplings t1 (October 2016) and t2 (September 2017) are provided in Rosenkranz et al. (2019). Because agromining efficiency depends on the Ni yield, which is controlled by Ni concentrations in shoots as well as the amount of harvested biomass (Kidd et al. 2018), *O. chalcidica*, which showed a better performance, was chosen as the experimental plant for the following year (2018). Besides cropping the most appropriate hyperaccumulator species, further agronomic practices (e.g. fertilization) might increase Ni yield while also improving soil quality (Bani et al., 2015a;

Kidd et al., 2018). Thus, in the second experimental year we studied the effects of cropping *O. chalcidica* in combination with agronomic practices, such as fertilization regime and planting density, on agromining efficiency and soil quality. As one part of the field trial 2018, the objective of this thesis was to study the effects of cropping hyperaccumulators on soil parameters (e.g. Ni-availability and soil quality), whereas the main results on plant performance can be found in Hipfinger et al. (2020).

In this thesis, following research questions were addressed:

- RQ1: How does agromining affect Ni-availability and soil quality?
- RQ2: What is the influence of the hyperaccumulator *O. chalcidica* and agronomic practices on soil quality and Ni-availability?
- RQ3: Which treatment was most effective for increasing Ni-availability and soil quality?

These research questions were based on following hypotheses:

- H1: Fertilization increases biological activity in soil and this effect is higher under organic amendments.
- H2: Fertilization improves indicators for soil quality (plant-available K, N and P, CEC, TC) and induces higher Ni availability.

2. MATERIAL & METHODS

2.1 Austrian field site

A field experiment was set up in the province of Burgenland (municipality of Bernstein *47° 24' N, 16° 15' O)*, in the East of Austria at 620 m a.s.l., with a slight inclination to SSW (Kidd et al., 2018). The climate is Pannonian, with a continental influence. Mean annual precipitation is moderate, with around 718 mm. The mean annual temperature is approx. 8 °C with hot summers and moderately cold winters (ZAMG, 2002).

Fig. 2.1: *Study site, Bernstein in Burgenland (modified after https://pixabay.com/illustrations/austria-map-regions-land-borders-2434253/).*

The area was chosen, as it represents one of the biggest outcrops of serpentinite in Austria. Geologically it belongs to the Rechnitzer Einheit, a geological window that was formed during the Penninikum. Different to the surroundings, the Rechnitzer Einheit, is mainly composed of greenschist and serpentinite, metamorphic rocks formed due to the orogeny of the Alps. During orogenesis, former igneous rocks were pressed deeply underground and went under metamorphose, before they appeared again on the surface on a view spots, building a so-called geological window, like the Rechnitzer Einheit (Michalek et al., 2015). Furthermore, the area is the most important spot of serpentine vegetation in Austria. Hyperaccumulation of Zn and Ni can be found in various plants in the area around Bernstein-Redschlag, such as the endemic Ni-hyperaccumulator *Noccaea goesingensis* (Schönlaub, 2000).

2.1.1 Experimental set up

The experiment was set up in fall 2016 on a former agricultural field on a Regosol (sandy loam), with a mean total Ni concentration of 1,450 mg kg^{-1}. Further initial soil properties are listed in Kidd et al. (2018). The experimental design of 2017 has been explained in chapter 1.5. It is important to mention, that the sulphur application in 2017 substantially influenced the plots (6_CM, 8_NPK, 10_PM, 15_OM) in 2018 due to an acidification, which resulted in a drop of 1 pH unit compared to non-sulphur-amended plots.

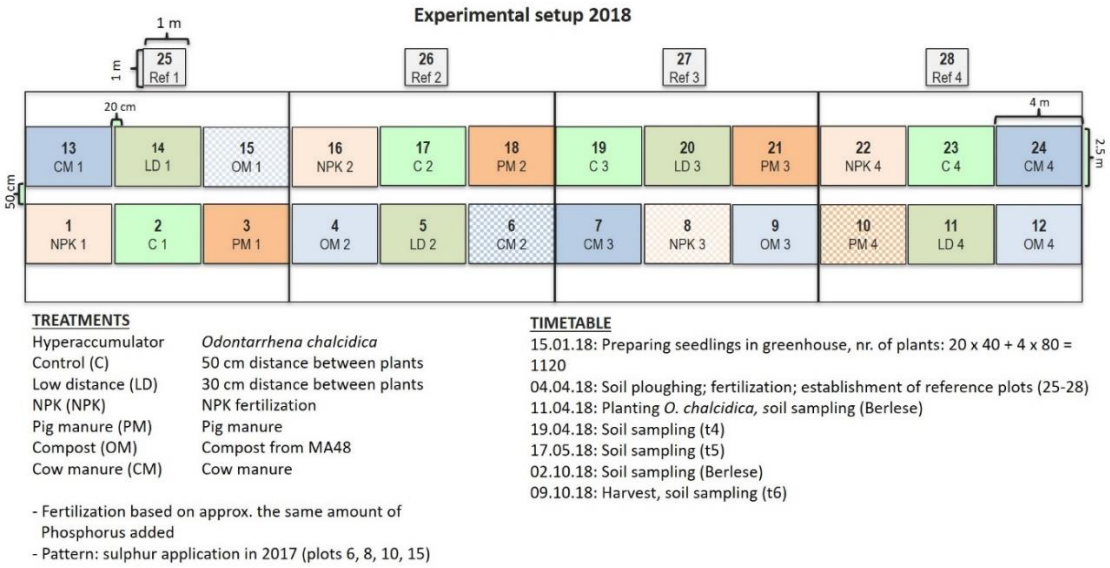

***Fig. 2.2**: Experimental setup in 2018.*

The arrangement of plots in 2018 was the same as in 2017, consisting of 24 plots à $10m^2$ (fig. 2.2). In 2018, inorganic and organic fertilizers were applied to the experimental site, to assess the most suitable amendment for improving soil quality and Ni-phytomining efficiency. The experimental crop was the hyperaccumulator *O. chalcidica*. The seeds derived from Albania (Pojskë) and were in the greenhouse in a mixture (50:50) of serpentine soil from the experimental site in Bernstein and plotting substrate. After that they grew in the greenhouse for 3 months from January to April 2018. In April (04.04.2018), the site was ploughed to ~ 10 cm depth and 6 treatments, each with 4 replicates (n=4), were established: i) control (C, no fertilizer), ii) NPK inorganic fertilizer (Substral® Osmocote; 80:18:69 kg NPK ha^{-1}), iii) cow manure (CM, 401:84:616 kg NPK ha^{-1}, fresh and from conventional farming), iv) pig manure (PM, 158:89:175 kg NPK ha^{-1}, fresh and from conventional farming), v) organic matter (OM, 212:23:12 kg NPK ha^{-1}, compost from Vienna City Administration) and vi) low distance plantation (9.6 individuals per m^2, no fertilizer). The applied manure was solid and mixed with straw from the stables, which made homogenization a challenging task. The amount of fertilizer applied was calculated to apply a comparable amount of P, while N contents differed more than P contents.

tillage & fertilization (April) early plant development (April) late plant development (August)

Fig. 2.3*: Experimental site with Block 1 in the front and block 4 at the bottom of the slope. Pictures show plots close to the street, plot 1 (right) and plot 13 (left).*

Table 2.1 gives an overview of nutrient contents in the fertilizers applied to field. Lowest amounts of N were measured in inorganic fertilizer (NPK; 80 kg N ha^{-1}), while CM contained the highest amount of nitrogen (401 kg N ha^{-1}), followed by OM (212 kg N ha^{-1}). Besides that, the carbon concentrations in organic fertilizers were: i) CM (27.8 g kg^{-1}), ii) PM (18.3 g kg^{-1}) and iii) OM (17.6 g kg^{-1}).

Table 2.1: *Amounts of nitrogen, phosphorus and potassium in the applied fertilizers.*

Fertilizer [kg NPK ha^{-1}]	Nitrogen [N]	Phosphorus [P]	Potassium [K]
NPK	80	18	69
CM	401	84	616
PM	158	89	175
OM	212	23	123

The seedlings of *O. chalcidia* were transferred to the experimental site one week after tillage and manure application (11. 04. 2018). All seedlings were planted within 50 cm distance (except for LD treatment), as recommended by Bani et al. (2015b), who reported max. Ni yield of *O. chalcidica* under a planting density of 4 plants per m^2. Additionally, unplanted reference plots (Ref) were established on the remaining agricultural soil, just next to the fence of the site. This was done to compare the soil conditions of the planted experimental plots with initial soil

conditions of the site, without hyperaccumulator influence. Throughout the growing period, manual weeding and irrigation was needed to ensure plant survival.

2.1.2 Soil sampling

An initial soil sampling was carried out 3 weeks before planting (t3), to calculate the required amount of inorganic and organic fertilizers applied to the field. In order to detect changes of soil parameters over the whole growing period, soil samples were furthermore taken two weeks after application of fertilizers (one week after planting *O. chalcidica;* t4, 19. 04. 2018) and 4 weeks after fertilization in May (t5, 17. 05. 2018) to study the initial effects of the fertilizers, with a negligible influence of the hyperaccumulator. Finally, 6 months after plantation at the harvest of the hyperaccumulators in October (t6, 09. 10. 2018). All soil samples were taken with an auger from the topsoil of each plot (0 - 20 cm). From each plot 12 subsamples were taken in ~ 0.5 m distance of the plot edges and mixed to one composite per plot. The composite soil was collected in a plastic bag, labelled and transferred to the laboratory for analyses. Sample preparation differed depending on the parameter. For the determination of soluble N-$NH4^+$ and N-$NO3^-$ concentrations (Nmin) (Austrian Standards, 1999), soil samples were cooled before the fresh soil was sieved to 4 mm, using a stainless-steel sieve. For the remaining analyses soil was air-dried, sieved to 2 mm using a stainless-steel sieve; a subsample was also oven dried by 105 °C for 24 h and ground to a fine powder using a ball mill. For mesofauna determination, additional soil samples were taken only from the first 10 cm of soil, at two different time points. The first samples were taken 1 week after fertilizer application in spring (11. 04. 2018) and second, 1 week before harvest in autumn (02. 10. 2018).

2.2 Soil physicochemical parameters

For all parameters, certified or in-house reference materials, as well as blanks were used for quality control. Furthermore, recovery rates of ± 10% of the target concentrations were considered as acceptable.

2.2.1 Ni-availability

There are several methods to assess Ni availability in serpentine soils, including: i) single chemical extractions using salt solutions (e.g. $CaCl_2$, $Sr(NO_3)_2$) or chelating agents (e.g. DTPA, EDTA); ii) sequential chemical extraction procedures and iii) isotopic exchange and dilution

techniques (IEK) (Echevarria, 2018). For this study, plant available Ni was assessed using DTPA and $Sr(NO_3)_2$ extraction solutions.

2.2.1.1 DTPA extractable trace elements

Metal availability (e.g. Ni-availability) was measured using a DTPA-extraction (diethylene triamine pentaacetic acid). The Ni extracted with DTPA explains the Ni that is exchangeable or complexed to surfaces (e.g. clay minerals, organic matter) and hence available for plant uptake (Echevarria, 2018). To assess DTPA-extractable trace elements according to Lindsay and Norwell (1978), 10 g of air-dried soil (< 2 mm) were weighed into 50 ml centrifugal vials and 20 ml of extraction solution (DTPA solution adjusted to pH ± 7.3) were added. The suspensions were shaken for exactly 20 h at 20 rpm (revolutions per minute), before being centrifuged at 6000 rpm for 5 min. After that, samples were immediately filtrated. Finally, filtrates were acidified using (2% subboiled 65% HNO_3) for preservation. For the final measurement, samples were diluted twice. First, in HQ (dilution 1:25) and second, acidified with 2% HNO_3 (dilution 1:10). Finally, Indium (110 ppb) was added as an internal standard and metal concentrations were measured on the ICP-MS (Inductively Coupled Plasma – Mass Spectrometry, Perkin Elmer Elan 9000 DRCe).

2.2.1.2 $Sr(NO_3)_2$ - extractable trace elements

Following the procedure of Pardo et al. (2018), the extractable trace elements and especially plant-available Ni, were measured using a 10 mM strontium nitrate ($Sr(NO_3)_2$) extraction solution. Therefore, 40 ml of extraction solution were added to 10 g of air-dried soil (< 2 mm). The suspension was mixed well by hand and transferred to an overhead shaker for 2 h at 20 rpm and at room temperature. After that, samples were given 20 min time to settle before filtration. An aliquot of the filtrate was directly pipetted into ICP-tubes and acidified with 65% HNO_3, to obtain a 2% acid-solution. Indium was added as internal standard for the measurement of plant-available Ni-fraction and other metals on the ICP-MS (Perkin Elmer Elan 9000 DRCe).

2.2.2 Cation exchange capacity

The effective cation exchange capacity (CEC) was measured following a modified version of ÖNORM L 1086-89 (Austrian Standards, 2014). 100 ml of 0.1 M $BaCl_2$-solution (non-buffered) were added to 5 g of air-dried soil (< 2 mm) and mixed, to ensure contact of the whole

sample with solution. The samples were left overnight and put on an overhead shaker for 2 h at 20 rpm at room temperature. Soil particles were given 20 min to settle, before being filtrated using folded paper filters (150 mm Munktell 14/N). Part of the filtrate was directly pipetted into ICP-tubes, acidified (2% HNO_3) and an internal standard (Yttrium) was added for measuring Al, Ca, Fe, K, Mg and Na on the ICP-OES (Inductively Coupled Plasma – Optical Emission Spectrometry, Perkin Elmer Optima 8300). The rest was stored in the freezer at - 20°C. Finally, CEC was measured by summing up the concentrations of Ca, K and Mg in $mmol_c\ kg^{-1}$ of soil.

2.2.3 N-Min

For the determination of mineral nitrogen (Nmin = $N\text{-}NH4^+$ + $N\text{-}NO3^-$), the soil was cooled after sampling, before sieved to 4 mm one day after sampling. Following Austrian Standards (1999), 15 g of the fresh soil were mixed with 60 ml extraction solution (0.0125 M $CaCl_2.2H_20$) and shaken at 20 rpm for 30 min at room temperature. After letting the suspensions settle for 20 min, they were filtered through folded paper filters (150 mm Munktell 14/N) and stored at - 20 °C in the freezer until further analyses. Additionally, the water content of fresh soil samples was determined to refer to the dry mass, for the calculation of the amount of inorganic nitrogen per hectare of soil. For the analyses of ammonium, samples were pipetted into microtiter plates, incubated at 25 °C for 30 min and absorbance was measured at a wavelength of 660 nm on a spectrophotometer (Agilent Cary 8454 UV Visible). The same was done for measuring nitrate, except incubation was at 37 °C for 30 min and wavelength was set to 540 nm. The resulting concentrations of $N\text{-}NH4^+$ and $N\text{-}NO3^-$ ($\mu g\ ml^{-1}$), were converted to Nmin in ($kg\ ha^{-1}$), using (Eq. 2.1) with a respective Bd of 1.3 $kg\ dm^{-1}$ and sampling depth of 20 dm:

$$\text{Nmin [kg ha}^{-1}\text{]} = \frac{\text{[concentration c (N-NH4}^+\text{+ N-NO3}^-\text{) (µg ml}^{-1}\text{) * vol. extraction solution (ml)]}}{\text{[dry mass sample (g) * depth (dm) * bulk density (kg dm}^{-3}\text{)]}}$$

Eq. 2.1: *Calculation of mineral nitrogen content in soil.*

2.2.4 Olsen-P

The Olsen-P (Olsen et al., 1954) is a method to measure the plant-available fraction of phosphorus in a weak sodium bicarbonate solution ($NaHCO_3$), appropriate in samples with a pH > 6.2. The fraction of P soluble in $NaHCO_3$ is closely related to a form of phosphorus, which highly correlates with the amount of plant- available phosphorus. Following the procedure of Olsen et al. (1954), 2.25 g of air-dried soil (< 2 mm) and 0.1 g of solid carbon were mixed with

45 ml extraction solution (0.5 M $NaHCO_3$ adjusted to pH 8.5). Carbon was added to remove dissolved organic matter from the extractions. The suspensions were shaken for 30 min at 25 rpm and room temperature. Coarse particles were given 20 min time to settle, before being filtrated through folded paper filters. Filtrates were kept in the fridge only for one night to avoid microbial activity. The next day, 5 ml of the Olsen-P extract were mixed with 5.25 ml sulphuric acid (0.25 M H_2SO_4), to maintain a dilution factor of 2.05. Some samples had to be diluted in a further step to stay under the limit of detection. Finally, 2.05 ml of a staining reagent for acidified samples were added to trigger a colour reaction, following the procedure of molybdate blue colorimetry method (Murphy and Riley, 1962). After a reaction time of 15 - 20 min, samples were analyzed under a UV/VIS spectrophotometer, set to a wavelength of 881 nm. The measured absorbance in the samples was blank corrected and the P concentration was calculated in (mg L^{-1}). Finally, P concentrations in soil were converted to (mg P kg^{-1} soil), following Eq. 2.2:

$$\text{mg P kg}^{-1}\text{ soil} = \text{P concentration [mg L}^{-1}\text{]} * \text{extraction volume [ml]} * \frac{1}{\text{kg dry soil}}$$

Eq. 2.2: *Calculation of plant-available phosphorus in soil.*

2.2.5 Soil pH

The soil pH was measured following the ÖNORM L 1083-89, using ProLab 4000 (SCHOTT instruments, Germany). 10 g of air-dried soil (< 2 mm) were weighed in a 50 ml bottle and 25 ml of 0.01 M $CaCl_2$ solution were added. The suspensions were shaken well by hand and left to equilibrate for 2 h. After that, samples were stirred again. Once the soil settled to the bottom, an electrode was inserted into the liquid to the same depth for every sample. Before measurement, the pH meter was calibrated to pH 4, 7 and 10. Finally, pH values were transformed into concentrations of mol H^+ L^{-1} for further calculations but transformed back into pH values for data visualization.

2.2.6 TC & TN

For measuring total contents of soil carbon and nitrogen, air-dried soil sieved to 2 mm, was milled (ball mill, Retsch). Afterwards, soil samples were transferred to an oven and dried at 105°C for 48 h. After cooling samples down in an exsiccator, 0.2 g of the homogenized subsample were weighed in a tin foil. Total carbon (TC) and total nitrogen (TN) concentrations

were measured in the furnace exhaust during dry combustion (Vario Macro Cube, Elementar, Germany). Inorganic carbon concentrations were negligible (data not shown). Thus, carbon concentrations (TC) were assumed being equivalent to soil organic carbon (SOC). The amount of total nitrogen in soil was interpreted as an indicator of the relative potential of the soil to supply nitrogen over time, but not understood as current nitrogen availability. Nitrogen availability was assessed using the N-min method.

2.2.7 Soil water content (gravimetric)

To determine the gravimetric soil water content, 2 - 5 g of soil were weighed in a porcelain cup and the exact mass was noted to 3 decimals. The samples were then oven-dried at 105°C, cooled down in an exsiccator and cooled samples were weighed again. The measured dry weight was used to calculate the mass of water lost, as a percentage of the mass of the dried soil. The gravimetric water content was calculated using Eq. 2.3:

$$\text{Soil Water (\%)} = \frac{[\text{weight of wet soil (g)} - \text{weight of dry soil (g)}]}{\text{weight of dry soil (g)}} \times 100$$

Eq. 2.3: *Calculation of gravimetric soil water content in (%).*

2.3 Biological parameters

For biological quality assessment, we chose one parameters for overall microbial activity. Second, mesofauna was extracted from soil samples and further interpreted using biological indices cited in literature (Menhinick, 1964; Parisi et al., 2005).

2.3.1 Microbial Activity

The hydrolysis of fluorescein diacetate [3´, 6´-diacetylfluorescein, FDA] is a method to measure overall microbial activity in soils (Green et al., 2006). For sample preparation 1 g of air-dried soil (< 2 mm) was mixed with 50 ml of 60 mM sodium phosphate buffer (adjusted to pH 7.6) and 0.50 ml of 4.9 mM FDA lipase substrate solution. The suspensions were swirled for a few seconds and placed in an incubator for 3 h , at 37 °C and under static conditions. After reaction time, 2 ml acetone were added to the solution, to slow down the process. Suspensions were let to settle for 5 min. After an aliquot of the solution was passed through a folded filter paper,

absorbance of the samples was measured immediately on a spectrophotometer, set to a wavelength of 490 nm. Finally, concentrations were converted to FDA values (fluorescein in mg kg^{-1} soil $3h^{-1}$). It is assumed, that with increasing hydrolyse activity the fluorescein solution colours more intensively, resulting in higher values measured by the spectrophotometer. Higher FDA values are understood as an indication for higher microbial activity. A higher microbial activity is generally seen as an indicator for a better soil quality, even though it is not clear which microbes are activated. Thus, within this study the FDA method is seen more as a possible indication than a proof.

2.3.2 Soil Mesofauna

Soil microarthropods were extracted immediately after collection, following the Berlese-Tullgren funnel method. Before soil sampling, litter and vegetation were removed from the soil surface. According to a standardized procedure, sampling started in approx. 0.5 m distance from the plot edges and 5 samples were taken in two rows, with 1 m distance in between. Soil samples were taken from the upper 10 cm of the soil with an auger. Thus, 10 subsamples were mixed to one composite for each plot. The samples were stored in a plastic bag and cooled overnight. The next morning the Berlese-Tullgren extraction was set up. For each plot, the composite of soil samples was homogenized and filled into a beaker up to a volume of 250 ml. Roots, leaves and bigger soil animals were taken out and the sample was carefully transferred to a pot with a 2 mm mesh underneath. Soil lost during handling, was put back into the pot. Sample vials with 40 ml volume, were filled with 10 ml of preservative liquid (70% Ethanol) and inserted beneath the funnel. The construction was then set under light bulbs (30 Watt) at 30 cm distance. The pots filled with soil were gently put on top of the funnel, to avoid soil falling through the mesh and hindering later visual determination. Light was switched on for 5 whole days. The principle behind, is that the light and heat of the bulbs creates a dry soil layer that is hostile for soil mesofauna, which forces them to migrate downwards the funnel, where they eventually fall into a collection vial, containing a preservation solution. The set-up of the Berlese-Tullgren-extraction is also visualized in (fig. 2.4). After the extraction time (120 h), light was switched-off, pots with dry soil were carefully removed and vials were closed and stored under room temperature until analysation. The extracted specimens were visually analyzed and photographed using a wide zoom stereo microscope (Olympus SZX10, magnification range of 6.3 x to 63 x), in the same preservative liquid (70% Ethanol).

Fig. 2.4: *Soil mesofauna extraction using the Berlese-Tullgren apparatus.*

2.3.2.1 Classification

For visual determination, the whole sample was filled on petri-dishes and put under the microscope. Soil microarthropods were identified on class or order level. Nematoda are no arthropods, but belong to mesofauna, due to their body size. Thus, frequencies of Nematoda were recorded and included in the evaluation of total mesofauna abundance. The number of identified mites and springtails, as well as all other taxa in the sample, were counted and recorded in total numbers for each taxon. Despite that, mites were identified on suborder level (e.g. Mesostigmata, Oribatida, Prostigmata), to obtain an overview of possible trends in patterns of Acari family abundance between treatments. The identification of the eco-morphological form of arthropods (soil adaption level) was performed after Parisi et al. (2005). The identified life forms were used to calculate the soil biological quality index (QBS), according to Parisi et al. (2005), which will be explained in detail in chapter (2.3.3.3). The identification and classification of soil arthropods was conducted with the aid of standard keys, provided by the Institute of Agronomy, BOKU, Tulln. In additions, identification of soil adaption levels of Collembola, was conducted using Salamon (s.a) and WWU (s.a.).

2.3.3 Biological indices

The results of the mesofauna extraction were reported for each treatment, as density (indiv. m^{-2} soil) and total abundance (total number of indiv.). Furthermore, soil arthropods were evaluated based on following indicators: i) Menhinick index, ii) C:A ratio and iii) QBS-index.

2.3.3.1 Relative Abundance of Acari and Collembola

The Menhinick index (M) is used to describe the species richness according to Menhinick (1964 cited in Santorufo et al., 2012). We modified this index to emphasize the relative abundance of our key arthropods (e.g. Acari and Collembola), in relation to the total number of

individuals in a sample. Thus, we evaluated the dominance (evenness) of the taxa in each sample. For calculating the relative abundance, the total number of the respective taxa (e.g. Acari or Collembola), was divided by the square root of the total number of individuals in the sample (Eq. 2.4):

$$\text{Relative Abundance (ind. m}^{-2}) = \frac{\text{number of individuals per taxa}}{\sqrt{\text{(total number of organisms)}}}$$

Eq. 2.4: *Abundance of Acari or Collembola in relation to total mesofauna abundance.*

2.3.3.2 C:A ratio

Depending on the total abundance of Collembola and Acari, a ratio was calculated by dividing the total abundance of Collembola by the total abundance of Acari, according to (Bachelier, 1986 cited in Parisi et al., 2005). To avoid a division by 0, a value of 0.1 was allocated to plots where no individual was recorded.

2.3.3.3 Soil biological quality index (QBS)

After extracted specimens were classified at order level, EMI scores (Ecological-Morphological Index*)* were assigned to every individual, to compute the final QBS index for each sample. The QBS index sums up the EMI scores and thereby characterizes the community of soil microarthropods (Parisi et al., 2005). Each microarthropod observed in a sample, received an EMI score between [1 = no adaption to soil] and [20 = maximum level of edaphic adaption]. Table 2.2 gives an overview of microarthropod groups and their eco-morphological indices. Some microarthropod groups (e.g. Acari) only have one possible EMI value [20], because all species belonging to these taxa are similarly adapted to soil (fig. 2.6). Other groups, like Collembola or Coleoptera, show a range of EMI scores [1 - 20], depending on the adaption level of the species observed in the sample (fig. 2.5 and 2.7). As a rule of thumb, eu-edaphic (e.g. deep soil living) life forms score the highest EMI values [20], followed by hemi-edaphic (intermediate) forms with scores proportionate to their degree of adaption. Finally, epi-edaphic (surface-living) forms achieve the lowest value [1]. If more than one eco-morphological form is present in the same taxon, the final score is determined by the highest EMI (fig. 2.5). Hence, the most adapted microarthropod determines the overall EMI score for that group. A greater number of groups well adapted to the soil [higher EMIs], results in a higher QBS value, which is according to Parisi et al. (2005) an indication of a better soil quality.

***Table 2.2**: List of relevant soil microarthropods and their eco-morphological indices (EMI scores), modified after (Parisi et al., 2005).*

Group	EMI
Protura	20
Collembola	1 - 20
Coleoptera (larvae)	1 - 20
Coleoptera (adult)	1 - 20
Diptera (larvae)	10
Diptera (adult)	10 - 20
Acari	20
Araneae	1 – 5
Chilopoda	10 - 20
Diplopoda	10 - 20
Symphyla	20

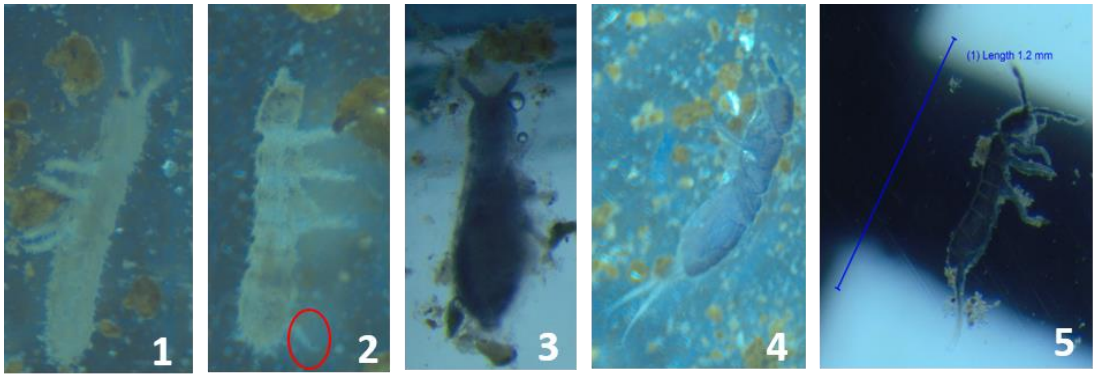

***Fig. 2.5**: Examples of Collembola belonging to different morphological groups and EMI-classes. Calculation of QBS according to Parisi et al. (2005): 1.) clearly eu-edaphic, no furca, pigmentation or eyes [score 20]; 2.) slightly eu-edaphic with ocelli, reduced furca and pigmentation [score 10]; 3.) hemi-edaphic with pigmentation [score 6]; 4.) epigeous with pigmentation in violet, well developed ocelli, small body size limited to litter [score 4]; 5.) more developed epigeous form [score 2].*

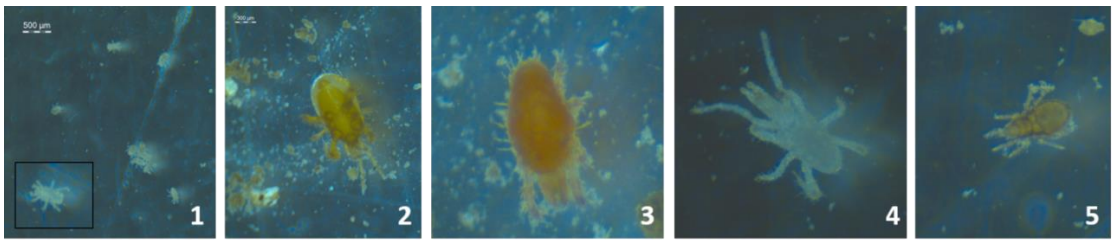

***Fig. 2.6**: Examples of Acari: 1.) Prostigmata, 2.) Mesostigmata, 3.) Prostigmata, 4.) Mesostigmata (Gamasina, 400 µm), 5.) Oribatida (Oppioidea, 200 µm. All Acari received EMI score [20], according to Parisi et al. (2005).*

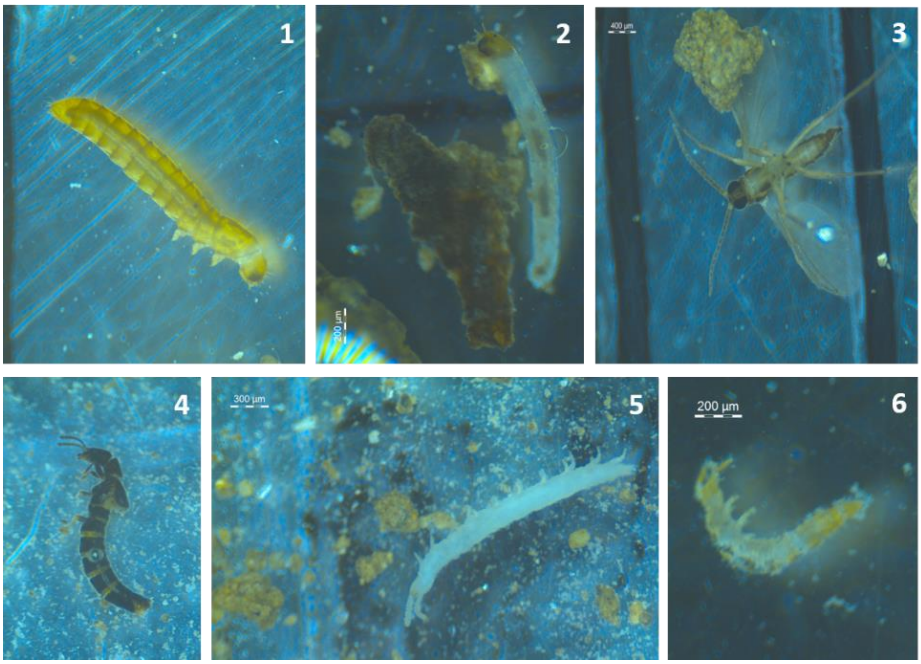

Fig. 2.7: *Examples of other edaphic microarthropods and assigned EMI scores, according to Parisi et al. (2005): 1.) Coleoptera Larva [10], 2.) Diptera Larva [10], 3.) Diptera Adult [1], 4.) Coleoptera Adult [10], 5.) Symphyla [20], 6.) Protura [20].*

2.3.4 Set of soil quality indicators

For soil quality evaluation we chose a minimum set of specific indicators (table 2.3), which relate to soil functions that are influenced by the management practices conducted (e.g. cropping hyperaccumulators, fertilizers). Because physical properties are mostly inherent and do not change immediately after agronomic practices (Bünemann et al., 2018), only dynamic parameters (chemical and biological), which are considered being more sensitive to management practices, were analysed.

Table 2.3: *Selected soil quality indicators, in relation to soil functions and influence by agronomic practices (USDA, 2001, Bünemann et al. 2018, Karlen et al. 1997, Karlen et al., 2008; Moldenke, s.a). In this study SOC is equivalent to TC.*

	Indicator	Role in soil functioning	Agronomic measure
biological	Microbial activity [FDA]	Decomposition (humus production, nutrient cycling) Soil physical stability (soil aggregation)	Fertilization
	Mesofauna [C:A ratio, abundance; QBS]	Decomposition (breakdown of organic matter) Soil structure (microarthropod moder) Stimulation of microbial activity and -growth	Fertilization Tillage Crop management
chemical	Soil Organic Carbon [TC = SOC]	Crop production Growth and support of microorganism and soil fauna Cycling and supplying essential nutrients (N, P, S) Filter, buffer and degradation of contaminants Carbon – and water storage Soil physical stability	Fertilization (organic) Tillage Crop management (diversity, intensity, crop residues)
	Total Nitrogen [TN]	Crop production Stimulation of microbial activity and -growth	Fertilization (organic)
	pH	Nutrient availability (e.g. P, Zn) Toxicity and deficiency of trace elements Ammonification and nitrification Microbial habitat Plant root growth and -development Absorption or mobilization of contaminants Carbon storage	Fertilization Liming
	Available P & K [P-Olsen, $BaCl_2$-K]	Supports plant growth (essential macronutrients)	Fertilization Maintaining pH (6 - 6.5)
	Mineral Nitrogen [Nmin, $NO3^-$, $NH4^+$]	Biological activity (N mineralizable by MO) Plant growth	Fertilization Crop rotation using legumes

2.4 Statistics

The sulphur treatment from 2017 still influenced site properties and significantly affected pH. However, plots treated with sulphur in 2017 (6_CM, 8_NPK, 10_PM, 15_OM) were not generally excluded from the statistical analysis, as we did not find any outlier in sulphur plots. Despite that, Plot 1 receiving NPK fertilizer was excluded from the statistical evaluation of the C:A ratio at t6, as it was an extreme outlier (caused by the fact that no specimen of Acari was found in this plot). A 2-way ANOVA (2 factors: treatment, block) was conducted to i) detect differences between treatments (fixed factor) at each time point (t4, t5, t6), and ii) include the influence of block position (random factor; complete randomized block design), followed by a Tukey HSD post-hoc test. Normal distribution was tested using the residuals of the model. In addition, a 3-way ANOVA (fixed factors: treatment and time, random factor: block) was performed to compare differences between time points (t4, t5, t6). First, to evaluate the changes of parameters over one growing period from spring (t4, April) to fall (t6, October). Second, to detect effects of fertilization by comparing t4 (April) with t5 (May), assuming that one month after plantation (at the early growth stage of *O. chalcidica*) the influence of the hyperaccumulator on soil parameters is negligible. Third, to detect changes in soil characteristics, as a result of hyperaccumulator cultivation (t5, t6). Differences of significance were reported for all tests based on the alpha level ($p < 0.05$). Unfortunately, we could not test for interactions between factors time and treatment, as sample size for treatments were too small (n=4). Finally, a covariance analyses was performed, to test the influence of continuous covariates (e.g. pH) on dependent variables (e.g. DTPA-Ni concentration), while having the treatment as fixed factor and block as random factor. The influence of the covariate was reported significant if ($p < 0.05$). A positive or negative influence was interpreted according to the b - value. Statistical analysis was performed using IBM SPSS Statistics 24.

3. RESULTS

A summary of all soil physicochemical and biological parameters as well as results of covariance analyses are listed in the appendix.

3.1 Effect of agromining on Ni availability

To detect changes in soil Ni-concentrations before and after cropping the hyperaccumulator *O. chalcidica*, a comparison between the soil conditions at t5 (early growth stage) and t6 (harvest) was conducted.

Potentially bioavailable Nickel (DTPA-Ni). At t5, there were no significant differences between fertilized (NPK, CM, PM, OM) and unfertilized plots (C, LD) planted with *O. chalcidica* ($p > 0.05$), as well as no differences compared to Ref ($p = 0.315$). On the contrary, significant differences were observed at harvest, t6 ($p = 0.011$). Except PM, all treatments showed significantly lower DTPA-Ni concentrations compared to Ref (see fig. 3.1 and table A.7). The depletion of the potentially bioavailable Ni-pool was significant between t5 and t6 ($p < 0.001^{***}$) and was significantly different between the treatments ($p = 0.001^{**}$). Furthermore, DTPA-Ni levels at t5 and t6 were influenced by the block position ($p < 0.05$). Thus, lowest DTPA-Ni concentrations were observed in block 1.

The DTPA-Ni Pool increased significantly with $Sr(NO_3)_2$-extractable Ni at t5 ($p < 0.001^{***}$, b = + 10.167) and t6 ($p < 0.001^{***}$, b = + 15.689). Highest values were observed in 3 out of 4 sulphur plots (6_CM, 8_NPK, 10_PM) at t5, while at t6 also Ref showed similarly high values as sulphur plots. Furthermore, we observed a significant influence of the block position on Ni-concentrations in soil ($p < 0.001^{***}$).

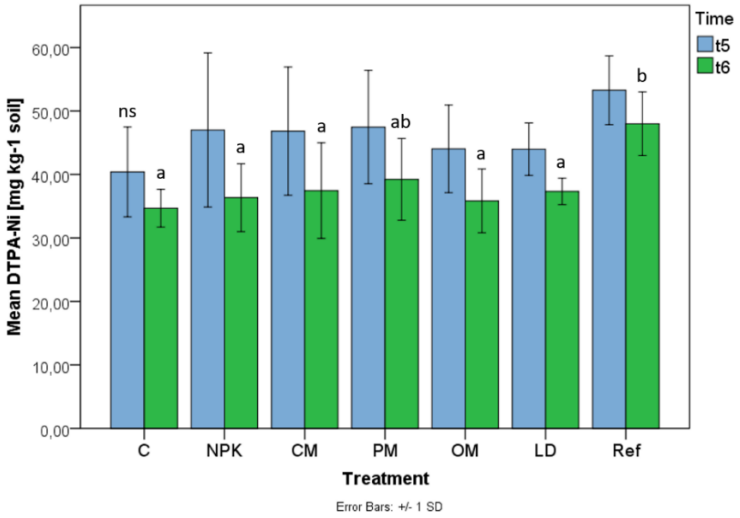

***Fig. 3.1**: DTPA-Ni in mg kg^{-1} (mean ± standard deviation; n=4) for t5 and t6. Differences between treatments (C, NPK, CM, PM, OM, LD, Ref) at t6 are indicated with lowercase letters ($p < 0.05$). No significant differences between treatments at t5 ($p > 0.05$).*

All plots with the lowest DTPA-values, despite higher levels of $Sr(NO_3)_2$-Ni, were situated in block 1. Similarly, DTPA-Fe and DTPA-Mn were lowest in block 1 and increased down the slope ($p < 0.05$). No significant differences in heavy metal concentrations in DTPA-extracts (Cu, Co, Fe, Mn) between treatments were recorded (table A.7). Despite that, concentrations of Ni, Co and Fe in DTPA-extracts significantly decreased with a higher pH at t5 and t6 and for DTPA-Cu at t6 (fig. A.1). This trend was especially pronounced in sulphur plots of CM, PM and NPK with lower pH, where metal concentrations were substantially higher than in other plots of the same treatment. Additionally, DTPA-extractable Ni significantly increased with Cu ($p = 0.003^{*}$, b = + 16.720), Co ($p < 0.001^{***}$, b = + 21.375) and Fe ($p < 0.001^{***}$, b = + 0.379) at t5. At harvest (t6), this was still the case for Co and Fe, but not for Cu in DTPA-extracts. The trend of higher DTPA-Ni with DTPA-Cu at t5, occurred in all treatments ($p = 0.003$, b = +16.720) except Ref, where DTPA-Ni concentrations varied with block position, but independently of DTPA-Cu concentrations.

Furthermore, the DTPA-Ni pool decreased with greater CEC ($p = 0.008$, b = - 0.594) and Ca concentrations ($p = 0.005$, b = - 0.627), a trend also observed for DTPA-Co and DTPA-Cu. This effect was only observed at t5. Moreover, DTPA-Ni increased with TC ($p = 0.010$, b = + 2.153) and TN ($p = 0.006$, b = + 21.964) at t5 as well as available P at t5 ($p = 0.006$, b = + 0.680) and t6 ($p = 0.036$, b = + 0.510). Besides that, no further changes of soil physicochemical parameters (e.g. Mg, K, Nmin) or soil biological parameters (e.g. FDA, QBS), with the DTPA-Ni pool ($p > 0.05$) were noticed.

Exchangeable Nickel ($Sr(NO_3)_2$). There were no marked differences in the exchangeable Ni fraction between treatments at the beginning of the season (t5, $p = 0.074$), or at harvest, after 6 months cropping hyperaccumulator *O. chalcidica,* (t6, $p = 0.088$). A 3-factorial ANOVA showed that concentrations of exchangeable Ni, assessed by $Sr(NO_3)_2$ extraction, varied significantly over the growing period ($p = 0.006$), as well as in between treatments. We observed a general trend of decreasing $Sr(NO_3)_2$-Ni over time, which was differently pronounced among treatments. Values tended to be higher in Ref and inorganic fertilized plots (NPK), compared to C and OM. At t5 maximum values were reported in NPK (1.83 ± 0.83 mg kg^{-1}), while at t6 Sr(NO3)-Ni was highest in Ref (1.16 ± 0.11 mg kg^{-1}). Over the whole vegetation period, the lowest $Sr(NO_3)_2$-Ni levels were reported in OM (~ 0.62 mg kg^{-1}).

Similar to DTPA-Ni, the $Sr(NO_3)_2$-extractable Ni decreased with a higher pH at t5 ($p < 0.001^{***}$, b = - 1.578) and t6 ($p < 0.001^{***}$, b = - 1.217). This effect was significantly influenced by treatments, while the impact of block position was only significant at t6 ($p < 0.05$). Thus, with respect to pH influence, OM and CM showed the lowest $Sr(NO_3)_2$-Ni levels, followed by C and LD plots. On the contrary, Ni levels in NPK and Ref tended to be higher. Furthermore, sulphur plots (6_CM, 8_NPK, 10_PM, 15_OM) showed considerably higher $Sr(NO_3)_2$-Ni concentrations. Just as DTPA-Ni, $Sr(NO_3)_2$-extractable Ni decreased with higher amounts of exchangeable Ca ($p = 0.015$, b = - 0.209) at t5, with exception of 4 plots (3 sulphur plots 6_CM, 8_NPK, 10_PM and one additional NPK-plot). Furthermore, $Sr(NO_3)_2$-Ni decreased with a greater Ca:Mg ratio ($p = 0.042$, b = - 7.417) and CEC ($p = 0.033$, b = - 0.187) at t5. On the contrary, there was a generally positive trend of increasing $Sr(NO_3)_2$-Ni with exchangeable Mg concentrations, in all treatments at t6 ($p = 0.054$, b = + 0.031). In addition, $Sr(NO_3)_2$-Ni decreased with available P ($p < 0.001$, b = - 1.217) and increased with Nmin ($p = 0.018$, b = + 0.055), at t5; while no significant effects with TC, TN, available K or biological parameters (e.g. mesofauna-indices, FDA) were observed.

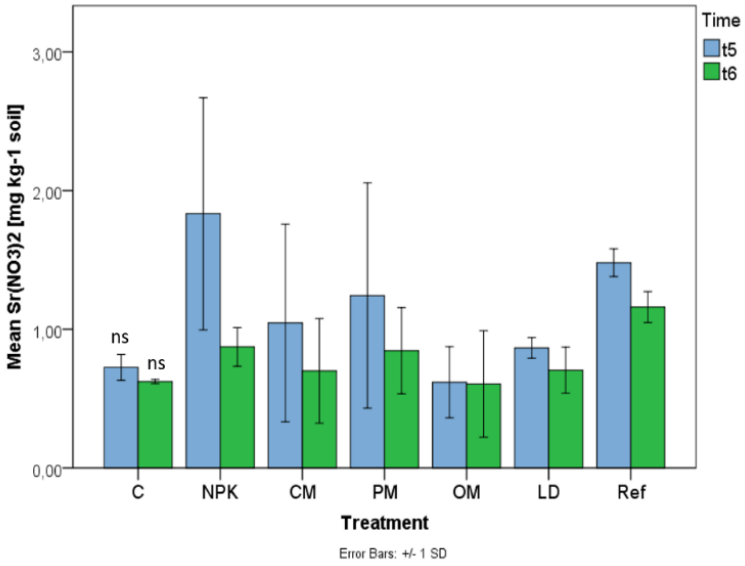

Fig. 3.2: *Ni extracted with $Sr(NO_3)^2$ in $mg.kg^{-1}$ (mean ± standard deviation; n=4) for t5 and t6. No significant difference between treatments ($p > 0.05$).*

Sulphur. Results of ANOVA changed considerably, after excluding sulphur plots, especially for $Sr(NO_3)_2$-Ni. The DTPA-Ni concentrations were sign. higher in Ref, compared to all other treatments at t5 ($p < 0.001^{***}$) and t6 ($p < 0.001^{***}$), while other treatments did not differ in DTPA-Ni concentrations. At t5, $Sr(NO_3)_2$-Ni was significantly greater in NPK and Ref ($p < 0.05$). At t6, significantly highest $Sr(NO_3)_2$-Ni was recorded in Ref, followed by NPK and were significantly higher than in CM and OM. The significant lowest concentration of $Sr(NO_3)_2$-Ni was detected in OM at t6. Moreover, the exclusion of sulphur plots had further severe consequences on results of covariance analyses, which are indicated in the regarding scatterplot for each covariance (fig. A.1). DTPA-Ni still significantly increased with DTPA-Co

(p = 0.023, b = + 19.224) and DTPA-Fe (p = 0.028, b = + 0.180) at t5. $Sr(NO_3)_2$-Ni significantly decreased with pH at t5 (p = 0.049, b = - 1.293). All other covariances were non-significant (p > 0.05).

3.2 Effect of agromining on soil physicochemical parameters

For the soil physicochemical parameters a comparison between time points t4 and t5 was conducted for mineral nitrogen fractions ($N\text{-}NO3^-$, $N\text{-}NH4^+$) as well as between t5 and t6 for pH, exchangeable cations (Ca, Mg, K) and cation exchange capacity (CEC). Total carbon and nitrogen (TC, TN) and available phosphorus (Olsen-P) were analysed over the whole vegetation period (t4, t5, t6).

3.2.1 pH

pH ($CaCl_2$). The pH did not differ significantly between treatments and blocks at t5 and t6 (p > 0.05). Thus, neither fertilization nor cropping hyperaccumulators significantly influenced the pH in soil. However, 4 plots were clearly affected by the sulphur treatment in 2017. These plots were distributed between 4 treatments, namely CM (plot 6), NPK (plot 8), PM (plot 10) and OM (plot 15). As visualized in fig. 3.3, pH substantially decreased in those plots, which might also be one reason for the higher standard deviations in NPK, CM, PM and OM. Furthermore, we observed different effects of pH on soil parameters (e.g. $Sr(NO_3)_2$-Ni, Ca, CEC, TN) in the sulphur plots. Consequently, statistical analyses were repeated without sulphur plots (excl. S) to compare results inclusive and exclusive sulphur influence. Thus, differences of pH between treatments became significant at t5 ($p < 0.001^{***}$) and t6 ($p = 0.002^{*}$).

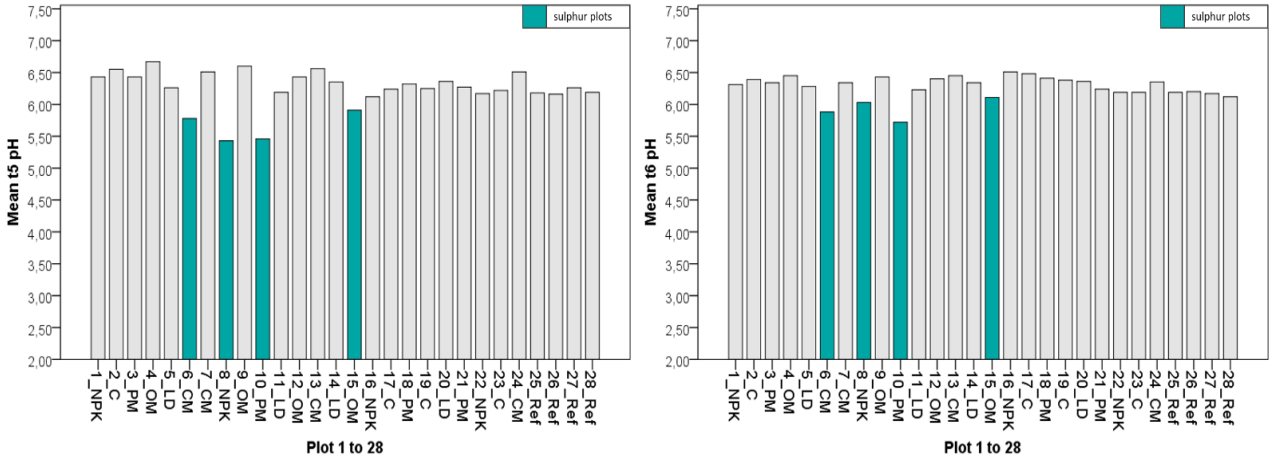

***Fig. 3.3**: Comparison of pH ($CaCl_2$) for each plot and treatment (C, NPK, CM, PM, OM, LD, Ref) at t5 and t6. Plots treated with sulphur in 2017 are coloured in turquoise.*

Exclusive sulphur plots, pH was lowest in Ref (6.19 ± 0.04) at t5, which was significantly different to C (6.32 ± 0.16), PM (6.34 ± 0.03), CM (6.53 ± 0.12) and OM (6.57 ± 0.12). Moreover, pH was significantly higher in OM than in PM, C, LD (6.29 ± 0.08), NPK (6.24 ± 0.17) and Ref, at t5. At t6, lowest pH was again observed in Ref (6.17 ± 0.04) and was significantly below pH in C (6.36 ± 0.12), CM (6.38 ± 0.06) and OM (6.43 ± 0.03). The pH in OM was significantly higher than in NPK (6.34 ± 0.10), PM (6.33 ± 0.85), LD (6.30 ± 0.06) and Ref. Generally, the influence of sulphur application on pH, seemed more pronounced six weeks after fertilization (t5), than at harvest (t6). Covariance analyses including sulphur plots, indicated increasing pH with Ca ($p = 0.004$, b= + 0.030) and CEC ($p = 0.003$, b= + 0.030) at t5, as well as a decrease of pH with higher Mg concentration at t6 ($p = 0.016$, b= + 0.717). Interestingly, all 4 sulphur plots contained almost the same level of Ca and CEC at t5, while showing different pH values. Excl. sulphur plots, covariances were not at a significant level ($p < 0.05$).

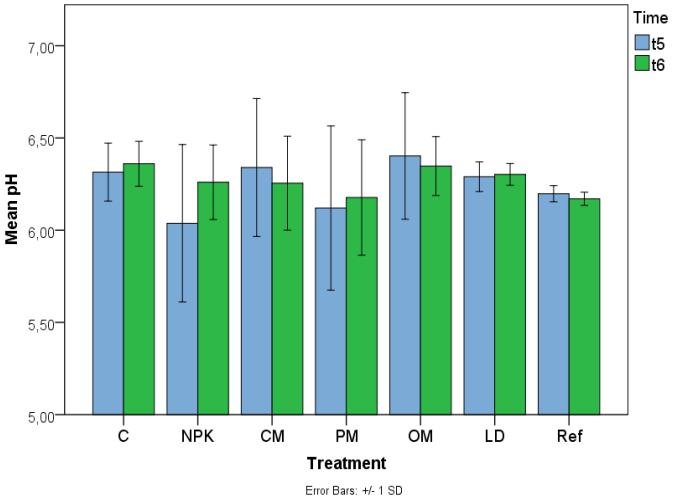

Fig. 3.4: *Mean pH ($CaCl_2$) incl. S (mean ± standard deviation; n=4) at t5 and t6. No significant differences between treatments (C, NPK, CM, PM, OM, LD, Ref) and time points (p>0.05).*

3.2.2 Exchangeable Cations & Cation Exchange Capacity

Calcium (Ca-$BaCl_2$). At t5 and t6, amounts of Ca were significantly different between treatments and blocks ($p < 0.001^{***}$). At t5, highest Ca concentrations were recorded in OM (130 ± 21) and were significantly higher than in all other treatments ($p < 0.05$). In contrast, lowest amounts of Ca were measured in Ref (90 ± 8), followed by NPK (102 ± 14). Furthermore, lowest Ca concentrations were observed in block 1 and increased down the slope ($p < 0.001^{***}$). At t6, differences between OM and other treatments diminished, as Ca concentrations in OM tended to decline, while they increased in other fertilized plots. Thus, at t6 greater concentrations of Ca in OM and LD were significantly different to NPK and Ref ($p < 0.05$). In Ref, Ca levels did not change at all over the growing period (90 ± 8). Differences

in Ca concentrations between t5 and t6 were not significant ($p = 0.255$). Moreover, the exclusion of sulphur plots did not show considerably different results at t5 and t6, except that at t6 Ca in OM (131 ± 3) was also significantly higher than in C (110 ± 10), besides NPK (97 ± 18) and Ref (90 ± 8).

Magnesium (Mg-$BaCl_2$). In contrast to Ca, the amount of Mg in Ref was significantly higher than in all other treatments ($p < 0.001^{***}$) at t5. At t6, Mg contents were still the highest in Ref, but also increased in PM (table A.7). The differences in Mg concentrations between t5 and t6 were highly significant ($p < 0.001^{***}$), however Mg increased differently between treatments and blocks ($p < 0.001^{***}$). The block influence was quite contrary to other soil parameters, as highest Mg levels were observed in block 1 and decreased down the gradient with lowest contents in block 4. Moreover, Mg concentrations (excl. S) were significantly highest in Ref at t5 and t6, compared to all other treatments. The remaining treatments did not show significant differences in Mg concentration when excluding sulphur plots ($p > 0.05$). However, lowest Mg concentration (excl. S) was observed in PM (53.2 ± 2.3) at t5 and increased to (59.1 ± 1.2) until harvest. Thus, at t6 lowest Mg content was measured in C (57.0 ± 5.5).

Ca:Mg ratio. At t5, the Ca:Mg ratio was significantly lowest in Ref (1.36 ± 0.16), with respect to all other treatments ($p < 0.016$). The highest Ca:Mg ratio was recorded in OM (2.26 ± 0.37). The ratio of Ca:Mg in OM was significantly higher than in Ref (1.36 ± 0.16, $p < 0.001^{***}$) and NPK (1.83 ± 0.40, $p = 0.029$). The change of Ca:Mg ratio over the growing period was highly significant ($p = 0.002^{*}$) and significantly different among treatments and blocks ($p < 0.001^{***}$). The Ca:Mg ratio decreased with higher Mg contents in all treatments. This significant influence of Mg is also visible when looking at post-hoc results. At t6, Ref showed significantly lower Ca:Mg ratio than all other treatments, while containing significantly highest Mg concentrations ($p < 0.05$). Similar results were conducted when excluding sulphur plots. At t5 and t6, the Ca:Mg ratio (excl. S) was significantly lowest in Ref, compared to all others. Furthermore, Ca:Mg ratio in OM was significantly higher than in NPK and Ref.

Potassium (K-$BaCl_2$). Significant differences were recorded among treatments at t5 and t6 for K ($p < 0.001^{***}$), without block influence ($p > 0.05$). Fertilization with CM significantly increased K concentrations, compared to all other treatments at t5 and t6 ($p < 0.001^{***}$), inclusive and exclusive sulphur plots.

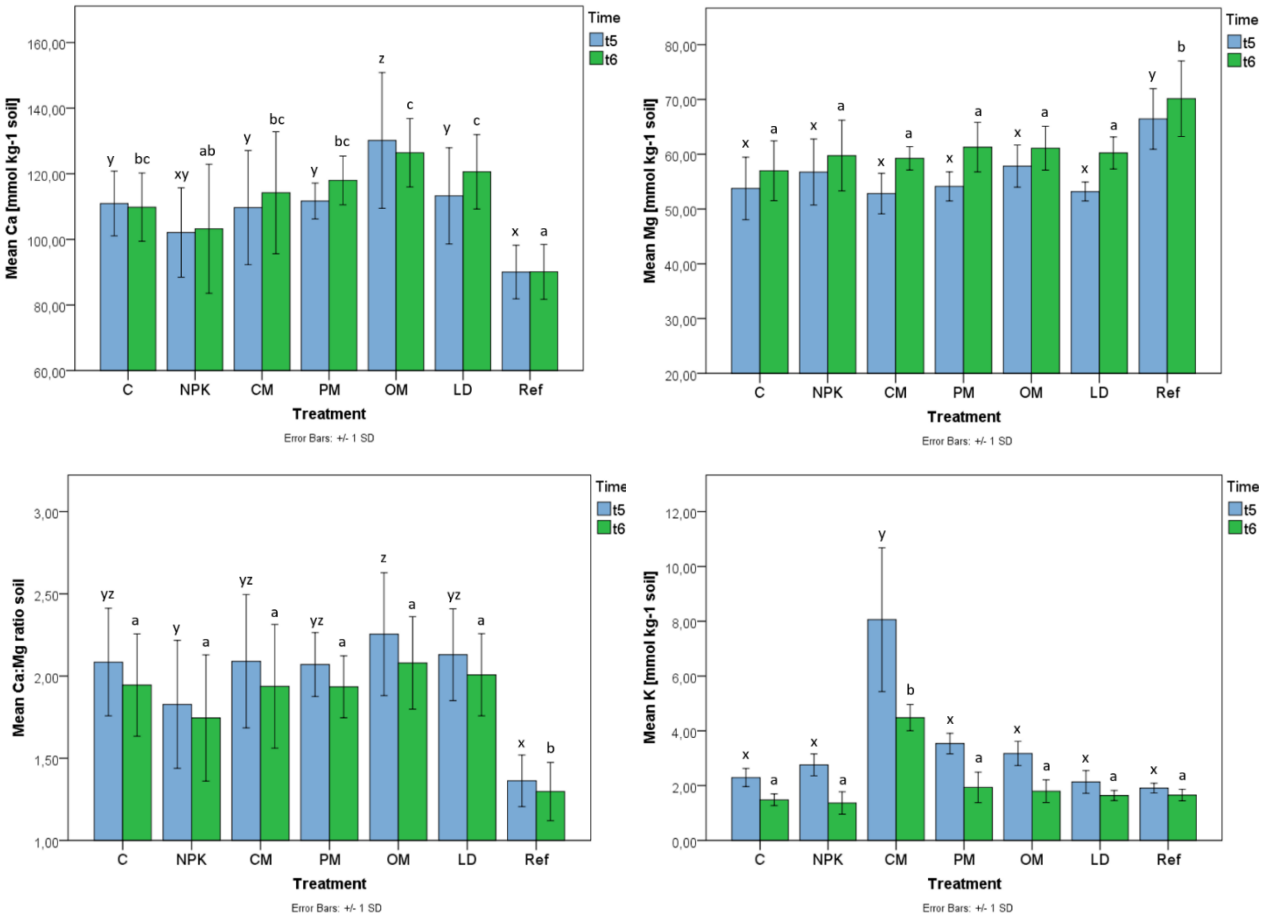

Fig. 3.5:*Concentrations of Ca, Mg and K in mmol$_c$ kg^{-1} soil and Ca:Mg ratio incl. sulphur plots (mean ± standard deviation; n=4). Differences between treatments (C, NPK, CM, PM, OM, LD, Ref) at t5 and t6 are indicated with lowercase letters (p < 0.05).*

Cation Exchange Capacity (CEC). The CEC was significantly different between treatments and blocks at t5 and t6 ($p < 0.05$). At t5, CEC was highest in OM (191 ± 22). At t6, CEC was still significantly greater in OM compared to C, Ref and NPK, but not different to other organic fertilizers (PM, CM) and LD ($p > 0.05$). We observed a significant increase of CEC between t5 and t6 ($p = 0.002^{*}$), however differently between treatments and blocks ($p < 0.001^{***}$). Covariance analyses (incl. S) showed a significant increase of Ca ($p = 0.004$, $b = + 13.431$) and CEC ($p = 0.003$, $b = + 13.843$) with pH, at t5. Furthermore, CEC (incl. S) significantly decreased with higher labile ($Sr(NO_3)_2$)-Ni at t5 ($p = 0.003$, $b = - 6.160$). While these trends were non-significant after excluding sulphur plots from analyses, the increase of CEC with higher contents of Ca and Mg was significant with and without sulphur plots (fig. A.1).

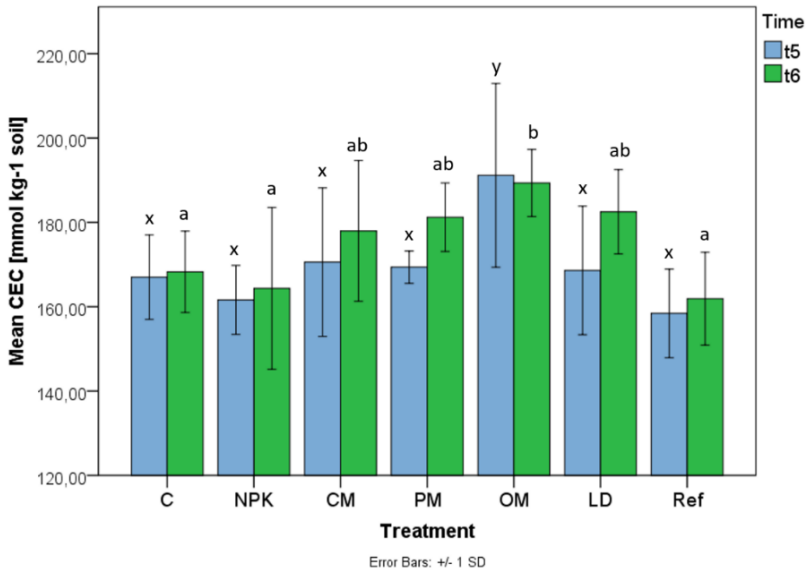

Fig. 3.6: *Mean CEC incl. S (mean ± standard deviation; n=4). Differences between treatments (C, NPK, CM, PM, OM, LD, Ref) at t5 and t6 are indicated with lowercase letters ($p < 0.05$). Differences between t5 and t6 were significant ($p < 0.05$).*

3.2.3 Total Carbon

Total Carbon (TC). Over the whole vegetation period we observed first a rise of total carbon contents within one month of fertilization from t4 to t5, followed by a decline until harvest at t6. This trend was observed in organically fertilized (CM, PM, OM) and non-fertilized treatments (C, LD), vegetated with the hyperaccumulator. In contrast, TC tended to decrease in Ref. TC contents did not differ significantly between time points (t4, t5, t6), but among blocks ($p < 0.001^{***}$). Treatments in block 1 showed the lowest TC values, while values increased downwards the slope from block 2 to 3 and finally with block 4 having the highest TC contents in all treatments; a significant trend observed over the whole growing period ($p < 0.05$).

Differences in TC contents between treatments were non-significant at t4, just two weeks after fertilization ($p = 0.286$). One month later (t5), first significant differences between treatments due to a fertilization effect, were recorded ($p = 0.006^{*}$). TC was significantly lower in NPK, compared to CM ($p = 0.049$) and OM ($p = 0.013$). These differences were still detectable at t6. At harvest (t6), highest TC contents were observed in CM (24.3 ± 4.5) and OM (24.1 ± 4.1), while the lowest TC contents occurred in Ref (20.4 ± 4.0) and NPK (20.6 ± 7.2). Total amounts of carbon were considerably lower in Ref than in CM ($p = 0.054$) and OM ($p = 0.072$). Furthermore, inorganic fertilization was the least effective in improving soil TC, with respect to CM ($p = 0.064$) and OM ($p = 0.085$).

Besides that, soil TC increased significantly with TN at t4 ($p < 0.001^{***}$, b = + 6.772) in all treatments, but differently among blocks ($p < 0.05$). At t5, six weeks after fertilization, we observed again higher TC contents with increasing TN levels ($p < 0.001^{***}$, b = + 9.076). This trend was however different between treatments and blocks ($p < 0.05$). Thus, we observed a clear influence of block position on TC levels two (t4) and six weeks (t5) after fertilization, with lowest values in block 1 and increasing contents of TN together with TC down the slope. At t6, TC still increased with TN ($p < 0.001$, b = + 10.771), but without block influence ($p > 0.05$).

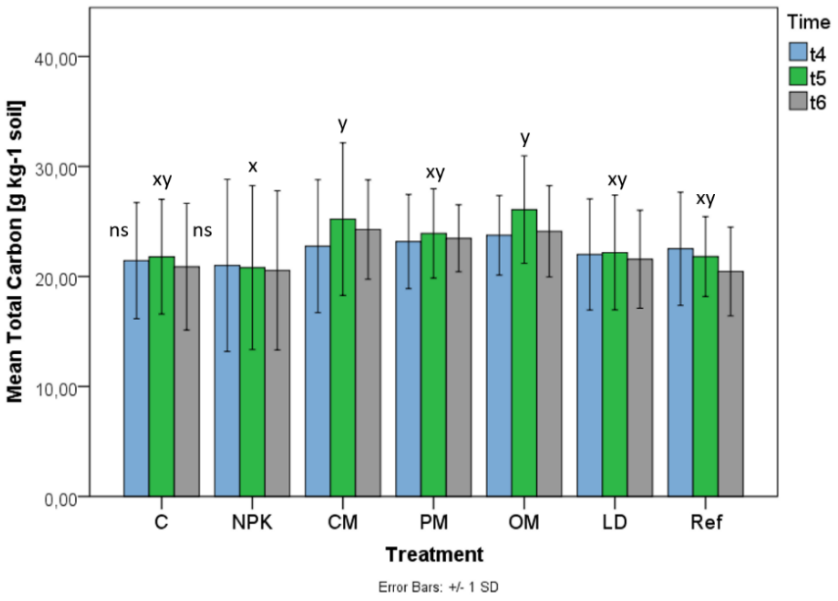

Fig. 3.7: *Total carbon contents in soil in g kg^{-1} (mean ± standard deviation; n=4). Differences between treatments (C, NPK, CM, PM, OM, LD, Ref) at t5 are indicated with lowercase letters ($p < 0.05$). No significant differences at t4 and t6 and between time points t4, t5 and t6 ($p > 0.05$).*

Furthermore, we recorded significantly higher TC with increasing contents of available P incl. sulphur plots ($p < 0.05$), over the whole growing period. Like TN, TC increased first with rising P in all treatments at t4 ($p = 0.012$, b = + 0.164), t5 ($p < 0.001^{***}$, b = + 0.262) and t6 ($p < 0.001^{***}$, b = + 0.262). This positive trend was significantly different between treatments and blocks ($p < 0.05$). Thus, except for Ref (no fertilizer, no hyperaccumulator), the TC contents increased with higher P. Furthermore, lower values were again observed in block 1, with an increase of nutrient contents down the slope until block 4, containing highest amounts of TC together with P ($p < 0.05$). Additionally, TC increased with higher concentrations of available K ($p = 0.029$, b = + 0.909) at t5, with significant differences between treatments and blocks ($p < 0.05$). This trend was not observed at t6. Furthermore, TC decreased with C:N ratio at

t4 ($p = 0.010^{*}$, b = - 1.093) and t5 (p = 0.028, b = - 2.027), while there was a tendency of increased TC with higher C:N ratio at t6 (p = 0.065, b = - 2.462). Despite that, we did not discover a significant covariance of soil TC with any other soil parameter (e.g. Ca, Mg, Ca:Mg ratio, CEC, pH).

Repeating analyses exclusive sulphur plots revealed different results. At t5, the concentration of TC in OM was significantly higher than in all other treatments. In addition, CM and PM showed significantly higher TC contents than NPK ($p < 0.05$). At t6, highest TC was still recorded in OM and was significantly greater compared to C, LD, Ref and NPK ($p < 0.05$). Furthermore, TC significantly raised with TN. In contrast, trends for TC and C:N ratio, available P and -K became non-significant ($p > 0.05$). However, the increase of TC with Ca was significant (p = 0.0.15, b = + 0.146), after excluding sulphur plots from covariance analyses.

3.2.4 Total Nitrogen

Total Nitrogen (TN). In contrast to total carbon, total nitrogen contents tended to increase between fertilization and plantation of *O. chalcidica* (t4) and harvest (t6) (p = 0.059). This trend was significantly different between treatments and blocks ($p < 0.001^{***}$). While differences between t4 and t5 were non-significant (p = 0.992), the rise of TN between t5 and t6 was significant (p = 0.045). At t4, no significant differences in TN between treatments and blocks were detected (p = 0.429). One month later (t5), TN levels differed considerably. The highest amount of TN was recorded in OM (2.10 ± 0.46), which was considerably greater compared to NPK (1.69 ± 0.65, p = 0.090). This trend was again observed at harvest (t6), with substantially lower amounts of TN in NPK, compared to CM (p = 0.067) and OM (p = 0.098). The influence of the block position on TN contents at all three time points (t4, t5, t6), was at a highly significant level ($p < 0.001^{***}$).

Like TC, TN decreased with higher C:N ratio at t4 and t5, incl. and excl. S (fig. A.1). TN increased with higher P contents at t5 ($p < 0.001^{***}$, b = + 0.023) and t6 ($p = 0.006^{*}$, b = + 0.020), but differently between treatments and blocks ($p < 0.05$). Again, TN contents in Ref varied due to block position (lowest in block 1), but independent of P concentrations in soil. A trend of increasing TN with available P was recorded when sulphur plots were excluded from statistics, but only for t6 (p = 0.017, b = + 0.018). Furthermore, TN seemed to increase with Nmin (p = 0.035, b = +0.017) and $N\text{-}NO3^{-}$ (p = 0.034, b = - 0.021) at t5, with significant differences between treatments and blocks ($p < 0.05$). However, no such trend was detected

excl. sulphur plots. Like available P, TN increased with higher amounts of available K at t5 ($p = 0.023$, $b = + 0.098$). Even though available K was significantly higher in CM, no significant differences between treatments were recorded ($p > 0.05$). Additionally, a considerable trend of higher TN with increasing Ca concentrations reached a significant level after excluding sulphur plots from covariance analyses ($p = 0.024$, $b = + 0.015$). Furthermore, we observed a tendency of lower TN contents in plots with higher pH ($p = 0.047$, $b = - 0.277$), depending on treatment and block position ($p < 0.05$). In Ref, TN varied again with block position. This trend of decreasing TN with higher pH was also observed at t5, excl. S ($p = 0.075$, $b = - 0.764$). Repeating ANOVA excl. sulphur plots revealed significant differences between treatments for t5 ($p = 0.007$) and t6 ($p = 0.028$). At t5, the amount of TN in OM was sign. higher compared to C Ref, LD and NPK. All organic amendments increased soil TN significantly compared to inorganic fertilization NPK, but TN in CM and PM was not sign. higher than C. At t6, TN was still the highest in OM, but only sign. higher than Ref and NPK.

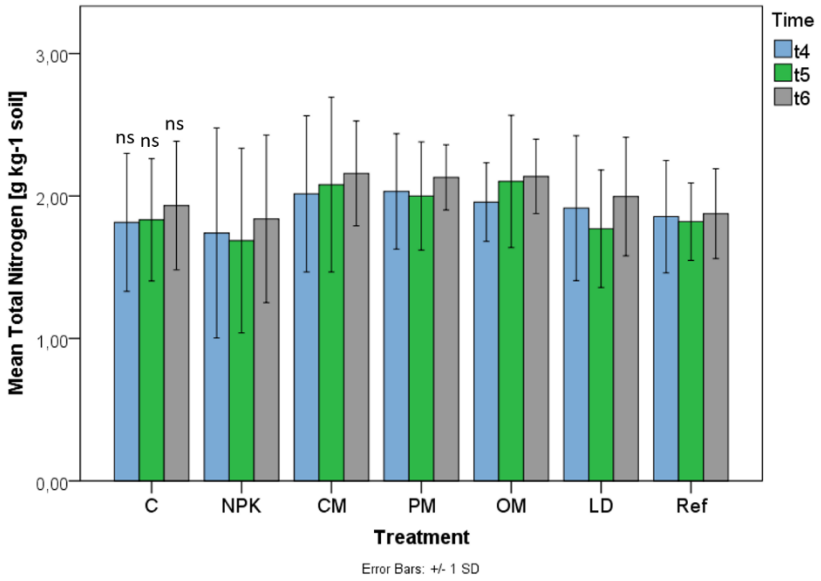

Fig. 3.8: *Total nitrogen contents in soil in g kg^{-1} (mean ± standard deviation; n=4). No significant differences between treatments (C, NPK, CM, PM, OM, LD, Ref) at t4, t5 and t6. Changes in TN were significant between time points t5 and t6 ($p < 0.05$).*

3.2.5 Mineral Nitrogen

Soil mineral nitrogen (Nmin) is an indicator for the readily available nitrogen for plant uptake. As shown in table A.6, the mineral nitrogen fraction was dominated by nitrate ($N\text{-}NO3^-$), while ammonium ($N\text{-}NH4^+$) was very low and sometimes not even detectable, except for the inorganic

fertilizer (NPK), showing high N-$NH4^+$ values (4.1 ± 2.3) at t5. Furthermore, mineral nitrogen decreased for all treatments from t4 to t5, except for NPK.

Nitrate (N-$NO3^-$). At t4, two weeks after fertilization, significant differences between treatments (p = 0.011) were observed. Nitrate concentrations were significantly lower in OM (6.9 ± 3.0), compared to PM (18.5 ± 6.8) with the highest N-$NO3^-$ concentrations (p = 0.029). Furthermore, PM showed considerably higher N-$NO3^-$ concentrations than LD (p = 0.055) and C (p = 0.058). At t5, six weeks after fertilization, this changed. The highest level of N-$NO3^-$ was detected in NPK, which was considerably greater than in OM (p = 0.053) and significantly higher than in unfertilized hyperaccumulator treatments (C and LD, p < 0.05). Differences between animal manure (CM, PM) and NPK as well as C and LD were non-significant (p > 0.05). Similarly, highest concentrations were noticed in NPK (excl. S), which were significantly greater than in Ref, OM, C and LD, with smallest amount of N-$NO3^-$ in LD.

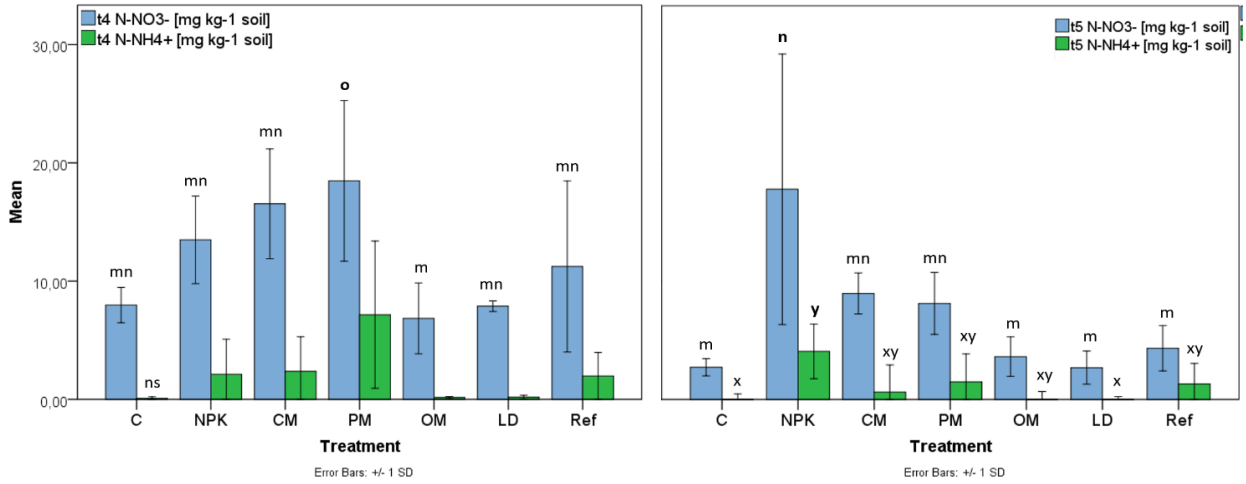

***Fig. 3.9**: Comparison of average ammonium (N-$NH4^+$) and nitrate (N-$NO3^-$)concentrations in mg kg-1 soil (mean ± standard deviation; n=4). Differences between treatments at t4 and t5 (C, NPK, CM, PM, OM, LD, Ref) are indicated with lowercase letters (p<0.05).*

Ammonium (N-$NH4^+$). Concentrations did not differ significantly among treatments (p = 0.058) at t4. Nevertheless, we observed a trend of higher N-$NH4^+$ in PM compared to C (p = 0.061). At t5, N-$NH4^+$ concentrations were lower in OM (p = 0.053) and significantly lower in LD (p = 0.035) and C (p=0.046), compared to the highest amount of N-$NH4^+$ in NPK. Exclusion of sulphur plots revealed no significant differences between treatments for t4 (p = 0.119), but t5 (p = 0.005*). At t5, the concentration of ammonium in NPK was significantly higher than in all other treatments, except for PM (p = 0.065) and Ref (p = 0.366).

Mineral nitrogen (Nmin). Significant differences between treatments at t4 and t5 ($p < 0.05$). At t4, PM showed significantly higher contents of Nmin, compared to C ($p = 0.002^{*}$). Furthermore, Nmin was lowest after fertilization with OM (7.0 ± 3.0), compared to significantly higher concentrations in PM (25.6 ± 3.4, $p = 0.001^{**}$) and CM (18.9 ± 3.5, $p = 0.046$). In contrast, highest amounts of Nmin were observed in NPK (21.9 ± 13.5) at t5. The amount of Nmin in NPK was significantly higher than in unfertilized plots (LD, C, Ref) and OM. Treatments with animal manure (PM, CM) were at the same level at t5 (9.6 mg kg^{-1}). Exclusive sulphur plots, NPK was still significantly higher than Ref, OM, C and LD, but did not differ significantly to animal manure CM ($p = 0.107$) and PM ($p = 0.127$).

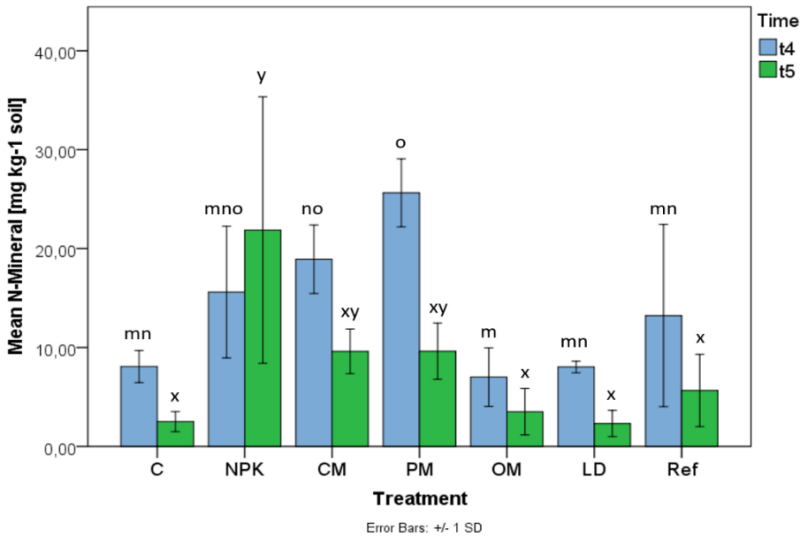

Fig. 3.10: *Concentrations of mineral nitrogen in mg kg^{-1} soil (mean ± standard deviation, n=4). Differences between treatments at t4 and t5 (C, NPK, CM, PM, OM, LD, Ref) are indicated with lowercase letters ($p < 0.05$).*

Overall, Nmin changed significantly between t4 and t5 ($p < 0.001^{***}$), with significant differences between treatments ($p = 0.001^{**}$). Except for NPK, Nmin decreased within one month after fertilization. The high value in NPK could be explained by two plots, which were no outliers but showed extremely high NO3-N^{-} concentrations, causing a high standard deviation. Despite that, no influence of block position on Nmin concentrations was noticed.

At t4, Nmin raised significantly with N-$NO3^{-}$ in all treatments and blocks ($p < 0.001^{***}$, b = + 0.871). Furthermore, Nmin tended to increase with higher amounts of N-$NH4^{+}$ ($p=0.057$, b= +0.717), however with significant differences between treatments ($p = 0.031$). Both trends were also observed when sulphur plots were excluded from statistics. At t5, trends of higher Nmin with increasing N-$NO3^{-}$ ($p < 0.001$, b = + 1.145) and N-$NH4^{+}$

(p = 0.004, b = + 1.931) were at a significant level but showed no differences among treatments and blocks (p > 0.05). Again, these trends were also significant when sulphur plots were excluded (fig. A.1). A significant increase of N-$NO3^-$ with N-$NH4^+$ concentrations has not been noticed. Furthermore, effects of pH, available P, CEC and Ca on Nmin, N-$NO3^-$ or $NH4^+$, were at a significant level incl. S, while covariance analyses were non-significant excl. S. Second, those effects seemed visually not reasonable (fig. A.1). Despite that, we did not observe any changes of Nmin with biological parameters (e.g. QBS, mesofauna density, C:A ratio).

3.2.6 Phosphorus

Plant-available phosphorus (Olsen-P). Over the whole growing period (t4, t5, t6), the amount of P was significantly lower in Ref, compared to all other treatments (p < 0.05). While no further differences between treatments were detectable at t4, we observed a trend of higher P concentrations in PM, than in C (p = 0.086) and LD (p = 0.098) six weeks after fertilization (t5). At harvest (t6), available P was still considerably higher in PM, compared to C (p = 0.055) and LD (p = 0.058). Exclusive sulphur plots, the amount of available P in PM was significantly greater than in all other treatments at t5 (p < 0.05). At t6, available P in PM was not different to CM (p = 0.284) but significantly higher than in other treatments (p < 0.05). Concentrations in Ref were significantly smaller than in all other treatments at t5 and t6 (p < 0.05).

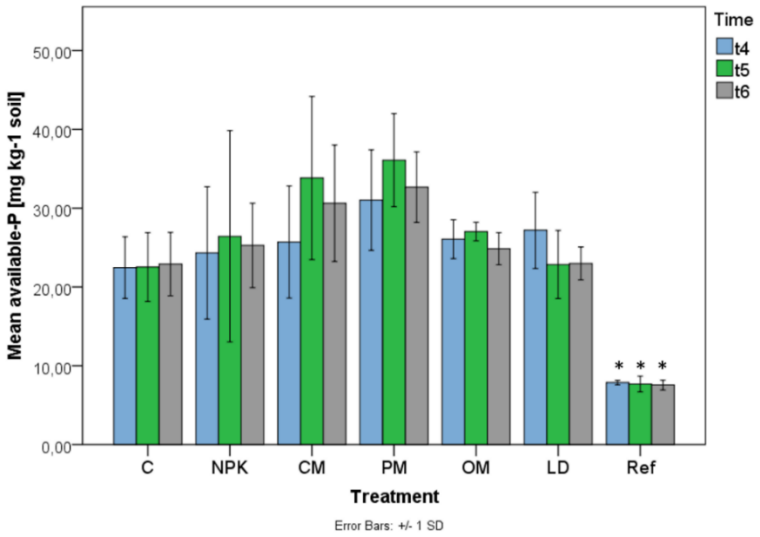

Fig. 3.11: *Concentrations of available phosphorus (Olsen-P) in mg kg^{-1} soil (mean ± standard deviation; n=4). Amounts in Ref were sign. different to all other treatments (C, NPK, CM, PM, OM, LD) at t4, t5 and t6 and indicated with a star * (p < 0.05). Differences between other treatments and time points were non-significant (p > 0.05).*

Differences between time points (t4, t5, t6) were non-significant incl. or excl. sulphur plots, while the amount of available P in soil varied significantly between blocks over the whole vegetation period ($p < 0.05$). Thus, block position also influenced P values between treatments. We observed for C, LD, NPK and CM the lowest values in block 1 and 4. However, this trend was not detectable for PM and OM. Furthermore, the amount of P was at the same low level in Ref for all blocks, explaining the small standard deviation in comparison to other treatments.

Contradictive results were recorded for covariance analyses of available P with DTPA-Co at t5. When sulphur plots were included, the increase of P with DTPA-Co was positive ($p = 0.035$, $b = + 10.198$), while being negative excl. S ($p = 0.046$, $b = - 27.645$). However, a negative trend seems visually more reasonable, as the positive trend was probably caused by very high concentrations of DTPA-Co in sulphur plots (6_CM, 8_NPK and 10_PM). Besides that, increasing P with available K, DTPA-Cu, DTPA-Mn and DTPA-Fe as well as lower P values at higher pH, were non-significant exclusive sulphur plots.

3.3 Effect of agromining on soil biological parameters

The effect of agromining and agronomic practices on biological soil quality, was assessed by comparing mesofauna abundance, C:A ratio, QBS-index and FDA-enzyme activity between treatments. A close look was paid to changes between fertilizer regimes (NPK, CM, PM, OM), planting density (LD) and seasons (t4, t6). In addition, plots cultivated with hyperaccumulator *O. chalcidica* were compared to unplanted plots (C to Ref).

3.3.1 Total Abundance of Mesofauna

All together 10 invertebrate taxa were extracted with a minimum of no individual recorded in C and a maximum of 7 taxa observed in PM, both at t6. The most abundant taxa were Acari, Collembola, Nematoda, Diptera larvae and Protura, accounting for approximately 89% of collected samples at t4. The remaining 11% comprised Coleoptera adults and -larvae, Diptera larvae, Symphyla, Araneae, Chilopoda (centipede), Diplopoda (millipede), while no adult stages of Diptera were observed. At t6 mesofauna composition changed, with 89% including Acari, Collembola and larvae of Coleoptera and Diptera. The rest (11%) consisted of Protura, adult stages of Coleoptera and Diptera, Symphyla and Nematoda. Table A.2 lists all individuals sampled at t4 and t6. Important to note is the remarkable increase of the total number of Acari at t6, especially in CM and PM. Furthermore, there was one plot in PM with an outstanding

number of Diptera larvae and prostigmatic mites (plot 18). A further exception at t6, was the very high number of Collembola (11 specimen) in CM (plot 24), compared to average number of Collembola (2 indiv. per plot). On the contrary, Collembola abundance in C was low, with one specimen collected at t4 and none at t6. At t6, soil mesofauna numbers ranged from 0 to 56 per sample, corresponding to a density of 0 to 6 indiv. m^{-2} of soil, with the highest density observed in PM (table A.1). Overall, mesofauna density significantly increased between t4 and t6, from 15 to 41 indiv. m^{-2} ($p < 0.001^{***}$). As indicated in fig. 3.12, mesofauna density was significantly lower in PM and LD at t4 ($p = 0.01^{*}$), compared to Ref (no fertilization, no tillage). At t6, we didn`t observe significant differences between treatments ($p > 0.05$).

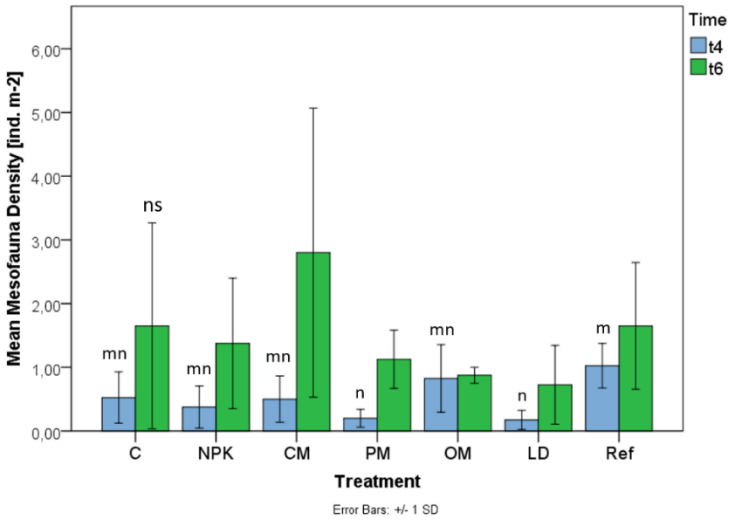

Fig. 3.12: *Mesofauna density in indiv. m^{-2} of soil (mean ± standard deviation, n=4). Differences between treatments at t4 (C, NPK, CM, PM, OM, LD, Ref) are indicated with lowercase letters ($p < 0.05$). No significant differences between treatments at t6 ($p > 0.05$).*

While no influence of block position was observed at t4 ($p > 0.05$), mesofauna density was with exception of OM lowest in block 1 ($p = 0.028$). Nevertheless, we did not detect any effects of soil nutrient concentrations (e.g. TC, TN, Nmin, Olsen-P, Ca, Mg), overall microbial activity (FDA) or lower pH in sulphur plots, on mesofauna density. However, there was a trend of higher mesofauna density with higher DTPA-Co ($p = 0.082$, $b = + 3.966$) and DTPA-Fe ($p = 0.102$, $b = + 0.031$) concentrations at t6, which showed higher significance levels excl. sulphur plots for DTPA-Fe ($p = 0.061$, $b = + 0.103$) and became significant for DTPA-Co ($p = 0.014$, $b = + 14.184$), see also with fig. A.1.

The abundance of mesofauna was dominated by Acari and Collembola at t4 (61%) and t6 (70%). The total number of Collembola and Acari in all plots, increased from 89 (t4) to 286 (t6) individuals. The average number of Collembola (2 indiv. per plot) stayed

equal throughout the growing period, while the mean number of Acari increased from 1 (t4) to 8 specimens per plot at t6. For the total number of Collembola as well as Acari no significant differences were reported between treatments or blocks at t4 ($p > 0.05$). At t6, significantly more Collembola were recorded in CM ($p = 0.028$), as well as a trend of higher abundance in PM ($p = 0.109$). Exclusive sulphur plots, total abundance of Collembola was significantly greater in CM compared to C ($p = 0.040$), while no trend of higher abundance in PM was observed ($p > 0.05$). Besides that, the number of Collembola in C declined to zero at t6 (table A.1). The total number of Collembola did not differ significantly between t4 and t6. Nevertheless, there seemed to be a trend of increasing abundance in CM and PM at t6. Plots 24 (CM) and 10 (PM) showed a considerably greater number of Collembola. Since both plots were no statistical outliers, we didn`t exclude them from statistics and considered them being artefacts. Still, both plots might be one reason for high standard deviations in CM and PM.

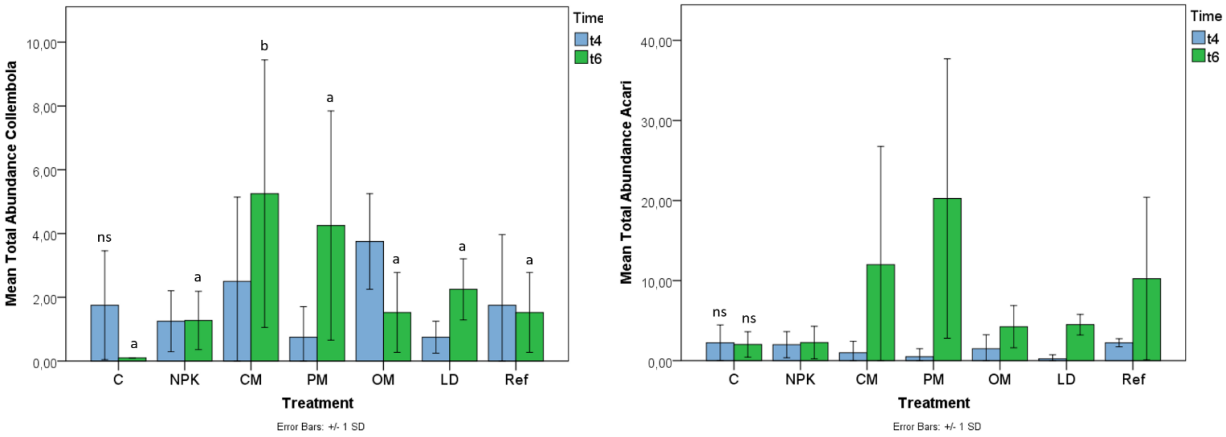

Fig. 3.13: *Total number of individuals of Collembola and Acari per treatment (mean ± standard deviation, n=4). Differences between treatments (C, NPK, CM, PM, OM, LD, Ref) are indicated with lowercase letters ($p < 0.05$).*

In contrast, trends for Acari were quite diverse. The total abundance of Acari sign. increased between t4 and t6 ($p = 0.001^{*}$). No significant differences were noticed between treatments at t4 ($p = 0.256$) or t6 ($p = 0.089$), even though Acari abundance tended to be higher in CM, PM and Ref, compared to C (fig. 3.13). However, those 3 treatments showed especially high standard deviations, caused by agglomerations of prostigmatic mites in plots of CM, PM and Ref (7_CM, 18_PM, 21_PM, 26_Ref) at t6. Thus, we observed a shift in Acari species composition over the season. At t4, the Acari community was dominated by Mesostigmata (77 %), while 23 % of recorded mites belonged to Prostigmata. In contrast, at t6 only 14 % of

sampled Acari belonged to Mesostigmata, but 68 % to Prostigmata. Furthermore, 4% of belonged to Oribatida at t6, while no individual was recorded at t4 (table A.3).

3.3.2 Relative abundance of Collembola and Acari

The dominance of Acari and Collembola above other soil microarthropods (relative abundance) didn`t differ significantly between treatments at t4. At t6, a tendency of higher abundance of Acari compared to other soil microarthropods was observed in PM, however not at a statistically significant level ($p = 0.155$). Furthermore, no significant differences were observed excl. sulphur plots at t4 ($p = 0.925$) or t6 ($p = 0.173$). Despite that, relative abundance of Acari sign. increased between t4 and t6 ($p = 0.001^{**}$). Testing the relative abundance of Collembola at t6 (2-way ANOVA) resulted in an alpha level of ($p = 0.065$) incl. and ($p = 0.129$) excl. sulphur plots. However, we noticed a trend of higher relative abundance of Collembola in CM compared to C. Furthermore, relative abundance of Collembola tended to decrease between t4 and t6, except for CM where one plot with exceptionally high numbers of Collembola outstood this trend, but not at a statistically significant level ($p > 0.05$). Relative and total abundance of Collembola or Acari didn`t change significantly with varying nutrient contents (TC, TN, K, Ca, Mg), metal availability (Ni, Co, Cu, Fe, Mn) or microbial activity (FDA). However, relative abundance of Collembola was considerably lower with higher amounts of available P at t6 (incl. S: $p = 0.067$, $b = -0.047$; excl. S: $p = 0.102$, $b = -0.062$). Furthermore, total number of Collembola increased sign. with rising P concentrations (incl. S: $p = 0.031$, $b = -0.234$; excl. S: $p = 0.048$, $b = -0.331$).

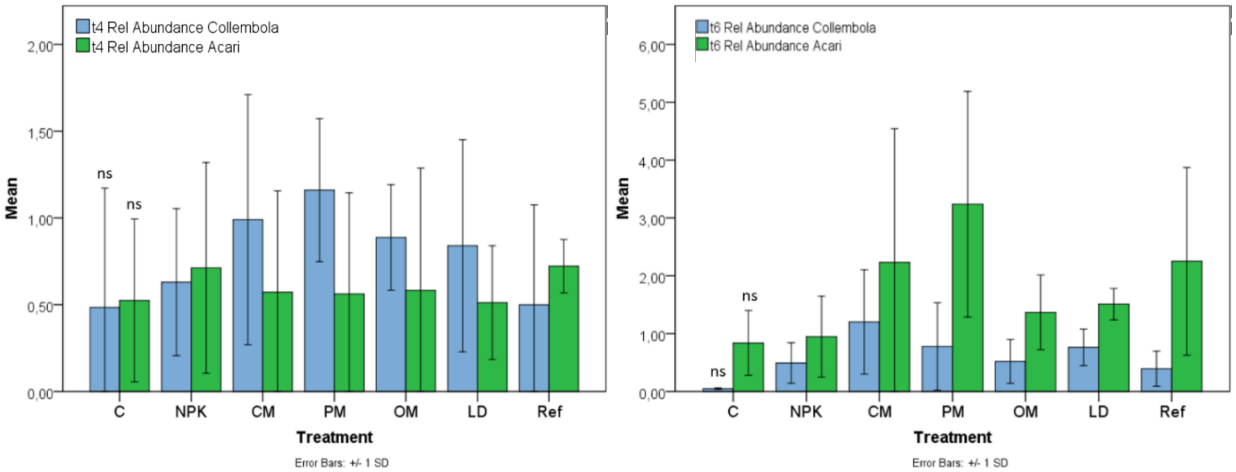

Fig. 3.14: *Abundance of Collembola and Acari relative to total numbers of soil arthropods per treatment (mean ± standard deviation n=4). No sign. differences between treatments (C, NPK, CM, PM, OM, LD, Ref) at t4 or t6 ($p < 0.05$). Differences between t4 and t6 were non-significant for Collembola ($p = 0.155$), but sign. for Acari ($p = 0.001^{**}$).*

3.3.3 C:A ratio

The C:A ratio was not sign. different between treatments and blocks at t4 or t6 (p >0.05). We observed very high standard deviations, which were higher than the average for some treatments (e.g. C, NPK), compare with fig. 3.15 and table A.1. Despite that, the overall trend of lower C:A ratio at t6 compared to t4, was significant (p = 0.005*). Differences in C:A ratio between treatments were also non-significant excl. S (p = 0.360).

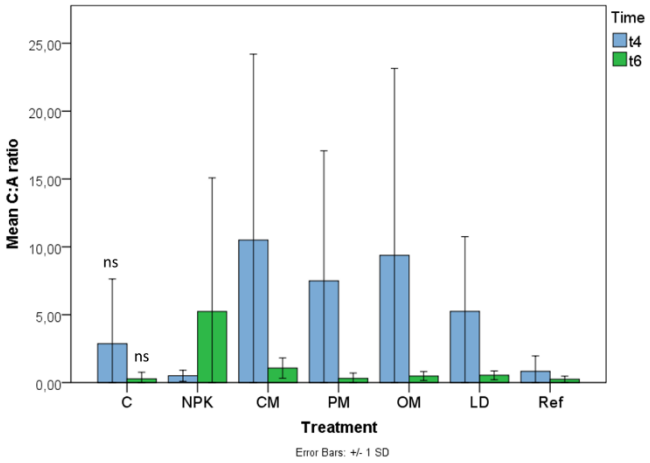

***Fig. 3.15**: Total number of Collembola relative to Acari (mean ± standard deviation, n=4). The C:A ratio was not sign. different between treatments, but time points t4 and t6 (p < 0.05).*

3.3.4 QBS-index

At t4, QBS values didn`t differ sign. between treatments (p = 0.317), or block position (p = 0.842). Still, QBS-index tended to be higher in Ref (62.5. ± 22.2) compared to C (15.0 ± 13.0), at t4. Moreover, QBS sign. increased between t4 and t6 (p = 0.002*). Higher QBS values at t6 were likely resulting from an increased abundance of larvae of Diptera and Coleoptera in almost every plot, rising the overall QBS-index in all treatments. Furthermore, at t6 Acari occurred in 26 out of 28 plots and a greater number of eu-edaphic forms of Collembola was recorded, compared to t4. Overall, QBS tended to increase at t6 for C, organic fertilizer treatments (CM, PM, OM) and plots with lower planting distance (LD), while remaining almost constant for NPK and Ref (table A.1). Thus, we observed significant differences between treatments (p = 0.012) and blocks (p = 0.020) at t6. Across all treatments, block 4 as well as block 3 showed the greatest QBS values. Furthermore, highest QBS at t6 was recorded in

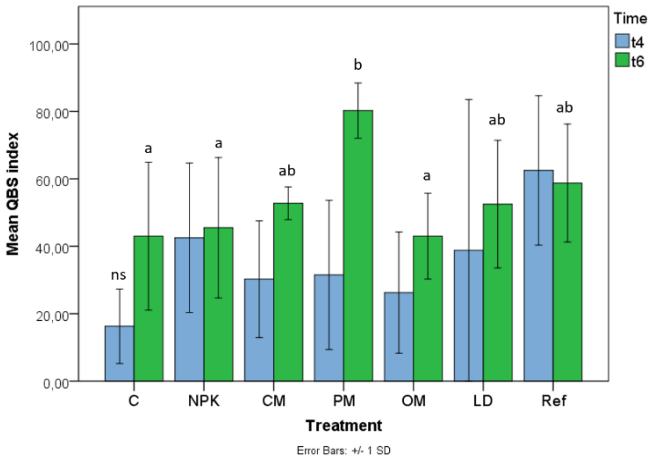

***Fig. 3.16:** Soil biological quality index (QBS) at t4 and t6 (mean ± standard deviation, n=4). Sign. differences between treatments (C, NPK, CM, PM, OM, LD, Ref) at t6 are indicated with lowercase letters (p < 0.05). QBS-index sign. changed between t4 and t6 (p = 0.002*).*

PM (80.3 ± 8.2), which was sign. greater than in C ($p = 0.014$), OM ($p = 0.014$) and NPK ($p = 0.024$) but did not differ sign. to LD, CM and Ref ($p > 0.05$). Excl. sulphur plots, significant differences between C and PM ($p = 0.027$) as well as among blocks ($p=0.014$) were noticed. Despite that, we didn`t observe any influence of soil physicochemical parameters (e.g. TC, TN, available P, exchangeable cations, CEC, pH, Nmin) or microbial enzyme activity (FDA) on QBS-index or C:A ratio.

3.3.5 FDA-enzyme activity

Differences in soil microbial activity between treatments at t6, assessed by the hydrolysis of fluorescein diacetate, were significant ($p = 0.009^{*}$). Furthermore, microbial activity was considerably influenced by the block position ($p = 0.013$), with lowest FDA values in block 1, except for CM showing an exceptionally high amount in block 1. A trend of lower FDA-enzyme activity in plots fertilized with NPK (117 ± 26), was significant when compared to CM ($p = 0.006$) and LD ($p = 0.038$), while not to C ($p = 0.584$). Besides that, neither fertilization nor higher planting density did significantly affect microbial activity, compared to C or Ref ($p > 0.05$). In contrast, C, OM, PM and Ref showed similar levels of FDA. However, excl. sulphur plots, FDA-enzyme activity measured in NPK was sign. lower than in C ($p = 0.022$), CM ($p = 0.001^{**}$), PM ($p = 0.003^{*}$) and LD ($p < 0.001^{***}$). Furthermore, FDA in NPK was considerably smaller than in Ref ($p = 0.050$) and OM ($p = 0.075$). Besides that, no further differences between treatments excl. sulphur plots were observed. According to covariance analyses, FDA-enzyme activity increased with higher contents of TC ($p = 0.031$, $b = + 5.704$), TN ($p = 0.036$, $b = + 64.72$), available P ($p = 0.017$, $b = + 2.424$) and elevated concentrations of Ni, Mn, Fe and Co in DTPA-extracts (fig. A.1). However, covariances were far above the significance level ($p < 0.05$) excl. sulphur plots.

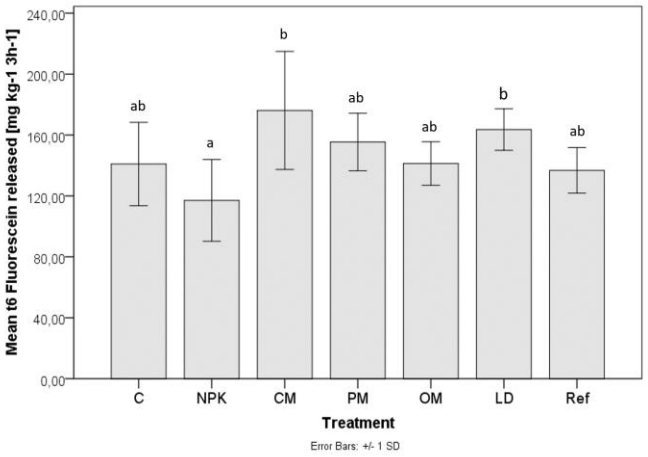

Fig. 3.17: *FDA enzyme activity, fluorescein released in mg kg^{-1} $3h^{-1}$ (mean ± standard deviation, n=4). Significant differences between treatments (C, NPK, CM, PM, OM, LD, Ref) at t6, are indicated with lowercase letters ($p < 0.05$).*

4. DISCUSSION

4.1 Effect of agromining on Ni-availability in soils

After one vegetation period, a significant decline of the DTPA-Ni pool was observed for all treatments. This agrees with Rosenkranz et al. (2019), who reported a decrease from 39.6 mg Ni kg^{-1} to 34.4 mg Ni kg^{-1} within one growing season in untreated control plots, in the previous experimental year (2017). In the following year, at the beginning of the growing period (t5), we detected 40.4 mg Ni kg^{-1} in those control plots. Thus, the DTPA-Ni pool was replenished over the winter season between September 2017 to May 2018. This could be explained by a) degradation and mineralization of Ni-rich biomass (e.g. roots from harvested hyperaccumulator plants) or b) weathering and release of Ni from Ni-bearing mineral fractions. Additionally, all plots cropped with the hyperaccumulator *O. chalcidica* showed significantly lower DTPA-Ni levels than Ref at harvest (t6, excl. S). A trend as well observed in the previous year, where DTPA-Ni decreased significantly in all treatments, except for plots that had received elemental sulphur amendments. Like Rosenkranz et al. (2019), we assume that the depletion of the DTPA-extractable Ni pool was mainly a result of Ni uptake by the hyperaccumulator *O. chalcidica*. We base this assumption on the fact, that other possibilities are less reasonable. One reason for the decline in DTPA-extractable Ni could be the incorporation into the crystal lattice of newly formed Fe-oxides (e.g. goethite) during weathering, making Ni unavailable and thus not assessable by the DTPA-extract. However, even though goethite is a known sink of Ni, this process probably becomes dominant at longer time scales than within one growing period (Chardot et al., 2007). Second, lithogenic metals, such as Ni weathered from serpentinite, are only slightly mobile and stable in soil solution, which is why their migration to groundwater is usually low (Kabata-Pendias, 1993; Echevarria et al., 2006). Hence, despite the fact that we did not calculate the balance between the amount of Ni in the biomass and the DTPA-pool, the assumption that DTPA-Ni pool decreased due to a depletion by the hyperaccumulator would agree with previous studies of Li et al. (2003b) and Echevarria et al. (2006). First, Li et al. (2003b), who conducted a greenhouse experiment with *O. chalcidica* (*A. murale*), reported that the amount of Ni phytoextracted, was ~ 39% similar to the DTPA-extractable Ni. Second, Echevarria et al. (2006) and Massoura et al. (2006) suggested, that the DTPA-extractable Ni fraction significantly correlated with the IEK (isotope exchange kinetics techniques) medium-

term pool (E 0 - 3 months), which includes elements fixed by sorption and surface complexation onto soil particles (Echevarria et al., 1998). Additionally, they detected that DTPA-Ni correlated with the isotopically labile Ni. Therefore, they proposed that first, DTPA-Ni can explain the isotopically exchangeable pool (Echevarria et al., 1998, 2006; Massoura et al., 2006; Shallari et al., 2011) and second, represents the Ni-source for plant uptake, as the medium-term pool declined after cropping the hyperaccumulator (Echevarria et al., 2006). This is as well confirmed by (Echevarria et al., 1998; Massoura et al., 2004 cited in Bani et al., 2015a; Shallari et al., 2011), who claim that plants in general as well as *O. chalcidica*, take up Ni from the isotopically-exchangeable pool.

The amount of Ni extracted by DTPA-solution represents Ni that is exchangeable and complexed to surfaces (e.g. organic matter, clay minerals). Hence, available for plant uptake. In contrast, $Sr(NO_3)^2$-extractions only assess Ni electrostatically bound to exchangeable sites (Echevarria et al., 1998). Like DTPA-Ni, the $Sr(NO_3)^2$-extractable Ni fractions decreased within the growing period. This decline of $Sr(NO_3)^2$-Ni was more pronounced in plots treated with elemental sulphur and was as well observed in the previous experimental year (2017). Exclusive sulphur plots, $Sr(NO_3)^2$-extractable Ni was significantly lower in OM. Simultaneously, we observed significantly higher contents of TC in OM. Thus, lower $Sr(NO_3)^2$-concentrations might be explained by a higher metal retention capacity of soils with increasing carbon contents, as more binding of cations to negatively charged surface or to organic ligands is possible. This would be in accordance with Álvarez-López (2016), who reported a decrease of extractable Ni after compost addition. However, a significant increase of $Sr(NO_3)^2$-Ni with higher TC was not statistically confirmed (fig. A.1). Even though DTPA-Ni increased with TC (incl. S), this trend was non-significant after excluding sulphur plots. Also, Chardot et al. (2007) and Alves et al. (2011) did not find a significant correlation of DTPA-extractable Ni and soil carbon contents. The amount of Ni extracted with DTPA-solutions was considerably higher, than in $Sr(NO_3)^2$. Thus, we assume that more Ni was complexed than electrostatically bound. This would agree with Alves et al. (2011), who investigated soils in Portugal and suggested that Ni in serpentine soils is mainly bound by specific adsorption, while exchangeable mechanisms are dominant in non-serpentine soils. In addition, we noticed that DTPA-Ni increased with the $Sr(NO_3)^2$-Ni, however this trend was not significant after excluding sulphur plots. Still this trend might be partially explained by the fact that DTPA-extractions also include the exchangeable Ni assessed with ($Sr(NO_3)^2$. In general, the Ni-fraction electrostatically adsorbed ($Sr(NO_3)^2$-

extractable) is through soil solution in equilibrium with the complexed Ni (DTPA). Hence, if Ni concentrations increase due to plant mobilisation processes or decrease after plant uptake, the equilibrium between the free Ni in soil solution and the Ni adsorbed or complexed to soil particles is changed, causing exchange processes because equilibrium always has to be maintained (Scheffer et al., 2010).

It is not clear which mobilisation processes are triggered by the hyperaccumulator to deplete the labile Ni pool and if this mobilisation is mainly affecting exchangeably bound Ni ($Sr(NO_3)^2$) or complexed Ni (DTPA). Generally, there are 3 possible plant-induced mechanism. First, depletion of the exchangeable pool through proton release by the hyperaccumulator, as protons substitute the Ni on the exchangeable binding sites, rising the amount of free Ni in soil solution, which is then ready for plant uptake (Scheffer et al., 2010). Second, ligand-induced mobilization, which comprises i) the release of carboxylate anions through root exudates, ii) mobilisation of Ni complexed with soil particles, and iii) complexation of Ni by carboxylate anions. However, only if the binding of the newly formed exudate-Ni-complex is stronger than the one between Ni and the soil-organic-ligand (Scheffer et al., 2010). However, scientist agree that hyperaccumulators only take up Ni in its free ionic form and not as a complex. The only complex that is known to be taken up by plants so far, are phytosiderophores, chelators for Fe (III). However, this strategy (II) is restricted to gramineous species. Since *O. chalcidica* does not possess this ability, the Ni-ligand-complex must dissociate before plant uptake (Broadley et al., 2012). The third mechanism is ligand induced co-dissolution, as for example investigated by Wenzel et al. (2003). They investigated the rhizosphere of the hyperaccumulator *N. goesingense* and proposed that the higher solubility of Ni in the rhizosphere, evaluated through higher amounts of water extractable Ni, Mg, Ca and K, was result of a ligand induced co-dissolution of Ni bearing minerals. Wenzel et al. (2003) observed enhanced metal mobilization due to increasing amounts of dissolved organic matter released via root exudates, causing chemical weathering of the mineral phase. Thus, proton release and root exudation are both mechanisms to mobilize Ni adsorbed to soil solid phase. Hence, a higher planting density might coincide with a higher depletion of Ni-pool, due to more protons and root exudates released by the plant for metal uptake. Despite that, metal uptake might also be influenced by rooting density and -activity, varying with root architecture and rooting depth. However, we did not find significant differences between Ni-pools of LD (9.6 plants m^{-2}) and C (4 plants m^{-2}). Neither fertilization, nor planting density did affect DTPA-Ni pool, while labile $Sr(NO_3)^2$-Ni

was more available in NPK and less in OM compared to C, exclusive sulphur plots. However, the experimental set up does not allow a well justified answer concerning a rejection or acceptance of the hypothesis that fertilization increases Ni-availability. First, because sulphur application considerably influenced the soil and second, different results for the fertilizer treatments were achieved. Probably only the fertilization with NPK increased the $Sr(NO_3)^2$-extractable Ni. Nevertheless, for a clear answer to this hypothesis further field experiments studying the effect of NPK on Ni-availability are needed.

Besides that, Ni availability is controlled by texture, pH, electrical conductivity (EC), organic carbon, CEC or sesquioxides (Massoura et al., 2006; Chardot et al., 2007; Kabata-Pendias, 2010). Massoura et al. (2006) and Chardot et al. (2007), reported a significantly positive correlation of Ni-DTPA with exchangeable Ca and CEC, as well as pH, clay content, total Mg and Fe, exchangeable Mg and free Fe oxides. In contrast, we recorded that DTPA-Ni decreased with higher values of CEC and Ca, however this trend was non-significant after excluding sulphur plots. Comparably, declining $Sr(NO_3)^2$-Ni with greater Ca and CEC were non-significant (excl. S). Nevertheless, we noticed that $Sr(NO_3)^2$-Ni was lowest in OM and that OM contained significantly highest amounts of TC and CEC (excl. S). The CEC was evaluated as the sum of cations analysed in the $BaCl_2$-extraction. Hence, it is first not an exact evaluation of all possible binding sites. Second, increasing Ca concentrations also rise CEC. One hypothesis of this contradictive results could be, that Ca antagonizes Ni at the exchangeable sites. Thus, free Ca (e.g. Ca^{2+}) in soil solution, substitutes Ni absorbed to exchangeable sites. The free Ni in soil solution is then taken up by the plant and thus decreasing the Ni-pool. Even if so, it is not clear why this was not the case for Mg. Unfortunately, we did not analyse the amount of Ni present in the $BaCl_2$-extraction to investigate this hypothesis. Besides that, this assumption would be in contrast to the usual trend of higher CEC and metal sorption capacity with increasing pH (Alves et al., 2011). First, surface charge is pH dependent. Thus, the metal retention onto soil solid phase (Ni-adsorption) rises with pH (Barman et al., 2013; Massoura et., 2006; Echevarria, 2018). Second, because Ni occurs primary in its cationic form, it is easily adsorbed to usually negatively charged surfaces in soil (Kabata-Pendias, 2010). However, we reported lower levels of DTPA-Ni and $Sr(NO_3)^2$-Ni with a higher pH. While the decline of DTPA-Ni with higher pH was non-significant (excl. S), the decrease of $Sr(NO_3)^2$-Ni with higher pH was significant (incl. and excl. S). Despite that, we observed a tendency of greater Ni-pools (labile and complexed) in sulphur plots at considerably lower pH. This trend agrees with studies

of Li et al. (2003a) and Echevarria et al. (2006). Echevarria et al. (2006) proposed that the higher solubility of Ni in acidic soils (pH range 4.5 - 7.0), promotes Ni uptake by the hyperaccumulator as Ni has to be continuously replenished by the labile pool during root uptake, to maintain equilibrium between the soil solid phase and soil solution. Li et al. (2003a) suggested that *Odontarrhena sp.* increase their Ni-uptake, if Ni-availability in soil is low, which might explain why Ni-pools were greater in more acidic plots. Thus, the mechanism of enhanced Ni mobilisation by the plant in soils with reduced Ni-availability might be more effective for Ni-hyperaccumulation as enhanced Ni solubility . This would explain why Ni pools were more depleted in soils with higher pH where we assumed a lower Ni-availability. Probably the hyperaccumulator plants adapted to this. Also, Li et al. (2003a) reported that Ni uptake by *Odontarrhena sp.* was higher with increasing soil pH, while being reduced at lower soil pH. While for normal plants (no Ni-hyperaccumulators) soil pH is the most important factor explaining Ni-availability in soil (Kabata-Pendias, 2010), several authors stress the role of Ni-bearing fractions in Ni-uptake by hyperaccumulator plants. According to Massoura et al. (2006), Bani et al. (2018) and Echevarria (2018), the Ni- bearing phase in serpentine soils controls substantially the availability of Ni, while the effect of pH is only limited. Thus, Ni availability in moderately weathered soils, just as present in our experimental site, is usually controlled by the association of Ni with amorphous Fe-oxides and secondary clay minerals with high-exchange capacity (Bani et al., 2018). Similar to organic matter, clay minerals and amorphous Fe-oxides, provide negatively charged surfaces, that adsorb metals. On the contrary, metals in crystalline forms (e.g. goethite) might be unavailable, due to incorporation into the crystal lattice (Echevarria et al., 2006; Pędziwiatr et al., 2018). The medium-term Ni-pool (DTPA) showed high correlations with amorphous Mn-oxides (Alves et al., 2011) and Fe-oxides (Massoura et al., 2006; Chardot et al., 2007). Hence, even though Ni availability might be affected by various soil properties at varying extents (e.g. CEC, clay- and carbon content, pH, amorphous Mn-/Fe-oxides), the authors concluded that amorphous oxides are the main Ni-bearing phase in serpentine soils.

In addition, we observed increasing DTPA-Ni with DTPA-Co and DTPA-Fe at t5 (incl. and excl. S). This could result from a fertilization effect, as fertilizers contained trace metals in different amounts (Hipfinger et al., 2020). On the contrary two points disagree with this hypothesis. First, this trend was also observed in non-fertilized C, LD as well as Ref. Second, Co and Fe concentrations in DTPA-extracts did not differ significantly between treatments.

Furthermore, the heavy metals in the DTPA-extracts also increased in Ref, which is why a mechanism by the hyperaccumulator might be also be excluded. Overall, it is also likely that the observed changes in DTPA-extractable Co and Fe fractions were fluctuating due to seasonal changes, including variations in soil moisture due to rainfall, soil temperature, microbial activity, and others.

4.2 Effect of agromining on soil quality

To evaluate the sustainability of agromining as well as the effectiveness of selected agronomic measures on soil quality, we measured a set of soil quality indicators and compared them with desired values (thresholds). We assumed that an indicator was at a sustainable level when exceeding a certain threshold.

4.2.1 Effect of agronomic practices on soil quality

The effects of agronomic measures (fertilization and planting density) on physicochemical and biological soil quality were interpreted by comparing results of selected soil indicators in fertilizer treatments (NPK, CM, PM, OM) as well as in plots with lower planting distance (LD), with control plots (C). The summarized evaluation of agronomic practices on soil quality is listed in table A.5. Furthermore, C was compared to Ref, to observe possible effects of hyperaccumulators on soil quality.

4.2.1.1 Physicochemical soil quality

pH and exchangeable cations (Ca, Mg, K, Ca:Mg, CEC). The pH of soil substantially influences plant growth and thus also the efficiency of phytomining. The optimum pH can vary between plant species, but also depends on biological and soil physicochemical parameters. The pH is an important indicator, as it influences the availability of macro– and micronutrients, the mobility of heavy metals, the rate of organic matter degradation (carbon storage) and the reproduction and species diversity of soil organisms (microbial habitat) (Scheffer et al., 2010). The optimum pH of agricultural soils can be assessed by their humus- and clay content. The soil of the experimental site in Bernstein contains on average 20.0 g kg^{-1} SOC, 30.2 g kg^{-1} humus and 21.0% clay. According to Scheffer et al. (2010), soils with an organic matter content < 4% and clay content > 13% have their pH optimum between 6.0 and 6.5. Furthermore, Baumgartner et al. (2017) suggested that in medium heavy soils with 15 - 25% clay content, pH should be

above 6. This specification can be explained by the fact that many plant macronutrients (N, P, K, S, Ca and Mg) show an optimum availability between pH 6.0 to 6.5. For example, availability of P might be limited at pH ($CaCl_2$) > 7.5, due to complexation of Ca with P. In contrast, Fe, Mn, B, Cu and Al are more available in more acidic conditions and Mo at alkaline pH levels (Lake, 2000). All plots of the experimental site are within this optimum pH for arable soils. The exclusion of sulphur plots revealed significantly highest pH in OM, followed by CM and lowest in Ref, compared to C. This order is similar to TC contents, with highest amounts in OM, followed by CM and lowest in Ref and NPK. While we did not analyse soil texture in different treatments, we assume that carbon content might be one crucial factor determining pH in our experiment. However, pH cannot be explained by TC contents in every treatment. For example, TC was third highest in PM and lowest in NPK. In contrast, pH in PM was lower than C and NPK. Besides that, we did not observe a significant covariance between pH and TC. Interestingly, sulphur plots with a lower pH also showed a lower amount of TC.

The pH range 5.5 to 6.5 represents the exchange buffer system, where pH is primarily controlled by the negatively charged surfaces of clay minerals and organic matter. The adsorption of protons to the negative exchange complex decreases the free protons in soil solution and thus increases pH (Baumgartner et al., 2017). Similarly, positively charged ions (cations) are electrostatically bound to negatively charged surfaces and thus plant available. Cations can be categorized into alkaline- (Ca, Mg, K and Na) and acidic ions (Fe, Mn and H). The sum of cations is representing the cation exchange capacity (CEC) of a soil, which is controlled by clay minerals, organic matter content and pH (Baumgartner et al., 2017). Van der Ent et al. (2018) reported increasing pH and CEC with higher amounts of exchangeable Mg and Ca. For Ca, we observed a similar trend with pH, as with TC. Highest concentrations of Ca and pH were reported in OM, while lowest Ca and pH were detected in Ref. Additionally, we noticed a significant increase of pH with Ca and CEC, however only at t5 and exclusive sulphur plots. In contrast, pH seemed to be unaffected by varying Mg levels in soil. Interestingly, Ca concentrations were in all 4 sulphur plots at the same level (~ 100 $mmol_c$ Ca kg^{-1} soil).

Besides their effect on pH, exchangeable cations (e.g. Ca, Mg, K) fulfil various services in soil. Calcium can enhance aggregate stability of medium heavy soils, as Ca is able to build bridges between soil colloids. Thus, Ca positively influences porosity and infiltration while decreasing erosion due to a better stability (Rowley et al., 2018). Magnesium is a component of chlorophyll and plays a role in phosphorus metabolism. Nevertheless, Mg deficiency does not substantially

inhibit plant growth, but might affect the nutritional quality of crops (Parnes, 2013). In contrast, deficient amounts of Ca can cause a dieback of growing tips in roots and leaves or reduction of cell membrane impermeability. Furthermore, potassium controls metabolic activities and plays a central role in the synthesis of proteins and starches. A deficiency in K might cause excessive accumulation of simple sugars and amino acids, which might retard photosynthesis (Parnes, 2013). Especially for K, the binding to the cation exchange complex is crucial because it is highly mobile and thus easily leached (Parnes, 2013). The ability of plants to take up electrostatically bound K is mainly controlled by the rooting density. Rooting density and depth can be restricted by subsurface compaction or poor soil structure, which might also limit K-uptake (Hartz, 2007). Besides that, a high amount of exchangeable K in soils, does not ensure sufficient K-nutrition, as uptake of electrostatically bound K can be antagonized by other exchangeable cations (e.g. Ca, Mg). According to Hartz (2007) the critical level of K-availability is a percentage of K > 2% on the total amount of exchangeable cations (CEC_{eff}). Below this threshold K-availability might be restricted due to cationic competition during plant uptake, while competition is no problem if the amount of K on CEC_{eff} is > 3%. Also, Baumgartner et al. (2017) stressed the importance of a good balance between cations on exchangeable sites to ensure sufficient nutrient supply. Thus, Baumgartner et al. (2017) recommended a share of Ca (75 - 90%), Mg (5 - 15%), K (2 - 5%) and Na (< 1%) on CEC_{eff}. All treatments in Bernstein showed a lower percentage of Ca and K, while almost twice the maximum of Mg. The percentage of Ca in relation to overall exchangeable cations (% of CEC_{eff}) ranged for all soils cropped with *O. chalcidica* between 63 and 67%, while being lower in Ref (~ 57%). The share of Mg on CEC_{eff} varied between 30% and 35% in hyperaccumulator treatments but was considerably higher in Ref (43%). Furthermore, the only agronomic measure improving K-availability in soil, was CM with 4.71% K on CEC_{eff} (t5) and 2.51% (t6), as well as PM at t5 (2.09%). For the other treatments, competition for K might be a problem with K < 2% (Hartz, 2007) and too high percentages of Ca and Mg on CEC_{eff}. In contrast to Hartz (2007) and Baumgartner et al. (2017), Parnes (2013) claimed that in recent years experiments have shown that yields are unaffected by the percentage of ions on CEC and thus questioning the hypothesis of cation balances on CEC. However, Parnes (2013) outlined the importance of a sufficient supply of each cation. When looking at the general amounts of exchangeable cations, the Bernstein soil shows quite a high amount of exchangeable Ca (Ref, 90 $mmol_c\ kg^{-1}$), compared to other serpentine soils with 5.0 $mmol_c\ kg^{-1}$ (Álvarez-López et al., 2016),

5.4 $mmol_c$ kg^{-1} (Bani et al., 2015a) and 17.5 mmolc kg^{-1} (Ghasemi et al. 2018). Compared to the non-hyperaccumulator treatment (Ref), the Ca content was significantly higher in organic amendments and LD but not in NPK. Interestingly, we reported no significant decline of Ca concentrations after growing Ni-hyperaccumulators, which was quite in contrast to what we expected. Usually Ni-hyperaccumulators take up large amounts of Ca during plant growth (Álvarez-López et al., 2016). Due to harvest and removal of biomass and plant residues, Ca is continuously removed which results in an even higher depletion of Ca in the already deficient serpentine soil. Therefore, Álvarez-López et al. (2016) suggested Ca-fertilization when cropping hyperaccumulators for agromining. In addition, Baumgartner et al. (2017) proposed addition of calcite, if the share of Ca on CEC_{eff} is < 60%, also in soils with pH > 6.

Usually, soil Ca levels are adjusted by adding calcite or gypsum. However, we observed high concentrations of Ca in compost, which significantly raised the amount of exchangeable Ca compared to C. The amounts of Ca in CM and PM were half of the Ca concentrations in OM, which was reflected in a way lower amount of available Ca in CM and PM treatments. Despite that, we observed that Ca concentrations also increased in unfertilized LD plots and were significantly higher than levels in C and Ref. This might indicate Ca mobilisation by the plant. Cerdeira-Pèrez et al. (2019) suggested that higher CEC in soils planted with hyperaccumulator *Odontarrhena sp.*, resulted from plant-induced mobilisation of exchangeable Ca and Mg. However, we observed no increase of Ca in C and NPK, also cropped with *O. chalcidica*, which would reject that hypothesis. Despite that, we observed a trend of increasing Mg levels in all treatments, also in non-vegetated Ref. This might indicate a general trend independent of hyperaccumulator influence. Thus, higher Mg levels might result from weathering. However, the release of cations during weathering processes usually occurs within longer time frames. Furthermore, increasing amounts of Ca and Mg concentrations, could also be linked to the mineralization of organic material. For example, Mg levels were lowest in PM at t5 but raised to the second highest amount at harvest (excl. sulphur). In summary, organic amendments could be an option to increase Ca concentrations in serpentine soils in order to enhance soil quality.

The availability of K decreased in all treatments between t5 and t6, which could be due to plant uptake. Due to the fact, that K is neither organically bound nor required to a great amount by soil organisms, K in organic amendments is quickly released during decomposition and thus easily plant available (Parnes, 2013). The available K declined to a quite similar level in all treatments, except for CM. In CM, K concentrations were considerably higher compared to all

other treatments at t5 and t6. One explanation of the elevated K-availability in CM might be, that the concentration of available K in the applied CM-fertilizer was nearly 4 times higher than in other fertilizers. Parnes (2013) recommend amendments of plant residues and animal manure, to sustain a sufficient supply of K for plants, while we only gained adequate nutrition after manure application. A high CEC is crucial to prevent K from immediate leaching during decomposition (Parnes, 2013). Besides that, soils with a high CEC that is also well balanced in cations, show a high buffer capacity that counteracts natural acidification (e.g. rain, soil respiration) (Parnes, 2013). According to Baumgartner et al. (2017), soils with a pH between 6.0 and 6.5 and a middle bulk density, should show an effective CEC between 170 and 180 $mmol_c\ kg^{-1}$. This threshold was achieved by organic amendments (CM, PM, OM) and low distance plantation (LD). However, only OM showed a sign. higher CEC compared to C. In contrast, inorganic fertilization (NPK), C and Ref showed a CEC below this threshold. This agrees with Ghasemi et al. (2018), who recorded a lower CEC after NPK application and a significantly higher CEC in plots treated with CM. Álvarez-López et al. (2016) reported 66 $mmol_c\ kg^{-1}$ CEC in untreated plots, 81 $mmol_c\ kg^{-1}$ in NPK, while in plots amended with compost CEC raised to 238 $mmol_c\ kg^{-1}$. Van der Ent et al. (2018) suggested that soils deriving from serpentinite at low altitude in temperate regions, have a high Mg:Ca ratio (5-25) and a very high CEC (150 - 250 $mmol_c\ kg^{-1}$). The untreated Ref plots in Bernstein showed a CEC within this range (162 $mmol_c\ kg^{-1}$), while the Mg:Ca ratio (0.75) was considerably below results of van der Ent et al. (2018). This can be explained by the fact, that even though Mg concentrations were elevated, also Ca concentrations were quite high (~ 68 $mmol_c\ kg^{-1}$) for serpentine soils that are usually deficient in Ca. Thus, Ca:Mg ratio was > 1, while a Ca:Mg ratio < 1 is often cited as a characteristic property of serpentine soils (Brooks, 1987). The Bernstein soil showed on average a Ca:Mg ratio of (1.91), which was significantly lower and closest to 1 in Ref (1.36). The low Ca:Mg ratio in Ref is probably a result of sign. higher Mg contents together with the lowest amount of Ca. Similarly, highest Ca:Mg ratio was detected in OM, with sign. highest Ca concentration. At t5, all treatments except for NPK and Ref, showed a Ca:Mg ratio > 2. Until harvest, the Ca:Mg ratio decreased in all plots as Mg contents increased to a greater extent than Ca. A low Ca:Mg ratio is often associated as one growth limiting factor of serpentine soils (Brooks, 1987). Usually higher Ca:Mg ratios are associated with a better soil structure, tilth and water infiltration rate, while a Ca:Mg ratio below < 2 may indicate a need of Ca-fertilization (e.g. lime, gypsum) (Schulte and Kelling, 1985). However, there is also criticism on only

looking at the Ca:Mg ratio. Schulte and Kelling (1985) outlined, that the Ca:Mg ratio can also be a misleading indicator, when not considering the amount of Ca and Mg in soil too. For example, a low Ca:Mg ratio can be due to low levels of Ca but normal levels of Mg, or a sufficient concentration of Ca but elevated Mg content. Furthermore, the amount of Ca and Mg in soil could be deficient, but still their ratio favourable. Additionally, Agroprofessional (2012) published a study, in which no relationship between Ca:Mg ratio and crop yield for several crops in soils in Kansas was detectable. Besides that, hyperaccumulators are naturally adapted to a low Ca:Mg ratio. Thus, the Ca:Mg ratio in Bernstein is likely adequate for cropping hyperaccumulators. However, for cultivation of "normal" crops in conventional agriculture, Ca-fertilization might be required to enhance plant growth and yields (Bani et al., 2018).

Another often cited characteristic of serpentine soils is a low amount of organic carbon, which is partly a result of scarce vegetation and thus low input of organic matter (Brooks, 1987). Despite clay minerals, humic substances have a great sorption potential for nutrients, which are present in a cationic form (e.g. Ca, Mg, K). Ca and Mg are attracted to the negatively charged sites of clay minerals and organic matter, preventing leaching from soils but still available for plant uptake (Schulte and Kelling, 1985). Thus, the CEC is increasing with the rate of humifications due to oxidation and thus formation of carboxyl groups, which can complex cations. Hence, especially soils poor in Ca require sufficient amounts of organic carbon to sustain a high CEC (Scheffer et al., 2010).

Total carbon. Many of soil biological and physicochemical properties are controlled by soil organic matter (Doran and Perkin, 1994), as it improves a set of soil parameters, such as soil structure, biological activity, water holding capacity, nutrient retention as well as filter- and buffer functions (Baumgartner et al., 2017). Thus, soil organic carbon (SOC) is essential to sustain soil productivity and fertility (Baumgartner et al., 2017). Similarly, Shukla et al. (2006) claimed that SOC is the most important indicator in soil quality assessment. The amount of carbon is often closely linked to soil texture, as soils dominated by smaller pore sizes (e.g. clay, silt) often show a higher humus content (Shukla et al., 2006). According to ÖNORM L 1050 (Baumgartner et al., 2017), medium heavy soils with a clay content between 15 - 25%, should contain 2.5 - 4.0% humus if used for agriculture. The Bernstein soil showed humus contents within this range, from 3.23% humus in Ref to 4.44% in OM (excl. S). Another threshold cited in literature is the percentage of soil organic carbon (SOC %), which should exceed 20 g kg^{-1} in agricultural soils (Baumgartner et al., 2017). The percentage of SOC was in all treatments above

20.0 g kg^{-1} (incl. S), also in non-fertilized C (20.9 g kg^{-1}), LD (21.6 g kg^{-1}), as well as Ref (20.4 g kg^{-1}). In contrast, the amount of SOC in NPK was slightly below the threshold (19.0 g kg^{-1}) exclusive sulphur plots. In summary, the Bernstein soil contained on average ~ 20 g kg^{-1} SOC, which is just sufficient for agricultural purposes but quite high for serpentine soils. For example, Bani et al. (2015a) reported a 10-fold lower SOC content in ultramafic Vertisols in Albania (2.74 g kg^{-1}). In contrast, Ghasemi et al. (2018) reported high amounts of total carbon with 47.1 g TC kg^{-1} soil and Álvarez-López et al. (2016) 30.3 g TC kg^{-1} soil in ultramafic soils in Spain. However, Bünemann et al. (2018) postulated that the effects of agronomic measures on total soil organic matter might be hard to detect, since the total pool is large and effects are only clearly visible after longer time frames. Furthermore, they claim that labile carbon is more sensitive to disturbances than total soil organic matter. Thus, they suggest to better measure particulate organic matter, hot water-extractable carbon, or dissolved organic carbon.

Besides that, we summarize that mineral fertilization (NPK) and a higher planting density (LD) did not significantly increase TC compared to C. In contrast, TC was considerably lower in NPK at t5. Moreover, we observed a higher amount of TC in organic amendments, with significantly highest values in OM and CM, in comparison to NPK but not to C and LD.-Similarly, TN was significantly lower in NPK compared to organic amendments (OM, CM). In addition, we noticed a significant increase of TC with TN over the whole season and in all treatments. This might be due to mineralization of organic matter and thus release of carbon and nitrogen, bound in the organic material. Generally, organic fertilization and especially OM-application were most effective in increasing TC and TN compared to inorganic fertilization or low distance plantation. Analyses of the OM-fertilizer before application to field showed, that OM contained the highest amount of Ca, Mg and some heavy metals, while containing less P and K in comparison to CM and PM. At t5 and t6, plots treated with OM showed significantly higher concentrations of Ca besides TC and TN. Interestingly, the amount of nitrogen in OM-fertilizer was half of the amount in CM-fertilizer and also TC was considerably lower. Furthermore, covariance analyses (excl. S) showed a significant increase of TC with Ca at t5, while this was not detectable for K, Mg or CEC. In addition, highest TC contents in OM and CM coincided with highest Ca concentrations. Rowley et al. (2018) observed a correlation between exchangeable Ca and SOC and claimed that cation bridging by Ca is a crucial component of SOC stabilization. Positive cations can bridge negatively charged surfaces of organic matter or clay minerals via inner- and outer sphere interactions (Rowley et al., 2018). Base cations, such

as Ca and Mg are part of the group A cations, indicating that they theoretically tend to form inner sphere complexes with oxygen-bearing ligands, such as carboxylate groups of organic matter (Rowley et al. 2018). In contrast, cations of group B have labile electrons which enable them to form complexes with nitrogen- or sulphur-bearing ligands via covalent bonding (Rowley et al. 2018). Furthermore, Rowley et al. (2018) postulated that the role of cations in enhancing soil structure is well understood, while their contribution to organic carbon stabilization is less investigated. They proposed that modelling interactions between organic carbon and Ca indicated, that SOC is stabilized by Ca via inner sphere as well as outer sphere processes (Rowley et al., 2018). Besides that, they cited recent studies (Rowley et al., 2018 after Kalinichev and Kirkpatrick, 2007; Wen et al., 2017), in which the authors suggested that SOC stronger associates with Ca than Mg. Hence, this might be one explanation why in Bernstein the TC significantly increased with Ca but no effects for Mg or K were detected. Thus, higher amounts of Ca in OM might be linked to greater TC contents as well as pH. Nevertheless, at a slightly acidic pH like in Bernstein, organic matter is usually bound to permanently charged clay minerals, building clay-humus-complexes (Scheffer et al., 2010).

Nitrogen. Besides carbon, nitrogen is an essential component of humic acids. During mineralization, carbon in organic matter is released as CO_2, while nitrogen is first incorporated into microbial biomass before being stabilized to 95% in organic complexes. For example, nitrogen contained in peptides can be stabilized in organic mineral complexes or $N\text{-}NH4^+$ is bound to clay minerals (Scheffer et al., 2010). The concentration of mineral nitrogen (Nmin) in soils depends on soil properties (e.g. humus- and clay content, pH, texture). The amount of Nmin in soil can change quickly in response to variations in temperature and soil moisture as well as plant uptake, leaching after heavy rainfall, volatilization, or immobilization (Baumgartner et al., 2017). In contrast to other soil nutrients, nitrogen in soil is mainly present in its plant-available form ($N\text{-}NH4^+$ and $N\text{-}NO3^-$). The amount of Nmin for sufficient plant nutrition depends on the crop species. In agricultural soils, mineral nitrogen is often dominated by nitrate, while ammonium is very low or sometimes not detectable (Hartz, 2007). This is due to the fact, that ammonium is a substrate for autotrophic nitrification and gets quickly oxidized to nitrate, especially in well aerated and dry soils (Hartz, 2007). Soil mineral nitrogen is measured shortly after crop establishment to estimate the amount of mineral nitrogen present in soil, which is readily available for plant uptake and growth (Hartz, 2007). The current nitrogen availability is often determined by the amount of $N\text{-}NO3^-$ as it usually dominates the mineral

nitrogen fraction in soil. Hence, Hartz (2007) suggested that if soil $N\text{-}NO3^-$ levels exceed 20 mg kg^{-1} soil, enough N is present to support plant growth for at least a few weeks. Depending on the N-demand of the crop, further fertilization might not be needed. In contrast, $N\text{-}NO3^-$ concentrations below 10 mg kg^{-1} soil indicate limited N-nutrition that requires fertilization according to Hartz (2007). However, we interpreted these thresholds for the total amount of mineral nitrogen (Nmin = $N\text{-}NO3^-$ + $N\text{-}NH4^+$), as both are plant available forms present in samples, even though nitrate dominated. At the beginning of the growing season (t5), the highest amount of Nmin was observed in NPK, which was the only treatment providing sufficient amounts of readily plant available nitrogen with 21.9 mg kg^{-1} (incl. S), even though amounts were below threshold when sulphur plots were excluded. Besides that, NPK sign. increased Nmin concentrations compared to C and Ref, while Nmin also tended to be higher in PM. The amount of Nmin was highest in PM at t4 but decreased within 4 weeks considerably. This negative trend was observed in all treatments except NPK, where Nmin concentrations increased. We assume that it took more time to mineralize nitrogen from the inorganic fertilizer, than organic material that was present in soil and mineralized in the unfertilized plots (C, LD, Ref) and those from the organic fertilizers. Thus, the decrease of Nmin might result from quicker mineralization of nitrogen, which was readily taken up by the plant. Hence, at the early stage of the growing season when crops have been planted, PM provided sufficient amounts of Nmin (25.6 mg kg-1 soil) but declined to 9.6 mg kg-1 soil within 4 weeks. However, in non-vegetated Ref the decline might result from leaching of $N\text{-}NO3^-$, as the Nmin fraction was dominated by negatively charged nitrate, which is easily leached in slightly acidic soils. In contrast, $N\text{-}NH4^+$ might have been exchangeably bound to organic or clay minerals, or immediately mineralized to $N\text{-}NO3^-$. However, we did not detect a sign. increase of $N\text{-}NO3^-$ with $N\text{-}NH4^+$. While NPK provided sufficient mineral nitrogen, application of other treatments might require a second N-fertilization. At t5 amendments with CM and PM showed mineral nitrogen contents just below the threshold (< 10 mg kg-1 soil), but both provided a good share of Nmin at plantation (t4). In contrast, in OM and LD a second N-fertilization might have been more plausible, as Nmin concentrations were even below levels in C and Ref. Especially the organic amendment with compost (OM) seemed to be an insufficient Nmin source, while the amount of total nitrogen (TN) was highest in OM. Besides that, NPK plots contained the lowest of amount of TN, that was even below the concentration of TN in Ref. When looking at the initial nutrient concentration (N, P, K) in applied fertilizers (table 2.1), we see that NPK-fertilizer contained

the lowest N amount and OM- and CM-fertilizer the highest amounts. Nevertheless, this cannot explain why TN in NPK was lower than in unfertilized C and LD, which were also cropped with hyperaccumulators. Since TC significantly increased with TN and vice versa, one reason might be the low amount of organic matter in NPK plots. Due to that, NPK fertilization provides less possibilities to bind mineral nitrogen ($N\text{-}NH4^{+}$). On the other hand, it could also be that mineral nitrogen was readily taken up by the plants and thus did not increase the total soil nitrogen pool. Hipfinger et al. (2020) reported highest biomass in NPK and PM (1.9 t ha^{-1} respectively), that was 3.7 times higher than in unfertilized C. Interestingly Nmin contents were significantly higher in NPK and considerably higher in PM compared to C at harvest (excl. S).

The soil nitrogen mineralization potential is defined as the ability of a soil to convert organic N, which cannot be taken up by the plant, to mineral N. Thus, N is made plant available. Even though the amount of TN is no direct indicator for N mineralization potential, a general rule is that it is limited in soils containing less than 0.7 g TN kg^{-1} soil. In contrast, concentrations above 1.5 g TN kg^{-1} soil are sufficient for N mineralization in a following crop cycle (Hartz, 2007). All treatments in Bernstein showed total nitrogen contents above this threshold, ranging from 1.8 g kg^{-1} to 2.2 g kg^{-1}, theoretically indicating an adequate nitrogen mineralization potential.

Available Phosphorus. Besides N and K, a low P content is often reason for limited plant growth in serpentine soils (Pędziwiatr et al., 2018). Phosphorus is vital for various processes, such as energy generation and transfer, photosynthesis, carbohydrate metabolism, N-fixation and translocation of nutrients within the plant (Manschadi et al., 2014). Furthermore, it stimulates root development. Hence, a sufficient P supply is essential in the early growth stage of plants (Parnes, 2013). Sims (2000) claimed, that an optimum level of P, assessed by the Olsen-extraction, is around 10 mg P kg^{-1} soil. Except for Ref, all treatments showed P concentrations considerably above this threshold, at the beginning of the growing season as well as at harvest. Thus, all plots cropped with hyperaccumulator *O. chalcidica* contained enough P for plant development, while in Ref soil was deficient in P. As shown in fig. 3.11, Ref plots showed over the whole vegetation period P contents below this threshold. At the beginning of the season (t4), P contents in hyperaccumulator treatments varied between 22.5 mg P kg^{-1} in C and 31.0 mg P kg^{-1} in PM, while it was about a third lower in Ref (~ 7.7 mg P kg^{-1}) and remained at an almost constant level over the whole season. The P concentration in untreated Ref was similar to Ghasemi et al. (2018), who reported 4.6 mg P kg^{-1} soil in untreated control plots in an ultramafic soil in Spain. Given the fact, that except for Ref, all other plots were cropped in the

previous experimental year with hyperaccumulators (*O. chalcidica* and *N. goesingense*), one explanation could be a plant-induced mobilization of P. This hypothesis might be confirmed by results of Cerdeira-Pèrez et al. (2019), who recorded significantly higher extractable P concentrations in soil, after planting *O. muralis* (1.8 fold) and *O. serpyllifolia* (1.7 fold). However, there are two points against this hypothesis. First, P contents in C remained almost at the same level through the growing period. Second, we also did not detect significant differences between time points for P, which would indicate depletion or mobilization of P.

The highest concentration of P was recorded in PM, which was significantly higher than in other fertilizer treatments (CM, OM, NPK), LD, C and Ref (excl. S). The availability of P in soil can be affected by pH and biological activity (Parnes, 2013). At lower pH, biological activity might be reduced, which also limits the release of phosphorus by soil organisms. In contrast, at higher pH, P-availability might decline due to precipitation of P with Ca. We did not observe a significant decrease of P-availability with higher Ca concentrations. Furthermore, there was also no tendency of lower P concentrations in soils with higher Ca levels, such as OM or LD. Anyhow, precipitation by Ca is more a risk at alkaline pH, while our treatments showed a slightly acidic pH (~ 6.3), which is close to the optimum pH range for P-availability (6.5 to 6.8) (Parnes, 2013). However, Brooks (1987) postulated that even though serpentine soils usually are on average within the optimum pH, P is often limited due to a high affinity of soluble phosphates for serpentine clay minerals (Brooks, 1987). In summary, we postulate that PM was the most effective fertilizer treatment to increase P-availability in soil. Interestingly, PM was also the treatment with a significant higher QBS-index (biological soil quality index). The QBS is an indicator for the adaption level of soil organisms to edaphic conditions. In contrast, microbial activity assessed by FDA-enzyme activity was only slightly higher in PM compared to C. Moreover, FDA-enzyme activity was highest in CM, which showed the second highest amount of P after PM. Parnes (2013) claimed an increase of biological activity with higher available P concentrations in soil. Even though we did not find significant covariances between P contents and biological parameters, there might be a linkage as we reported a higher abundance of Collembola and Acari, mesofauna density, QBS-index and FDA-enzyme activity in manure treatments (CM, PM).

4.2.1.2 Biological soil quality

Soil organisms play a key role in OM degradation, nutrient cycling and bioturbation, which is why they contribute substantially to soil quality, especially in nutrient-limited ecosystems (Scheffer et al., 2010; Santorufo et al., 2012; Yan et al., 2012). Human activities, such as agronomic practices (e.g. fertilization, ploughing) can however negatively affect species richness and abundance of soil mesofauna. Thus, the investigation of mesofauna community is a tool often suggested to monitor soil degradation. We chose Collembola and Acari as key indicator organisms, as they accounted for 62% (t4) and 70% (t6) of soil arthropod fauna and contribute substantially to decomposition and mineralization of dead organic matter and microbial population control. Second, due to the fact that they live with exception of some epigeous Collembola species, solemnly in existing pores in soil, they are highly affected by changes in soil properties. Thus, their population dynamics are an indicator for integrity of soil quality and can reflect natural or anthropogenically induced changes of soil (Gbarakoro and Zabbey, 2013; Roy et al., 2018; Scheffer et al., 2010).

Species composition. The average number of Collembola and Protura stayed at the same level over the vegetation period. Furthermore, we noticed one plot in PM with a high abundance of Acari as well as Diptera larvae. We assume this was mainly due to sampling directly in a manure hotspot, as fly larvae prefer moist situations and are important saprovores (Coleman et al., 2018). The total and relative abundance of Acari did not differ significantly between treatments. However, we observed a significant increase of Acari between t4 and t6, which was linked to agglomerations of prostigmatic mites in manure treatments (PM, CM) but also in Ref, at harvest. These agglomerations did not occur at the beginning of the season, shortly after ploughing and fertilization (t4). When looking closer into Acari species composition, we recorded a dominance of Mesostigmata (77%) at t4, but not at t6 (14%). However, this shift was not a result of a decline of Mesostigmata, as abundance stayed on average the same, but due to the considerable increase of Prostigmata, which dominated Acari at t6 (68%). Prostigmatic and mesostigmatic mites are fungivores and predators for Collembola and other microarthropods as well as Nematoda (Crossley et al., 1992). Thus, they control the population dynamics of those organisms. Even though C:A ratio significantly decreased within one growing season, this cannot only be explained by a higher abundance of predaceous mites, as the abundance of Collembola stayed on average at the same level over the growing period.

C:A ratio. The C:A ratio is interpreted differently in literature. On the one hand, some authors outlined the higher sensitivity of Acari to soil degradation (Jacomini et al., 2000), while Santorufo et al. (2012) observed decreasing abundance of Collembola in traffic-induced polluted soils with pH 6.8 to 7.3 and elevated concentrations of Cu, Pb and Zn. Since we are looking at possible negative effects of metal-hyperaccumulators on soil fauna, we decided to consider Collembola more sensitive to metal availability and thus interpreted a higher C:A ratio as an improvement of soil quality. We did not detect any significant differences or trends between treatments. However, the decrease in C:A ratio between t4 and t6 was significant, which might indicate a decrease in soil quality. However, as we already clarified, the lower C:A ratio wasn`t caused by a decline of Collembola, but a substantial increase of Prostigmata in some plots. Prostigmatic mites adapt quickly to changing soil properties after disturbances (e.g. tillage), which is why they are among the most frequent soil mites found in cultivated agricultural soils (Crossley et al., 1992). Generally, abundance and species richness of soil mesofauna is increasing with a higher porosity and organic matter content (Neher and Barbercheck, 1998). However, tillage destroys soils aggregates and pores and cracks are filled by smaller material, decreasing pore content and thus mesofauna abundance in top layers of cultivated soils. Moreover, Roy et al. (2018) outlined that organisms with high metabolic rates and short life cycles (k-specialists), are less affected by tillage, that usually disturbs the whole food web. Thus, tillage and other agronomic practices can cause multiplication of nematodes and some soil mites (e.g. Prostigmata, Astigmata, Mesostigmata) but reduces abundance of k-specialists (e.g Oribatida and Collembola) (Behan-Pelletier, 2003). In contrast, Crossley et al. (1992) claimed that cultivation caused a decrease of Oribatida and Mesostigmata but raised abundance of Collembola and Prostigmata. As mentioned before, we noticed that Mesostigmata dominated Acari population 1 week after tillage and fertilization (t4). Second, their average abundance did not decrease over the growing period. Thus, we suggest that cultivation did neither considerably decrease, nor enhance Mesostigmata population. Besides that, we noticed that the majority of observed mesostigmatic mites in hyperaccumulator treatments, belonged to the cohort of Gamasina, free living predaceous mites. According to Dervash et al. (2018), gamasid mites are good indicators for soil quality and ecotoxicology, due to a high sensitivity to external impacts. In addition, we observed some individuals of Oribatida in CM, LD and OM, but not in C, PM, NPK and Ref at t6, while no oribatid mite was recorded at t4. Indeed, oribatid mites are considered being sensitive and declining shortly after

cultivation, which is why they are often used as an indicator for soil quality. However, Gergocs and Hufnagel (2009) and Murvanidze et al. (2019) claimed that some groups of Oribatida are tolerant to agriculture activities. Maybe the Bernstein soil was recolonized from oribatid mites of the forest, where their abundance is usually highest. Due to the past history of agricultural management at the experimental site, the agriculture field might have been as well already inhabited by stress tolerant Oribatida. Despite that, cultivation had clearly a positive effect on abundance of Prostigmata. In accordance with Crossley et al. (1992) and Behan-Pelletier (2003), we propose that species composition was changed by agricultural practices, probably due to changing soil conditions. However, changing soil conditions, might also cause an elevated abundance of tolerant species and decline of sensitive species over a longer time frame (Gulvik, 2007; Parisi et al., 2005; Santorufo et al., 2012). Thus, one might risk interpreting a higher abundance with a better soil quality. However, we did not analyse mites to species level. Besides that, there was also a considerable rise of Prostigmata in uncultivated Ref at t6, which we did not observe in C and thus cannot be linked to soil cultivation. McIntyre et al. (2001) claimed amongst others, that due to their unsensitivity to a broad range of soil properties, the general abundances of Acari and Collembola are weak indicators to relate to soil quality. Thus, they outline the use of single species (e.g. oribatid mites, nematode, earthworms). On the contrary, Parisi et al. (2005) argued that focusing on species level requires higher knowledge in taxonomy. Despite that, Santorufo et al. (2012) noticed that changes in soil properties were reflected in abundance and density of invertebrates but didn´t considerably impact taxa richness.

Besides tillage, also the application of fertilizers can affect abundance and composition of soil mesofauna. In general, application of inorganic and organic fertilizers might influence soil organisms directly by adding nutrients and indirectly via better plant growth and thus accelerated root exudation (Scheffer et al., 2010). However, the response of mesofauna to fertilizer application is complex and is species dependent (Wang et al., 2016). Mineral fertilizers can decrease the abundance of Oribatida and Prostigmata or increase populations of Astigmata. Manure potentially accelerates mesofauna abundances by adding nitrogen and other nutrients as well as microbes and their food supply, both present in the organic fraction applied (Neher and Barbercheck, 1998). In addition, organic amendments improve the soil habitat by reducing soil compaction, temperature- and moisture fluctuations (Miyazawa et al., 2002). Also, Murray et al. (2006, cited in Wang et al. 2016) explained that a rise in soil fauna abundance after organic fertilization can be explained by increased detritus and nutrient availability as well as improved

micro- and macro-aggregation. Scheffer et al. (2010) postulated, that if organic and mineral fertilizers are applied with the same amount of nutrient contents (N, P, K), the activity of soil organism will still be higher in organic fertilizers. For example, urease and phosphatase activity is higher in manure compared to NPK. We observed a higher FDA enzyme activity in CM, which was significantly higher than in NPK. Interestingly, TN and P were also less contained in NPK compared to CM and PM. However, there was also way more N and P applied to soil via manure than in NPK per ha^{-1} soil.

Mesofauna density. Generally, the spatial and temporal distributions of soil microarthropods are controlled by food supply, temperature, and moisture conditions (Scheffer et al., 2010). Like higher plants, soil organisms require mineral nutrients (e.g. N, P, K, Ca, Mg, S, Mn, Fe, Cu and Zn) for growth (Scheffer et al., 2010). Thus, it`s reasonable that their abundance might increase with higher nutrient availability. Miyazawa et al. (2002) reported a correlation between mesofauna density and organic matter content, mineralized nitrogen and soil compaction. Furthermore, Cerdeira-Pèrez et al. (2019) claimed that exchangeable Ca and Mg, available P and -K influenced mesofauna abundance. We observed no influence of higher availability of P, K, Ca, Mg or Cu on mesofauna density. Even though covariance analyses showed a significant decrease of total and relative abundance with higher amounts of available P in NPK, OM, C, and PM (incl. and excl. S), the average abundance of Collembola was highest in CM and PM, that also showed highest concentrations of available P. These contradictive results might be explained by two plots (24_CM and 10_PM) that showed exceptionally higher numbers of Collembola than the average of the treatments. We considered both plots being artefacts. Furthermore, the significantly higher abundance of Collembola in CM cannot be explained by significantly higher amounts of available K in CM. Furthermore, no effects of nutrient concentrations Acari abundance was observed. In addition, we found no influence of TC, TN or Nmin on mesofauna density as well as abundance of Acari. Besides that, we observed a trend of higher relative abundance of Collembola in PM, while the total abundance of Collembola was highest in OM at t4 with lowest Nmin concentrations and mesofauna density highest in Ref with no fertilization but also no ploughing. Interestingly, the total abundance of Collembola tended to be higher in OM and quite low in PM at t4, while the relative abundance of Collembola was slightly higher in PM. This might be explained by the fact that at t4 mesofauna abundance tended to be in general higher in OM and Ref. Hence, we assumed that especially PM increased Collembola abundance (as the overall mesofauna density was quite low in PM at t4). In contrast,

relative abundance of collembola was highest in CM as well as mesofauna density. Quite in contrast to what we expected, mesofauna density and abundances of Collembola and Acari , tended to be lower in OM, which showed the highest amounts of TC and TN. However, also Wang et al. (2016) did not observed an increase of mesofauna abundance with higher TC and TN. Furthermore, a significantly higher concentration of Nmin at t4, was not reflected in mesofauna abundance.

Low sampling numbers. When looking at the overall abundance of mesofauna, we have to outline that the average mesofauna density of the experimental site is very low when compared to other studies. For example, Crossley et al. (1992) reported 50,000 – 100,000 indiv. per m^{-2} in annual cropping systems in Canada, while we observed an overall mesofauna density of 41 indiv. m^{-2} at harvest. Having in mind that the experimental site in Bernstein is on serpentine soil with low nutrient concentrations and high metal availability, we also compared mesofauna density with Santorufo et al. (2012), who reported 6,000 to 41,000 indiv. m^{-2} in an urban polluted soil (Cu, Pb, Zn) in Italy, which is still a factor 1000 higher than our records. Possible explanations for low sampling numbers are a) mistakes during sampling in field or b) during extraction. Regarding the later, one mistake might be a too short extraction time. However, we set up the Berlese-apparatus for 5 whole days and can ensure that the light bulbs were turned on the whole time. Furthermore, the soil was quite dry when sampled and setting up the apparatus. Thus, we claim that extraction time was long enough to force all soil organism to migrate down the profile and being conserved for further analyses. In addition, mistakes can also happen during soil sampling. First, samples in spring (t4) were taken just 1 week after tillage. Tillage is known to decrease mesofauna density substantially (Dervash et al., 2018). However, Miyazawa et al. (2002) reported a mesofauna abundance of 8,310 indiv. m^{-2} in a reduced tillage agricultural field. Even though we did not assess mesofauna abundance before tillage and fertilization, our average number of 41 indiv. m^{-2} is still far below numbers reported by Miyazawa et al. (2002). Furthermore, tillage is often followed by a change of species composition, where abundances of predatory mites rise, while k-specialists (e.g. oribatid mites and collembola) decline (Crossley et al., 1992). Indeed, a dominance of predatory mites was observed at t4. Thus, 77% of Acari were classified as Mesostigmata and the majority belonged to the suborder Gamasina, which are predatory mites. Additionally, no individuals belonging to Oribatida were sampled. Besides that, mesofauna density tended to be higher in the undisturbed Ref and was significantly greater than in PM and LD. Nevertheless, mesofauna density was still way lower in the undisturbed

Ref, compared to results by Miyazawa et al. (2002) amongst other studies. Tillage is a traditional agricultural practice to homogeneously distribute nutrients and organic substances in topsoil, improve aeration and thus enhance microbial growth. Reduced tillage, where soil is only loosed but not turned over can have positive effects, while conventional tillage is often reason for soil compaction and thus reduction of soil porosity. As mesofauna is living in soil pores, their abundance usually declines after cultivation (Neher and Barbercheck, 1998). Even though we did not intensively plough the soil, tillage might still have caused a reduction of soil porosity and as well as drying of the topsoil. Indeed, we noticed a very dry topsoil at t4 and t6. In addition, the second sampling (t6) was in October, where mesofauna abundance is usually starting to decline in topsoil due to higher temperature fluctuation during day and night. This is confirmed by McIntyre et al. (2001), who claimed that abundance of arthropods was highest in summer, lowest in winter and intermediate in spring and fall, which was linked to the average daily maximum and minimum air temperature. Thus, besides nutrient availability, also temperature and soil water content are essential factors possibly limiting biological activity (Scheffer et al., 2010). The soil in Bernstein has a high skeleton content, darker colour, low water holding capacity and is quite sandy. Thus, it can warm and dry up easily during the day, while mesofauna prefer a moist and cold environment. In fact, we sampled in the afternoon, where air and probably soil temperature was high, while the soil was dry due to a lack of precipitation. The high temperature fluctuation and dry soil conditions might have caused vertical migration of edaphic organisms, reducing their abundance in the first 10 cm of soil, where usually the highest biological activity occurs (Scheffer et al., 2010; Visoli et al., 2013; Menta et al. 2018). To limit temperature fluctuations and retain soil moisture, we applied mulch on the topsoil. Since we neither analysed temperature nor soil moisture, these are all just assumptions and attempts in trying to explain the low mesofauna density. Thus, further investigation is needed to relate abundance and species composition to soil moisture, temperature and serpentine properties in general.

QBS. Besides mesofauna abundance, we assessed the QBS-index as a further indicator of biological soil quality. The strength of this indicator is a general evaluation of the adaption of soil arthropods to the edaphic environment. As a result of this adaption, edaphic organisms are highly dependent on their immediate environment and respond quickly to changes in their habitat. Thus, soils with a good quality have a high number of groups well adapted to soil. Simultaneously, we assumed that a negative effect of cropping Ni-hyperaccumulators, would

be reflected in less edaphic forms and thus lower biological soil quality. Menta et al. (2018) investigated 41 published papers to clarify the relationship between soil quality and different land uses. They suggested that agricultural soils, urban parks and degraded soils (e.g. serpentine soil) generally showed the lowest QBS-values. Furthermore, they defined the overall mean of QBS of all land uses investigated as a threshold (QBS=93.7), to separate soils with high quality from poor soils. Despite that, QBS values for natural degraded soils ranged between 80–110, with an average of 90 and agricultural soils (55-101) on average 84.5. Considering the site in Bernstein as a naturally degraded agricultural field on serpentine substrate, our values are more or less in that range with an average QBS of 52.9 in hyperaccumulator treatments and 58.8 (QBS) in Ref, at harvest. Furthermore, the QBS was significantly higher in PM, while other fertilizers and low distance plantation did not increase QBS considerably compared to C or Ref. Even though none of the treatments reached a QBS value above the threshold (93.7), the QBS-index of PM increased from 31.5 (t4) to 80.3 (t6) within one growing season and was already quite close to a value of high soil quality according Menta et al. (2018). Due to the fact that we did not observe a significant lower QBS value in plots cropped with *O. chalcidica* compared to Ref and QBS increased in all hyperaccumulator treatments within one growth period, we propose that Ni-hyperaccumulation does not negatively influence the biological soil quality. To clearly propose an improvement of soil quality longer monitoring is needed. First, these are only results of the first year of application and second, we interpreted results from an one-time observation in April (t4) as well as October (t6).

Microbial Activity (FDA). Similarly to soil arthropods, changing soil conditions can also be reflected in the microbial community. Schloter et al. (2003) recommended for a minimum dataset in soil quality assessment, microbial biomass content and microbial activity rates incl. enzyme activities, despite soil physicochemical soil properties (e.g. organic carbon, pH). Soil enzymes as a soil quality indicator, can be useful to evaluate the sustainability of land management practices, as they reflect potential nutrient cycling processes (Schloter et al., 2003). However, Schloter et al. (2003) also pointed out, that interpretation is limited and that it is hard to decide which enzyme activity should be determined. For example, there are 500 enzymes that substantially influence cycling of C and N in soil. Thus, Schloter et al. (2003) proposed “benchmark” enzymes, which are able to inform early about changes in soil processes. For example, the biological activity in soil is closely linked to the amount of organic matter, as it is the basis for life for heterotrophic soil organisms (Scheffer et al., 2010). Dehydrogenase enzyme

activity, assessed by the FDA method, is involved in transportation of electrons during oxygen metabolism and thus an indicator of microbial oxidation of organic matter (Prasanthi et al., 2019). A higher enzyme activity directly influences organic matter formation and microbial growth, which also supports soil mesofauna populations. With the increase of faunal activity, the availability of organic carbon, phosphorus and exchangeable cations (e.g. Ca, Mg, K) raises (Prasanthi et al., 2019). However, to maintain biological activity at a high level, a continuous supply of organic matter as substitution of the used up organic material in soil is needed (Scheffer et al., 2010). Thus, in agricultural systems, were organic matter is continuously removed during harvest, fertilizers are required. The organic matter in soil is usually composed of dead biomass of plants (e.g. leaves) and roots, as well as root exudates, microorganism and dead soil organisms. A substantial part of soil organic matter is contributed by dead roots and rhizodeposition, which are low molecular and N-rich organic substances released by plant roots. Rhizodeposition contributes substantially to carbon content in soils. Root exudates contain a range of lower molecular components, like sugars, polysaccharides, organic acids, amino acids and peptides, which are immediately assimilated by soil microorganism. Thus, the density of microorganism is usually higher in the rhizosphere (Scheffer et al., 2010). Furthermore, Cerdeira-Pèrez et al. (2019) reported higher bacterial densities in soils cropped with hyperaccumulator, compared to non-planted soils and assumed that plants enhanced microbial abundance via root exudation. Additionally, Scheffer et al. (2010) postulated that with a higher planting density, the number of soil organisms increases due to a higher food supply via rhizodeposition, stimulating microbial growth. We observed a higher microbial activity in LD treatments with a higher rooting density, however not significantly different to C. Besides that, we did not record any influence of higher soil TC contents on enzyme activity, in contrast to Cerdeira-Pèrez et al. (2019). Organic amendments are rich in C and are known to increase soil fertility, structure, microbial enzyme activity as well as mesofauna abundance (Prasanthi et al., 2019). We noted highest microbial enzyme activity (FDA) in CM, but not significantly higher than in C or Ref. Furthermore, second highest FDA was measured in LD, followed by PM. The abundance of Collembola and Acari as well as QBS-index was also higher in CM and PM, followed by LD. In contrast to Prasanthi et al. (2019), who recorded a positive relation between mesofauna and microbial enzyme activity (e.g. dehydrogenase, acid and alkaline phosphatase and urease), we did not observe an increase of mesofauna abundance with higher FDA-enzyme activity. Even though FDA-enzyme activity was not significantly different between any

agronomic measure and C, the higher activity in LD and CM was significant when compared to NPK. This is also confirmed by Cerdeira-Pèrez et al. (2019), who reported significantly higher FDA-enzyme activity in organic amended soils (composted sewage sludge) compared to NPK, in planted as well as non-planted treatments. We observed 117 mg kg^{-1} $3h^{-1}$ in NPK, while Cerdeira-Pèrez et al. (2019), recorded around 60 mg kg^{-1} $3h^{-1}$ (NPK) and approximately 75 mg kg^{-1} $3h^{-1}$ in sewage sludge, which was less than half of the FDA-enzyme activity we recorded in organic amendments (CM, PM, OM) on average (150 mg kg^{-1} $3h^{-1}$). Thus, in the case of CM, higher microbial activity may be linked to the set of nutrients provided by the CM-fertilizer, which might support microbial growth better than a mineral fertilizer just containing inorganic N, P and K. Furthermore, CM contained the highest amount of N and K and was rich in P, Cu and Mn, compared to other organic amendments. Besides that, CM showed a significantly higher abundance of Collembola. According to Neher and Barbercheck (1998), application of compost or manure also adds microbes to the soil, which are a food source of soil arthropods. Hence, this might partly explain first the lower FDA-enzyme activity in mineral fertilizers (NPK) compared to CM, and second the higher abundance of Collembola and Acari in manure treatments (CM, PM). Furthermore, another reason for a higher microbial activity might be explained by the "microbial loop". During mineralization of organic matter, nitrogen is fixed in microbes. By grazing on microbes, soil mesofauna increases the nutrient availability in soils, because nutrients immobilized in microbes are released. In response plant growth and root exudation increases, which in turn boosts microbial growth and is in summary understood as the microbial loop (Neher and Barbercheck, 1998; Scheffer et al., 2010). Furthermore, droppings of Collembola and Acari enhance decomposition and nutrient availability, as mesofauna breaks down bigger organic material, moistens it and makes it available for microbial decay (Neher and Barbercheck 1998). In contrast, Moldenke (s.a.) stressed that a too high density of grazer populations might also decrease bacterial and fungal populations. Most springtails and some mites, graze on fungi and to some extent on bacteria (e.g. from roots). Due to that, Moldenke (s.a.) claimed that predatory arthropods are essential to keep grazer populations under control and to prevent over-grazing on microbes.

Organic matter showed the significantly highest amount of TC and TN. Still, biological activity (enzyme activity, QBS, abundance of Collembola and Acari) was not improved compared to C and Ref. One explanation might be the earlier explained complexation of organic substances by Ca. If excessive Ca stabilizes SOC, usually at more alkaline pH, microbial respiration can be

reduced, as only unstable organic substances are decayed by microbes (Parnes, 2013). However, we found no significant covariance between Ca and FDA. Furthermore, the pH in all plots treated with OM was slightly acidic. Interestingly, also the abundance of Collembola in OM declined between t4 and t6. Besides that, highest P was observed in PM and available K was significantly higher in CM at t6. Even though FDA was higher in CM, we did not observe a significant increase of FDA with K. Besides that, analyses of CM-fertilizer showed that cow manure contained more Cu, Fe, Mn, Ni as well as other heavy metals (Cd, Co, Cr, Pb). However, we did not notice a significant increase of FDA-enzyme activity with metal availability after excluding sulphur plots. Sulphur plots showed considerably lower pH. The pH is an important indicator controlling microorganism. Their activity usually rises with a higher pH. In contrast, FDA-enzyme activity was highest in 8_NPK (150.56 mg kg^{-1} $3h^{-1}$) showing a considerably lower pH, compared to the 3 other NPK plots with an average FDA-enzyme activity of around (84.91 – 117.19 mg kg^{-1} $3h^{-1}$). Furthermore, we also did not observe a significant covariance between pH and FDA. Nevertheless, there seemed to be an influence of TC, TN, P, Fe and Cu on enzyme activity. For example, contents of TC were considerably lower and even fell below the threshold of 20 g SOC kg^{-1} soil in NPK. The overall lowest FDA-enzyme activity was observed in NPK_1, which showed a considerably lower TC content (10.42 mg kg^{-1}), compared to the other NPK plots (20.35 - 26.24 mg TC kg^{-1}. Moreover, TN was lower in NPK_1, however less pronounced. Besides that, highest FDA-enzyme activity was not observed in OM, which showed sign. highest TC content. The carbon content in NPK was below threshold. Hence, a negative effect of too little organic carbon for microbial nutrition might have caused a decrease, while no influence of higher soil carbon contents on enzyme activity occurred, as proposed by Cerdeira-Pèrez et al. (2019). These are however just assumptions. In the end the interpretation of FDA-enzyme activity is limited, as it just measures overall microbial activity, while giving no information about which enzyme activity is increased (e.g. urease or phosphatase). Thus, it maybe just an indicator to get a first impression. Even though indicators are useful tools to detect changes in complex systems (Parisi et al., 2005), Bünemann et al. (2018) claimed that a direct relation of soil properties to biological indicators is often difficult to apply on soil fauna.

4.2.2 Summary Total Soil Quality Evaluation

Highest soil quality regarding soil physicochemical parameters was achieved by application of OM, followed by fertilization with manure (PM, CM), NPK and lowest soil quality in LD, as

no parameter was significantly increased when compared to C. Thus, organic amendments improved soil physiochemical properties better than mineral fertilization and cropping at a higher planting density. Similarly, also Álvarez-López et al. (2016), suggested that compost amendment had a higher growth-promoting effect than inorganic fertilisation. The addition of compost improved soil physicochemical properties, such as nutrient availability, CEC, or organic matter content. Organic amendments cannot only provide essential nutrients (which are usually limited in serpentine soils) but also improve soil structure, porosity and water holding capacity (Álvarez-López et al., 2016) and increase microbial activity (Cerdeira-Pèrez et al., 2019). Also, Nkrumah et al. (2016) proposed that the main positive effect of organic amendments is the improvement of physical properties in ultramafic soils. Especially, soil moisture is increased, which has a positive effect on Ni yield, as ultramafic soils usually have a low water retention (Nkrumah et al., 2016). Moreover, Ghasemi et al. (2018) recorded sign. higher biomass and Ni-yield of three Ni-hyperaccumulators (*Odontarhhena spp.*) as well as a higher nutrient availability and water retention capacity in ultramafic soils amended with CM compared to NPK.

The total soil quality (physicochemical and biological) was highest in manure treatments (CM and PM). When looking at the different results for biological- and physicochemical properties, it`s interesting to note that even though OM showed the highest physicochemical quality, biological activity was not increased. In contrast, highest biological soil quality was reported in CM and PM. In summary, relative abundance of Collembola and Acari, QBS and FDA-enzyme activity were higher in CM and PM, but didn`t increase in OM compared to C. This was quite surprising as microbes feed on carbon and nitrogen. Moreover, we observed that organic amendments improved soil properties better than mineral fertilizer. Fertilization with CM tended to induce higher TC, TN and K contents, while PM increased P. TC and TN, Ca and CEC were sign. higher in OM compared to C, while contents of Nmin and available K were below critical thresholds for plant nutrition. Besides that, fertilization with NPK was the only treatment increasing Nmin to a sufficient level for plant growth at the beginning of the growing season. Even though this might have positively affected plant growth, the application of NPK sign. decreased microbial activity compared to C (excl. S) and resulted in lowest biological soil quality. Thus, fertilization per se did not increase biological activity, but was higher in organic amendments (H1). Furthermore, TC in NPK was below the threshold for SOC. Besides that, lower planting distance (LD) didn`t sign. effect soil quality. Previous studies suggested

inorganic fertilization to increase Ni-phytomining efficiency (Bani et al. 2015a; Álvarez-López et al., 2016). They claimed NPK-fertilizers were better to improve soil properties in agromining because higher biomass production was achieved compared to organic amendments. Besides enhancing agromining efficiency, it is however questionable if amendments which do not only improve Ni-phytoextraction but also enhance soil quality and thus have positive environmental effects, might be better. Indeed, we found second lowest soil quality for NPK, while the biological quality was even lowest in mineral fertilized plots. In contrast, but confirming our hypotheses, organic fertilization was most effective in increasing biological and physicochemical soil quality indicators. Furthermore, Hipfinger et al. (2020) reported highest Ni-yields in CM (22.7 kg Ni ha^{-1}), followed by PM (21.3 kg Ni ha^{-1}) and NPK (20.6 kg Ni ha^{-1}), however not at a sign. level compared to C. In addition, highest biomass was observed equally in NPK and PM treatments (1.9 t ha^{-1}). Thus, in contrast to previous studies, we didn`t record a higher agromining potential of mineral- compared to organic fertilizers. Thus, with respect to the results of soil quality evaluation (table A.5), we recommend organic fertilization with CM or PM to improve soil quality in Ni-agromining.

4.3 Effect of hyperaccumulators on soil quality

Besides evaluating the effect of fertilization and planting distance, the influence of the hyperaccumulator itself on soil quality is interesting. Some authors claimed that agromining has a positive effect on the environment and suggested to introduce it as a strategy to improve soil fertility, while decreasing Ni availability in serpentine soils over a longer time period (Echevarria et al., 2018; van der Ent et al., 2015; Kidd et al., 2018). However, introducing non-native species might also cause negative effects on the soil ecosystem. Thus, agromining should be accompanied by risk assessment, such as monitoring soil quality indicators. Therefore, we paid a closer took to the influence of the hyperaccumulator *O. chalcidica* on physicochemical and biological properties. Furthermore, we tried to investigate possible effects of higher metal availability on mesofauna. Compared to C, available P, pH, Ca and Ca:Mg ratio were sign. lower in Ref. In contrast, Mg and DTPA-Ni were significantly higher in Ref compared to C and other hyperaccumulator treatments. Furthermore, $Sr(NO_3)^2$-Ni in Ref tended to be greater and TC lower than in C. Despite that, we observed no significant differences for available K, CEC, TN and Nmin. In summary, nutrient availability was higher in plots cropped with hyperaccumulator *O. chalcidica,* while metal concentrations decreased. This might indicate a positive influence of

the hyperaccumulator on physiochemical soil properties. Despite that, the mesofauna density and QBS-index tended to be lower in C compared to Ref, at t4. Mesofauna density increased over the vegetation period and reached almost the same level in C and Ref, at harvest. The QBS-index increased in C but stayed at an equal level in Ref. Besides that, abundances of Collembola and Acari tended to be higher in Ref, while no differences for microbial activity were observed. However, no specimen of Collembola was sampled in C at t6. Moreover, total abundance of Acari seemed to decrease in C but increase in Ref, where we noticed an exceptionally high accumulation of Prostigmata. Oribatida, did neither occur in Ref nor in C. Regarding the lower values of biological parameters in C compared to non-cultivated Ref at t4, we propose that this was an effect of tillage and fertilization, which might have influenced soil faunal species composition. Furthermore, we noticed an overall increase of mesofauna density and QBS until harvest. We postulate that cropping *O. chalcidica* doesn`t negatively affect the edaphic life.

4.4 Limitations & Outlook

Sulphur plots. Plots treated with sulphur in 2017 were not excluded from statistics because they didn`t contain outliers, which is why their exclusion would not have been statistically justified. However, during statistical data evaluation, it became obvious that sulphur amendments considerably impacted soil parameters. After excluding sulphur plots (6, 8, 10, 15) from statistics, ANOVA and covariance-analyses were repeated. As a result, for ANOVA already observed trends of differences between treatments became sign., while most of the covariances that were originally conducted with the inclusion of sulphur plots (fig. A.1) and at a sign. level became non-significant. Interestingly, sulphur application seemed to mainly effect soil physicochemical parameters, while not biological indicators (except for microbial activity). This might be due to the fact, that chemical soil parameters are substantially influenced by the pH. Since pH was sign. lower in sulphur plots, these plots showed very different values compared to plots of the same treatment (OM, CM, PM, NPK). In summary, the increase of TC with TN was still detectable over the whole growing period (t4, t5, t6), while for available P only a trend of increasing TN with available P at t6 was recorded. ($p = 0.107$). Furthermore, there was still a trend ($p < 0.1$) of decreasing TN levels with rising pH at t5 and t6. Hence, it was not only a matter of sulphur influence. Interestingly, the already observed trend of increasing TC and TN with Ca, became significant after excluding sulphur for TN, but not for TC. We concluded that the exclusion and comparison of results incl. and excl. sulphur plots were crucial for

interpretation. First, the often quite different concentrations in sulphur plots of NPK, CM, PM and OM increased standard deviations, which were already high due to the field variabilities (block influence). Consequently, results of ANOVA were blurred, as it was for example for $Sr(NO_3)^2$-Ni. Second, most covariances became non-significant excl. sulphur plots. Thus, only looking at results incl. sulphur plots might have caused a misleading interpretation of results.

Field variabilities. Besides the influence of sulphur application, also the natural variabilities/heterogeneity of the field sign. affected results and probably caused higher standard deviations, which also troubled interpretation of differences between treatments. The experimental site was located on a former agricultural soil with a slight inclination to SSW. Block 1 was located at the top of the slope, while block 4 was situated at the foot, in a flatter position. During soil sampling and field maintenance (e.g. weeding), we observed that soils on the top appeared drier and tended to have a coarser texture. In contrast, some plots in block 3 and 4 showed a better aggregate stability and seemed moister. Unfortunately, we did not measure aggregate stability, skeleton- or water content. Thus, we can only assume that due to the slope, physical properties varied in field and caused considerable field differences, which influenced chemical and biological soil properties. We included the block position as a random factor in statistics and noticed a sign. influence of the block position on soil conditions. The amounts of TC, TN, Ca, CEC and FDA were lowest in block 1. For TC, TN and Ca, we Furthermore, concentrations of TC, TN and Ca increased downwards the slope, with highest levels in block 4. In contrast to all soil parameters, highest Mg levels were observed in block 1 and decreased down the slope with lowest concentration in block 4. Interestingly, QBS was highest in block 3 and 4, where we also noticed a higher soil moisture. Furthermore, also DTPA-extractions of Ni, Mn and Fe, were lowest in block 1, while $Sr(NO_3)^2$-Ni tended to be higher in block 1 and decreased down the slope. Besides that, block influence was especially pronounced in the uncultivated reference plots. In Ref, the lowest values for all soil indicators (except Mg) were observed in block 1. Furthermore, available P was at the same low level in all blocks, which again showed that the block didn`t influence P concentrations. It`s interesting that even though TN contents were clearly influenced by the block position, this trend was not observed for $N\text{-}NH4^+$ and $N\text{-}NO3^-$. In addition, block position didn`t affect available K, pH, and the mesofauna abundance, mesofauna density and C:A ratio.

Recommendations. Unfortunately, natural disturbances can occur in field experiments and may cause large data variability. Hence, effects on significance levels of differences between

treatments are a risk. Consequently, often only trends are detectable, which require follow-up experiments to be validated. Besides that, there are a few recommendations for further studies. First, soil quality assessment should identify the multifunctionality of soils, as well as biological, chemical and physical aspects that control soil functions (Doran and Perkin, 1994). We stress that physical properties, especially soil temperature and soil moisture, would have been valuable indicators to better interpret results. Especially since temperature fluctuations and drought are substantially influencing mesofauna abundance (Barbercheck et al., 2009). Some typical properties of serpentine soils can be improved by agronomic measures to create a more favourable habitat for soil organisms. For example, we propose application of mulch to increase water infiltration and reduce evaporation and big temperature changes. Besides that, nutrient deficiency of serpentine soils, as well as the high demand of *Odontarrhena spp.* for Ca, P, N and K (Bani et al., 2015a; van der Ent et al., 2015), can limit agromining efficiency or negatively affect soil quality. Thus, a secondary fertilization of N and K, in addition to a primary P-rich manure application, might be recommendable within the scope of agromining. Moreover, if serpentine soils are returned to conventional agriculture with "normal" crops, additional Ca-fertilization (e.g. liming) can be crucial to ensure plant growth, while hyperaccumulators are usually adapted to low Ca concentrations. In addition, Bani et al. (2015a) used a single spraying anti-monocot herbicide to control weeds in a phytomining field trial in Albania cropping *O. chalcidica*. We decided for an ecological alternative by weeding by hand, however this is not economically feasible. Furthermore, with respect to low sampling numbers and the fact that agriculture measures may substantially influence soil organisms, we would sample soil mesofauna at different time points, such as shortly before and after soil cultivation (2 – 3 days). Second, we propose sampling in August or September, when most mesofauna in temperate climate show their highest abundance, while in October numbers are usually declining in topsoil. Finally, the concentration of heavy metals (e.g. Ni) in soil invertebrates might have been of interest, as it is a possible path for metals into the food web. For example, Peterson et al. (2003) recorded mean Ni concentrations exceeding 1300 ppm in several unidentified species of relatively large-bodied Heteroptera, flies and ants. Hence, Peterson et al. (2003) outlined the potential threat of spreading heavy metals through animal or human food chains at toxic levels, which is why they recommended monitoring of metal concentrations in invertebrate and vertebrate herbivores during agromining field trials. Since Ni is purified by the hyperaccumulator during uptake, Ni concentrations in leaves are way higher than in soil. Thus,

agromining sites should be fenced to prevent eating of leaves by animals, as food intake is the major route of Ni exposure (Iyaka, 2011). Besides that, in the case of Bernstein, a spread of the non-native hyperaccumulator *O. chalcidica* can be prevented by harvesting plants before they blossom.

5. CONCLUSION

Ni is a limited resource with a high commercial importance. Agromining is an innovative technology to recover Ni from low productive serpentine soils, with the potential benefit of improving soil quality and ecosystem services, while gaining higher revenues for the local farmers. Thus, it is not only an environmental-friendly alternative to traditional mining activities but can also provide biomass for local energy production and stimulate rural development of abandoned regions. The aim of this study was to evaluate the effects of cropping the hyperaccumulator *O. chalcidica* and accompanied agronomic practices on soil quality and Ni-availability, on a Ni-rich agricultural land in Austria. Evaluating the effects of management practices on soil quality is crucial when introducing new technologies and developing a sustainable soil management system, which doesn`t degrade but improve soil conditions and maintains ecosystem services. Therefore, the comparison of changes in key soil indicators over time can be an early warning device for soil degradation. So far, some experiments studied the effects of agromining (e.g. agronomic practices and cropping hyperaccumulators) on physicochemical soil quality, however little is known about possible impacts on soil fauna. In contrast to previous studies, mineral fertilization didn`t improve soil properties and agromining potential to a greater extent than organic amendments. The application of NPK decreased FDA-enzyme activity and was significantly lower than in CM. Besides that, we noticed a higher abundance of Collembola and Acari in manure treatments. Furthermore, QBS-index was highest in PM, while other fertilizers and LD did not significantly increase QBS-index compared to C or Ref. In addition, PM was the only treatment increasing QBS-index close to the threshold for high quality soils. Interestingly, mesofauna abundance and QBS-index didn´t rise with FDA-enzyme activity. Even though plots treated with OM contained highest soil physicochemical quality, biological activity was not considerably greater than in C or Ref. Since mesofauna abundance, FDA-enzyme activity and QBS were not significantly lower in hyperaccumulator

treatments compared to Ref, we postulate that cropping *O. chalcidica* doesn`t negatively impact biological activity. In contrast, soil quality was considerably improved in organically amended soils, especially with cow- and pig manure. We conclude that organic amendments improved soil properties better than mineral fertilization or low distance plantation. In addition, neither fertilization nor planting density did affect DTPA-Ni pool. On the contrary, labile $Sr(NO_3)^2$-Ni was more available in NPK and less in OM, exclusive sulphur plots. Furthermore, Ni-availability in soil was dominated by the DTPA-Ni pool and NPK only weakly contributed to soil quality. Thus, we recommend the application of manure because soil quality is improved while Ni-availability is not decreased. Despite that, cropping *O. chalcidica* reduced the DTPA-extractable Ni pool, which was also observed in the previous experimental year. In addition, we noted a replenishment of Ni over winter (between 2017 and 2018), which might ensure Ni-availability for continuous cropping of hyperaccumulators, a crucial factor in agromining applications. We propose that the use of hyperaccumulators on serpentine soils in combination with agronomic practices, can improve soil quality while limiting soil concentrations of phytotoxic Ni. Nevertheless, the results presented are based on one single growing period. A clear evaluation of the negative or positive effects of agromining and accompanied practices on soil quality, requires monitoring over a longer time frame (10-30 years).

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A. APPENDIX

Table A.1: Soil biological parameters at t4 (1 week after tillage and fertilization) and t6 (harvest). Significant differences between treatments (C, NPK, CM, PM, OM, LD, Ref) at each time point are indicated with lowercase letters ($p<0.05$). Non-significant differences are indicated with (ns, $p>0.05$). C-control, NPK-mineral fertilizer, CM-cow manure, PM-pig manure, OM-organic matter, LD-low distance plantation, Ref-reference.

		Mesofauna	Density	Collembola	Acari	C:A	Collembola	Acari	QBS	FDA
		(total abundance)	(ind. m^{-2})	(total abundance)	(total abundance)	ratio	(rel. abundance)	(rel. abundance)	Soil Quality Index	(mg kg-1 3h-1)
C	**t4**	5.25 ± 4.03 mn	0.53 ± 0.40 mn	1.75 ± 1.71 ns	2.25 ± 2.22 ns	7.79 ± 14.8 ns	0.49 ± 0.69 ns	0.53 ± 0.47 ns	15.0 ± 13.0 ns	nd
	t6	16.50 ± 8.09 ns	1.65 ± 1.62 ns	0.00 ± 0.00 a	2.03 ± 1.59 ns	0.28 ± 0.48 ns	0.48 ± 0.02 ns	0.84 ± 0.56 ns	43.0 ± 22.0 a	141 ± 27 ab
NPK	**t4**	3.75 ± 3.30 mn	0.38 ± 0.33 mn	1.25 ± 0.96	2.00 ± 1.63	3.40 ± 4.58	0.63 ± 0.42	0.71 ± 0.61	42.5 ± 22.2	nd
	t6	13.75 ± 10.24	1.38 ± 1.02	1.28 ± 0.91 a	2.28 ± 2.03	5.24 ± 9.84	0.49 ± 0.35	0.95 ± 0.70	45.5 ± 20.8 a	117 ± 27 a
CM	**t4**	5.00 ± 3.65 mn	0.50 ± 0.37 mn	2.25 ± 2.65	1.00 ± 1.41	5.63 ± 5.09	0.99 ± 0.72	0.57 ± 0.58	30.3 ± 17.3	nd
	t6	28.00 ± 11.35	2.80 ± 2.27	5.25 ± 4.19 b	12.00 ± 14.8	1.07 ± 0.75	1.20 ± 0.90	2.23 ± 2.31	52.8 ± 4.9 a	176 ± 39 b
PM	**t4**	2.00 ± 1.41 n	0.20 ± 0.14 n	0.75 ± 0.96	0.50 ± 1.00	12.9 ± 14.5	1.16 ± 0.41	0.56 ± 0.58	31.5 ± 22.1	nd
	t6	11.25 ± 2.29	1.13 ± 0.46	4.25 ± 3.59 a	20.30 ± 17.4	0.31 ± 0.39	0.78 ± 0.76	3.24 ± 1.95	80.3 ± 8.2 b	155 ± 19 ab
OM	**t4**	8.25 ± 5.32 mn	0.83 ± 0.53 mn	3.75 ± 1.50	1.50 ± 1.73	5.45 ± 5.25	0.89 ± 0.30	0.58 ± 0.70	26.3 ± 18.0	nd
	t6	8.75 ± 1.26	0.88 ± 0.13	1.53 ± 1.25 a	4.25 ± 2.63	0.48 ± 0.33	0.52 ± 0.38	1.37 ± 0.65	43.0 ± 12.7 a	141 ± 14 ab
LD	**t4**	1.75 ± 1.50 n	0.18 ± 0.15 n	0.75 ± 0.50	0.25 ± 0.50	1.63 ± 0.95	0.84 ± 0.61	0.51 ± 0.33	38.8 ± 44.8	nd
	t6	7.25 ± 6.18	0.73 ± 0.62	2.25 ± 0.96 a	4.50 ± 1.29	0.54 ± 0.33	0.76 ± 0.32	1.51 ± 0.27	52.5 ± 18.9 ab	164 ± 14 b
Ref	**t4**	10.25 ± 3.50 m	1.03 ± 0.35 m	1.75 ± 2.22	2.25 ± 0.50	0.85 ± 1.12	0.50 ± 0.57	0.72 ± 0.15	62.5 ± 22.2	nd
	t6	16.50 ± 9.95	1.65 ± 0.99	1.53 ± 1.25 a	10.30 ± 10.10	0.25 ± 0.23	0.40 ± 0.30	2.25 ± 1.62	58.8 ± 17.5 ab	137 ± 15 ab

Table A.2: Total number of each taxon detected in soil at t4 (1 week after ploughing and fertilization) and t6 (harvest). Numbers are listed for each plot (1 to 28) and treatment (C, NPK, CM, PM, OM, LD, Ref). C-control, NPK-inorganic fertilizer, CM-cow manure, PM-pig manure, OM-organic matter, LD-low distance plantation, Ref-reference.

Samples t4	Treatment	Collembola	Acari	Protura	Coleoptera Adults	Coleoptera Larvae	Diptera Adult	Diptera Larvae	Symphyla	Araneae	Chilopoda	Diplopoda	Nematoda	Total Mesofauna	Total Collembola & Acari
2	C	0	0	0	0	0	0	0	0	0	0	0	0	**0**	0
17	C	0	1	0	0	0	0	0	0	1	0	0	1	**3**	1
19	C	0	2	0	0	0	0	0	0	0	0	0	1	**3**	2
23	C	3	0	0	0	0	0	1	0	0	0	0	0	**4**	3
6	CM	1	0	0	0	0	0	0	0	0	0	0	0	**1**	1
7	CM	6	3	0	0	0	0	0	0	0	0	0	0	**9**	9
13	CM	1	0	3	0	1	0	0	0	1	0	0	1	**7**	1
24	CM	1	2	0	0	0	0	0	0	0	0	0	0	**3**	3
5	LD	6	4	1	0	0	0	4	0	1	0	0	0	**16**	10
11	LD	0	0	0	0	0	0	0	0	0	0	0	0	**0**	0
14	LD	0	0	0	0	0	0	0	0	0	0	0	0	**0**	0
20	LD	3	1	1	0	0	0	0	1	0	0	0	0	**6**	4
1	NPK	0	1	0	0	0	0	0	0	0	0	0	0	**1**	1
8	NPK	1	0	0	0	0	0	0	0	0	1	0	1	**3**	1
16	NPK	3	1	0	1	1	0	1	0	0	0	0	0	**7**	4
22	NPK	2	4	1	0	0	0	0	0	0	0	0	1	**8**	6
4	OM	1	0	0	0	0	0	0	0	0	0	0	0	**1**	1
9	OM	1	0	0	0	0	0	0	0	1	0	0	0	**2**	1
12	OM	4	5	0	0	0	0	0	0	0	0	1	0	**10**	9
15	OM	1	1	0	0	0	0	0	0	0	0	0	1	**3**	2
3	PM	2	0	1	0	0	0	0	0	0	0	0	0	**3**	2
10	PM	2	2	0	0	0	0	0	0	0	0	0	0	**4**	4
18	PM	2	3	2	0	0	0	0	0	0	0	0	0	**7**	5
21	PM	3	0	0	0	0	0	0	0	0	0	0	0	**3**	3
25	Ref	1	2	0	0	1	0	2	1	0	0	0	2	**9**	3
26	Ref	0	2	0	0	0	0	1	0	0	0	0	3	**6**	2
27	Ref	5	2	0	0	0	0	0	1	0	0	1	5	**14**	7
28	Ref	1	3	0	0	0	0	2	1	0	0	1	4	**12**	4
SUM		**50**	**39**	**9**	**1**	**3**	**0**	**11**	**4**	**4**	**1**	**3**	**20**	**145**	**89**
MEAN		**1,8**	**1,4**	**0,3**	**0,0**	**0,1**	**0,0**	**0,4**	**0,1**	**0,1**	**0,0**	**0,1**	**0,7**	**5,2**	**3,2**
STDEV		**9,1**	**7,1**	**1,8**	**0,3**	**0,6**	**0,0**	**2,2**	**0,8**	**0,8**	**0,3**	**0,6**	**3,8**	**26,3**	**16,2**

Samples t6	Treatment	Collembola	Acari	Protura	Coleoptera Adults	Coleoptera Larvae	Diptera Adult	Diptera Larvae	Symphyla	Araneae	Chilopoda	Diplopoda	Nematoda	Total Mesofauna	Total Collembola & Acari
2	C	0	2	0	0	1	0	0	0	0	0	0	0	**3**	2
17	C	0	0	0	0	1	1	1	0	0	0	0	0	**3**	0
19	C	0	2	1	0	1	0	0	2	0	0	0	0	**6**	2
23	C	0	4	2	0	0	2	1	0	0	0	0	0	**9**	4
6	CM	4	4	0	0	1	0	5	0	0	0	0	2	**16**	8
7	CM	1	34	0	0	1	0	0	0	0	0	0	0	**36**	35
13	CM	5	3	0	0	3	2	2	0	0	0	0	0	**15**	8
24	CM	11	7	0	0	3	0	1	0	0	0	0	0	**22**	18
5	LD	1	4	0	0	1	0	1	0	0	0	0	0	**7**	5
11	LD	3	6	1	0	0	0	1	1	0	0	0	0	**12**	9
14	LD	3	3	0	0	1	0	0	0	0	0	0	0	**7**	6
20	LD	2	5	0	0	0	0	1	0	0	0	0	1	**9**	7
1	NPK	2	0	1	0	0	1	1	0	0	0	0	0	**5**	2
8	NPK	0	2	0	0	0	0	0	0	0	0	0	0	**2**	2
16	NPK	2	5	0	0	2	1	0	0	0	0	0	0	**10**	7
22	NPK	1	2	0	0	1	0	2	0	0	0	0	1	**7**	3
4	OM	0	8	0	0	2	0	2	0	0	0	0	0	**12**	8
9	OM	3	4	0	0	2	0	0	0	0	0	0	1	**10**	7
12	OM	2	3	2	0	3	1	0	0	0	0	0	0	**11**	5
15	OM	1	2	0	0	0	1	0	0	0	0	0	0	**4**	3
3	PM	1	9	3	0	1	1	1	0	0	0	0	1	**17**	10
10	PM	9	10	2	0	0	0	1	1	0	0	0	0	**23**	19
18	PM	2	16	0	0	2	0	19	1	0	0	0	0	**40**	18
21	PM	5	46	0	0	3	0	0	2	0	0	0	0	**56**	51
25	Ref	1	2	0	0	1	0	0	0	0	0	0	2	**6**	3
26	Ref	2	25	0	0	1	0	0	0	0	0	0	2	**30**	27
27	Ref	0	6	1	0	3	0	1	2	0	0	0	2	**15**	6
28	Ref	3	8	0	2	2	0	0	0	0	0	0	0	**15**	11
SUM		**64**	**222**	**13**	**2**	**36**	**10**	**40**	**9**	**0**	**0**	**0**	**12**	**408**	**286**
MEAN		**2,3**	**7,9**	**0,5**	**0,1**	**1,3**	**0,4**	**1,4**	**0,3**	**0,0**	**0,0**	**0,0**	**0,4**	**14,6**	**10,2**
STDEV		**11,7**	**41,1**	**2,5**	**0,5**	**6,5**	**1,9**	**8,0**	**1,7**	**0,0**	**0,0**	**0,0**	**2,3**	**74,1**	**52,4**

Table A.3: Classification of Acari to order level. Evaluation of total numbers of Mesostigmata, Prostigmata and Oribatida and their percentage on total Acari abundance at t4 and t6.

			Acari Suborder						Acari Suborder		
sample	treatment	Total Number Acari	Mesostigmata	Prostigmata	Oribatida	sample	treatment	Total Number Acari	Mesostigmata	Prostigmata	Oribatida
23	C	0				**23**	C	4	4		
2	C	0				**2**	C	2		2	
19	C	2	2			**19**	C	2	1	1	
17	C	1	1			**17**	C	0			
7	CM	3	3			**7**	CM	34		34	
13	CM	0				**13**	CM	3			3
6	CM	0				**6**	CM	4	4		
24	CM	2	2			**24**	CM	7	7		
20	LD	1	1			**20**	LD	5			
5	LD	4	4			**5**	LD	4	3		1
11	LD	0				**11**	LD	6		4	2
14	LD	0				**14**	LD	3			
16	NPK	1	1			**16**	NPK	5		5	
22	NPK	4	4			**22**	NPK	2			
1	NPK	1		1		**1**	NPK	0			
8	NPK	0				**8**	NPK	2	2		
4	OM	0				**4**	OM	8	4	4	
12	OM	5	2	3		**12**	OM	3	1		2
9	OM	0				**9**	OM	4			
15	OM	1	1			**15**	OM	2		2	
3	PM	0				**3**	PM	9		9	
18	PM	3	3			**18**	PM	16		16	
10	PM	2	2			**10**	PM	10			
21	PM	0				**21**	PM	46	3	43	
26	Ref	2	2			**26**	Ref	25	1	25	
25	Ref	2		2		**25**	Ref	2		2	
27	Ref	2	2			**27**	Ref	6			
28	Ref	3		3		**28**	Ref	8	1	7	
	SUM	**39**	**30**	**9**	**0**		**SUM**	**222**	**31**	**154**	**8**
	(%)		**77**	**23**	**0**		**(%)**		**14**	**69**	**4**

Table A.4: Soil microarthropods taxa, associated EMI scores and final QBS per plot for t4 (1 week after ploughing and fertilization) and t6 (harvest). Listed per plot (1 to 28) and treatment (C, NPK, CM, PM, OM, LD, Ref). C-control, NPK-inorganic fertilizer, CM-cow manure, PM-pig manure, OM-organic matter, LD-low distance plantation, Ref-reference.

Samples t4	Treatment	Collembola	Acari	Protura	Coleoptera Adults	Coleoptera Larvae	Diptera Adult	Diptera Larvae	Symphyla	Araneae	Chilopoda	Diplopoda	QBS
1	NPK	0	20	0	0	0	0	0	0	0	0	0	**20**
2	C	0	0	0	0	0	0	0	0	0	0	0	**0**
3	PM	10	0	20	0	0	0	0	0	0	0	0	**30**
4	OM	10	0	0	0	0	0	0	0	0	0	0	**10**
5	LD	20	20	20	0	0	0	10	0	5	0	0	**75**
6	CM	6	0	0	0	0	0	0	0	0	0	0	**6**
7	CM	20	20	0	0	0	0	0	0	0	0	0	**40**
8	NPK	10	0	0	0	0	0	0	0	0	20	0	**30**
9	OM	10	0	0	0	0	0	0	0	5	0	0	**15**
10	PM	10	20	0	0	0	0	0	0	0	0	0	**30**
11	LD	0	0	0	0	0	0	0	0	0	0	0	**0**
12	OM	10	20	0	0	0	0	0	0	0	0	20	**50**
13	CM	10	0	20	0	10	0	0	0	5	0	0	**45**
14	LD	0	0	0	0	0	0	0	0	0	0	0	**0**
15	OM	10	20	0	0	0	0	0	0	0	0	0	**30**
16	NPK	20	20	0	10	0	10	10	0	0	0	0	**70**
17	C	0	20	0	0	0	0	0	0	5	0	0	**25**
18	PM	20	20	20	0	0	0	0	0	0	0	0	**60**
19	C	0	20	0	0	0	0	0	0	0	0	0	**20**
20	LD	20	20	20	0	0	0	0	20	0	0	0	**80**
21	PM	6	0	0	0	0	0	0	0	0	0	0	**6**
22	NPK	10	20	20	0	0	0	0	0	0	0	0	**50**
23	C	10	0	0	0	0	0	10	0	0	0	0	**20**
24	CM	10	20	0	0	0	0	0	0	0	0	0	**30**
25	Ref	10	20	0	0	10	0	10	20	0	0	0	**70**
26	Ref	0	20	0	0	0	0	10	0	0	0	0	**30**
27	Ref	10	20	0	0	0	0	0	20	0	0	20	**70**
28	Ref	10	20	0	0	0	0	10	20	0	0	20	**80**
SUM		**252**	**340**	**120**	**10**	**20**	**10**	**60**	**80**	**20**	**20**	**60**	**992**
MEAN		**9**	**12**	**4**	**0**	**1**	**0**	**2**	**3**	**1**	**1**	**2**	**35**
STDEV		**7**	**10**	**8**	**2**	**3**	**2**	**4**	**7**	**2**	**4**	**6**	**26**

Samples t6	Treatment	Collembola	Acari	Protura	Coleoptera Adults	Coleoptera Larvae	Diptera Adult	Diptera Larvae	Symphyla	Araneae	Chilopoda	Diplopoda	QBS
1	NPK	10	0	20	0	0	1	10	0	0	0	0	**41**
2	C	0	20	0	0	10	0	0	0	0	0	0	**30**
3	PM	20	20	20	0	10	1	10	0	0	0	0	**81**
4	OM	0	20	0	0	10	0	10	0	0	0	0	**40**
5	LD	10	20	0	0	10	0	0	0	0	0	0	**40**
6	CM	10	20	0	0	10	0	10	0	0	0	0	**50**
7	CM	20	20	0	0	10	0	0	0	0	0	0	**50**
8	NPK	0	20	0	0	0	0	0	0	0	0	0	**20**
9	OM	10	20	0	0	10	0	0	0	0	0	0	**40**
10	PM	20	20	20	0	0	0	10	20	0	0	0	**90**
11	LD	10	20	20	0	0	0	10	20	0	0	0	**80**
12	OM	10	20	20	0	10	1	0	0	0	0	0	**61**
13	CM	20	20	0	0	10	1	0	0	0	0	0	**51**
14	LD	10	20	0	0	10	0	0	0	0	0	0	**40**
15	OM	10	20	0	0	0	1	0	0	0	0	0	**31**
16	NPK	20	20	0	0	10	1	0	0	0	0	0	**51**
17	C	0	0	0	0	10	1	10	0	0	0	0	**21**
18	PM	20	20	0	0	10	0	10	20	0	0	0	**80**
19	C	0	20	20	0	10	0	0	20	0	0	0	**70**
20	LD	20	20	0	0	0	0	10	0	0	0	0	**50**
21	PM	20	20	0	0	10	0	0	20	0	0	0	**70**
22	NPK	20	20	0	0	10	0	20	0	0	0	0	**70**
23	C	0	20	20	0	0	1	10	0	0	0	0	**51**
24	CM	20	20	0	0	10	0	10	0	0	0	0	**60**
25	Ref	20	20	0	0	10	0	0	0	0	0	0	**50**
26	Ref	10	20	0	0	10	0	0	0	0	0	0	**40**
27	Ref	0	20	20	0	10	0	10	20	0	0	0	**80**
28	Ref	20	20	0	15	10	0	0	0	0	0	0	**65**
SUM		**330**	**520**	**160**	**15**	**210**	**8**	**140**	**120**	**0**	**0**	**0**	**1503**
MEAN		**12**	**19**	**6**	**1**	**8**	**0**	**5**	**4**	**0**	**0**	**0**	**54**
STDEV		**8**	**5**	**9**	**3**	**4**	**0**	**6**	**8**	**0**	**0**	**0**	**19**

Table A.5: Soil Quality Evaluation: Agronomic practices (fertilization, low distance plantation) are compared to C-control plots. Statistics including sulphur plots (6-CM, 8-NPK, 10-PM, 15-OM) 1st rowand exclusive sulphur in 2nd row. Final soil quality score was evaluated by following criteria: **(+)***-significantly positive trend (+2), (+)-postive trend (+1), (-)-negative trend (-1), or (0)-neutral (0). The treatment with the greatest sum of scores achieved the highest soil quality. All values are displayed for time point t6, except for Nmin (t5).

Physicochemical Soil Quality

Agronomic practice	C_{total} (g kg-1)		SOC (%)	N_{total} (g kg-1)		% of soil DW	N_{min} (mg kg-1)		available-P (mg kg-1)		available-K % of CEC		pH $CaCl_2$		CEC ($mmol_c$ kg-1)		SQ-score
NPK	20.6 ± 7.2	p=1.000 (0)	2.06	1.8 ± 0.6	p=0.964 (0)	0.18	21.9 ± 13.5	p=0.002 (+)*	25.3 ± 5.4	p=0.983 (0)	0.85 %	p=0.999 (0)	6.26 ± 0.20	p=0.988 (0)	164 ± 19	p=0.995 (0)	+ 1.5
	19.0 ± 8.0	p=0.635 (0)	1.90 (-)	1.7 ± 0.7	p=0.359 (0)	0.17	15.5 ± 5.2	p<0.001 (+)*	22.8 ± 2.3	p=1.000 (0)	0.96 %	p=1.000 (0)	6.34 ± 0.16	p=0.999 (0)	157 ± 16	p=0.615 (0)	
CM	24.3 ± 4.5	p=0.110 (0)	2.43	2.2 ± 0.4	p=0.327 (0)	0.22	9.6 ± 2.3	p=0.555 (0)	30.6 ± 7.4	p=0.193 (0)	2.53 %	p<0.001 (+)*	6.26 ± 0.25	p=0.984 (0)	178 ± 17	p=0.720 (0)	+ 2.0
	23.7 ± 5.3	p=0.239 (0)	2.37	2.1 ± 0.4	p=0.497 (0)	0.22	8.7 ± 1.7	p=0.114 (0)	27.5 ± 4.9	p=0.522 (0)	2.44 %	p<0.001 (+)*	6.38 ± 0.06	p=1.000 (0)	180 ± 20	p=0.522 (0)	
PM	23.5 ± 3.0	p=0.341 (0)	2.35	2.1 ± 0.2	p=0.473 (0)	0.21	9.6 ± 2.8	p=0.552 (0)	32.7 ± 4.5	p=0.055 (+)	1.05 %	p=0.502 (0)	6.18 ± 0.31	p=0.819 (0)	181 ± 8	p=0.418 (0)	+ 2.0
	22.4 ± 2.7	p=0.802 (0)	2.24	2.1 ± 0.2	p=0.875 (0)	0.21	9.0 ± 3.1	p=0.092 (+)	33.7 ± 4.9	p=0.008 (+)*	1.23 %	p=0.117 (0)	6.33 ± 0.85	p=0.995 (0)	179 ± 8	p=0.680 (0)	
OM	24.1 ± 4.1	p=0.143 (0)	2.41	2.1 ± 0.3	p=0.431 (0)	0.21	3.5 ± 2.3	p=1.000 (0)	24.9 ± 2.0	p=0.994 (0)	0.95 %	p=0.830 (0)	6.35 ± 0.16	p=1.000 (0)	189 ± 8	p=0.046 (+)*	+ 3.5
	25.8 ± 2.9	p=0.008 (+)*	2.58	2.2 ± 0.2	p=0.080 (+)	0.22	3.4 ± 2.8	p=1.000 (0)	24.7 ± 2.5	p=0.987 (0)	0.88 %	p=0.947 (0)	6.43 ± 0.03	p=0.817 (0)	193 ± 4	p=0.023 (+)*	
LD	21.6 ± 4.5	p=0.997 (0)	2.16	1.9 ± 0.4	p=0.995 (0)	0.20	2.3 ± 1.3	p=1.000 (0)	23.0 ± 2.1	p=1.000 (0)	0.87 %	p=0.993 (0)	6.30 ± 0.06	p=0.999 (0)	183 ± 10	p=0.316 (0)	0
	21.6 ± 4.4	p=0.990 (0)	2.16	1.9 ± 0.4	p=0.992 (0)	0.20	2.3 ± 1.3	p=1.000 (0)	23.0 ± 2.1	p=1.000 (0)	0.87 %	p=0.988 (0)	6.30 ± 0.06	p=0.859 (0)	183 ± 10	p=0.268 (0)	

Biological Soil Quality

Agronomic practice	C:A ratio		Collembola (rel. abund.)		Acari (rel. abund.)		mesofauna density (ind. m^{-2})		QBS SQ-Index		microbial activity (mg kg-1 3h-1)		SQ-score
NPK	5.24 ± 9.84	p=0.509 (0)	0.49 ± 0.35	p=0.814 (0)	0.95 ± 0.70	p=1.000 (0)	1.38 ± 1.02	p=1.000 (0)	45.5 ± 20.8	p=1.000 (0)	117 ± 27	p=0.584 (0)	- 1.0
	6.97 ± 0.92	p=0.322 (0)	0.63 ± 0.02	p=0.642 (0)	0.79 ± 0.77	p=1.000 (0)	1.10 ± 1.06	p=0.989 (0)	54.0 ± 14.7	p=0.889 (0)	106 ± 27	p=0.022 (-)*	
CM	1.07 ± 0.75	p=1.000 (0)	1.20 ± 0.90	p=0.032 (+)*	2.23 ± 2.31	p=0.713 (0)	2.80 ± 2.27	p=0.695 (0)	52.8 ± 4.9	p=0.940 (0)	176 ± 39	p=0.183 (0)	+ 2.0
	1.09 ± 0.92	p=1.000 (0)	1.27 ± 1.09	p=0.043 (+)*	2.64 ± 2.65	p=0.577 (0)	2.53 ± 2.70	p=0.903 (0)	53.6 ± 5.5	p=0.902 (0)	158 ± 17	p=0.512 (0)	
PM	0.31 ± 0.39	p=1.000 (0)	0.78 ± 0.76	p=0.326 (0)	3.24 ± 1.95	p=0.163 (0)	1.10 ± 0.56	p=0.989 (0)	80.3 ± 8.2	p=0.014 (+)*	155 ± 19	p=0.930 (0)	+ 2.0
	0.12 ± 0.12	p=1.000 (0)	0.41 ± 0.23	p=0.937 (0)	3.62 ± 2.20	p=0.155 (0)	1.10 ± 0.56	p=0.989 (0)	77.0 ± 6.1	p=0.032 (+)*	154 ± 23	p=0.796 (0)	
OM	0.48 ± 0.33	p=1.000 (0)	0.52 ± 0.38	p=0.771 (0)	1.37 ± 0.65	p=0.996 (0)	0.88 ± 0.13	p=0.930 (0)	43.0 ± 12.7	p=1,000 (0)	141 ± 14	p=1,000 (0)	0
	0.48 ± 0.41	p=1.000 (0)	0.53 ± 0.46	p=0.809 (0)	1.49 ± 0.73	p=0.994 (0)	0.83 ± 0.12	p=0.931 (0)	47.0 ± 12.1	p=0.999 (0)	137 ± 13	p=0.999 (0)	
LD	0.54 ± 0.33	p=1.000 (0)	0.76 ± 0.32	p=0.348 (0)	1.51 ± 0.27	p=0.987 (0)	0.73 ± 0.62	p=0.856 (0)	52.5 ± 18.9	p=0.947) (0)	164 ± 14	p=0.631 (0)	0
	0.54 ± 0.33	p=1.000 (0)	0.76 ± 0.32	p=0.350 (0)	1.51 ± 0.27	p=0.989 (0)	0.73 ± 0.62	p=0.844 (0)	52.5 ± 18.9	p=0.917 (0)	164 ± 14	p=0.167 (0)	

Treatment	Final SQ-score
NPK	+ 0.5
CM	+ 4.0
PM	+ 4.0
OM	+ 3.5
LD	0

Table A.6: Soil concentrations of total carbon and nitrogen, mineral nitrogen and plant-available phosphorus 1 week (t4) and 4 weeks after fertilization (t5) and at harvest (t6) (mean ± standard deviation; n = 4). Significant differences between treatments (C, NPK, CM, PM, OM, LD, Ref) are indicated with lowercase letters ($p<0.05$). Non-significant differences are indicated with (ns, $p>0.05$). C-control, NPK-mineral fertilizer, CM-cow manure, PM-pig manure, OM-organic matter, LD-low distance plantation, Ref-reference.

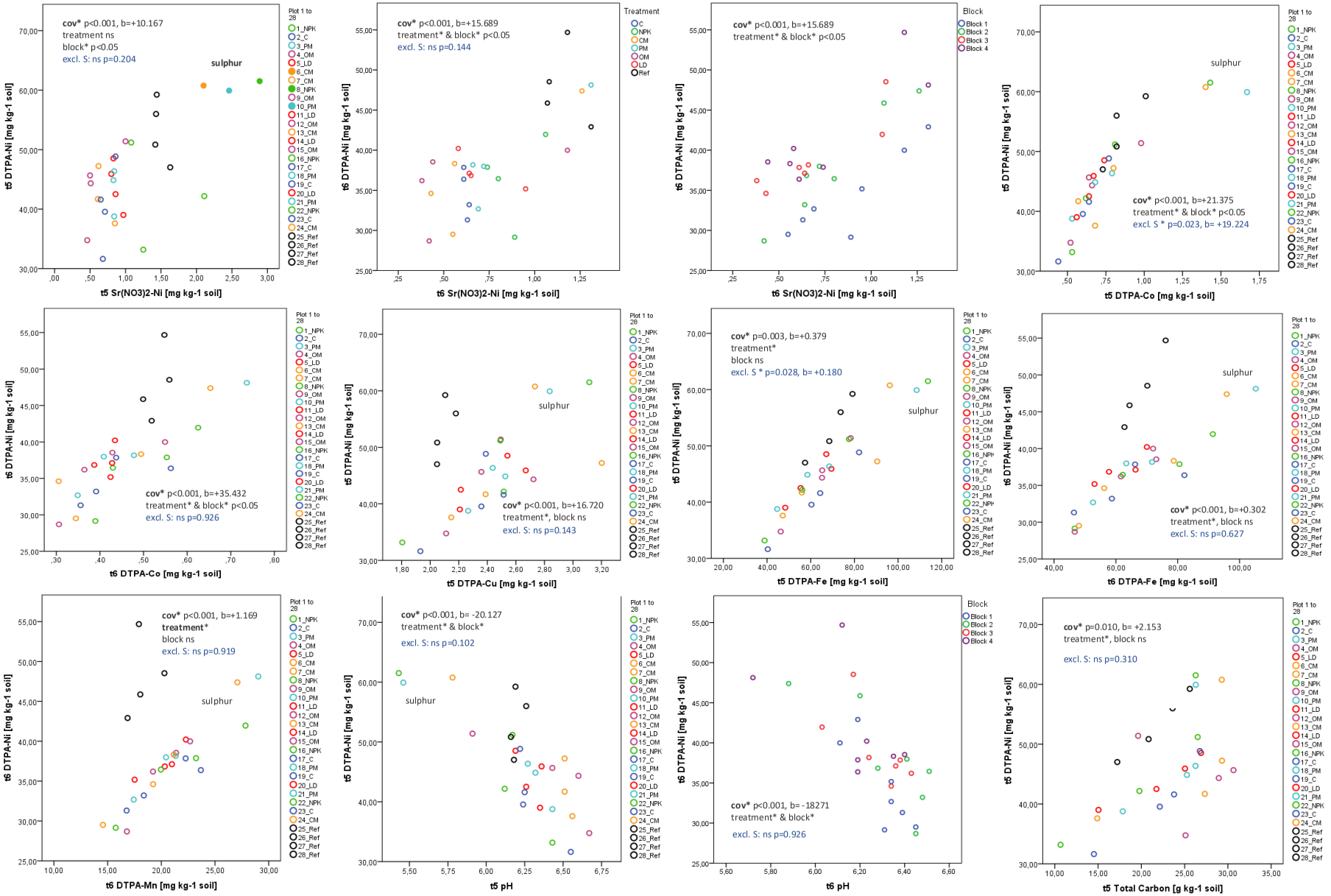
		C_{total} (g kg-1)	N_{total} (g kg-1)	N_{min} (mg kg-1)	NO_3-N (mg kg-1)	NH_4-N (mg kg-1)	Olsen-P (mg kg-1)
C	**t4**	21.4 ± 5.3 ns	1.82 ± 0.48 ns	8.1 ± 1.6 mn	8.0 ± 1.5 mn	0.10 ± 0.13 ns	22.5 ± 3.9 m
	t5	21.8 ± 5.2 xy	1.83 ± 0.43 ns	2.5 ± 1.0 x	2.7 ± 0.7 x	-0.22 ± 0.69 x	22.5 ± 4.4 x
	t6	20.9 ± 5.8 ns	1.93 ± 0.45 ns	nd	nd		22.9 ± 4.0 a
NPK	**t4**	21.0 ± 7.8	1.74 ± 0.74	5.6 ± 6.7 mno	13.5 ± 3.7 mn	2.12 ± 3.00	24.3 ± 8.4 m
	t5	20.8 ± 7.5 x	1.69 ± 0.65	21.9 ± 13.5 y	17.8 ± 11.5 y	4.10 ± 2.30 y	26.4 ± 13.4 x
	t6	20.6 ± 7.2	1.84 ± 0.59	nd	nd	nd	25.3 ± 5.4 a
CM	**t4**	22.8 ± 6.0	2.02 ± 0.55	18.9 ± 3.5 no	16.5 ± 4.6 mn	2.38 ± 2.90	25.7 ± 7.1 m
	t5	25.2 ± 6.9 y	2.08 ± 0.61	9.6 ± 2.3 xy	9.0 ± 1.7 xy	0.63 ± 2.30 xy	33.8 ± 10.4 x
	t6	24.3 ± 4.5	2.16 ± 0.37	nd	nd	nd	30.6 ± 7.4 a
PM	**t4**	23.2 ± 4.3	2.03 ± 0.41	25.6 ± 3.4 o	18.5 ± 6.8 n	7.16 ± 6.20	31.0 ± 6.4 m
	t5	23.9 ± 4.1 xy	2.00 ± 0.38	9.6 ± 2.8 xy	8.1 ± 2.6 xy	1.50 ± 2.40 xy	36.1 ± 5.9 x
	t6	23.5 ± 3.0	2.13 ± 0.23	nd	nd	nd	32.7 ± 4.5 a
OM	**t4**	23.7 ± 3.6	1.96 ± 0.28	7.0 ± 3.0 m	6.9 ± 3.0 m	0.16 ± 0.10	26.1 ± 2.5 m
	t5	26.1 ± 4.9 y	2.10 ± 0.46	3.5 ± 2.3 x	3.7 ± 1.7 x	-0.12 ± 0.80 xy	27.0 ± 1.2 x
	t6	24.1 ± 4.1	2.14 ± 0.26	nd	nd	nd	24.9 ± 2.0 a
LD	**t4**	22.0 ± 5.1	1.92 ± 0.51	8.0 ± 0.58 mn	7.9 ± 0.5 mn	0.19 ± 0.20	27.2 ± 4.8 m
	t5	22.2 ± 5.2 xy	1.77 ± 0.41	2.3 ± 1.3 x	2.7 ± 1.4 x	-0.39 ± 0.63 x	22.8 ± 4.3 x
	t6	21.6 ± 4.5	2.00 ± 0.42	nd	nd	nd	23.0 ± 2.1 a
Ref	**t4**	22.5 ± 5.1	1.86 ± 0.39	13.2 ± 9.2 mn	11.2 ± 7.2 mn	1.98 ± 2.00	7.90 ± 0.3 n
	t5	21.8 ± 3.6 xy	1.82 ± 0.27	5.7 ± 3.7 x	4.3 ± 1.9 x	1.30 ± 1.74 xy	7.68 ± 1.0 y
	t6	20.4 ± 4.0	1.88 ± 0.32	nd	nd	nd	7.60 ± 0.6 b

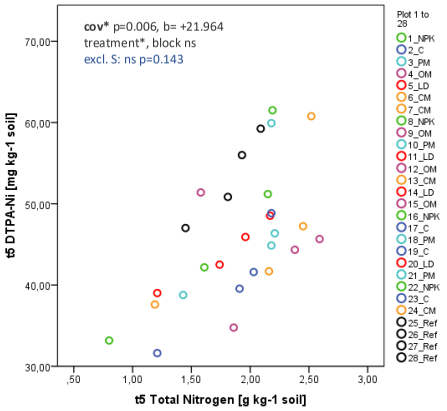
Table A.7: Soil physicochemical parameters 3 weeks after plantation and 4 weeks after fertilization (t5) and at harvest (t6) (mean ± standard deviation; n = 4). Significant differences between treatments (C, NPK, CM, PM, OM, LD, Ref) at each time point are indicated with lowercase letters ($p<0.05$). Non-significant differences are indicated with (ns, $p>0.05$). C-control, NPK-mineral fertilizer, CM-cow manure, PM-pig manure, OM-organic matter, LD-low distance plantation, Ref-reference.

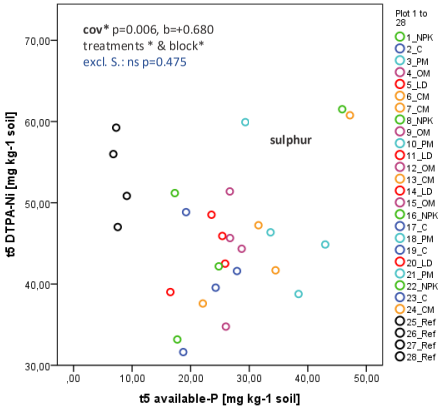
		Ca	Mg	K	Ca:Mg	CEC	Ca	Mg	K	pH	Ni-DTPA	Ni-Sr(NO_3)$_2$	Co-DTPA	Cu-DTPA	Fe-DTPA	Mn-DTPA
		($mmol_c$ kg^{-1})	($mmol_c$ kg^{-1})	($mmol_c$ kg^{-1})	ratio	($mmol_c$ kg^{-1})	% of CEC	% of CEC	% of CEC	$CaCl_2$	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
C	**t5**	111 ± 10 y	53.8 ± 5.7 x	2.30 ± 0.33 x	2.09 ± 0.33 xy	167 ± 10 x	66 %	32 %	1.38 %	6.32 ± 0.16 ns	40.4 ± 7.1 ns	0.73 ± 0.09 ns	0.61 ± 0.16 ns	2.30 ± 0.25 ns	61.8 ± 17.2 ns	nd
	t6	110 ± 10 bc	57.0 ± 5.5 a	1.48 ± 0.21 a	1.95 ± 0.31 a	168 ± 10 a	65 %	34 %	0.88 %	6.36 ± 0.12 ns	34.7 ± 3.0 a	0.62 ± 0.02 ns	0.44 ± 0.09 ns	2.14 ± 0.28 ns	63.4 ± 14.9 ns	20.3 ± 3.24 ns
NPK	**t5**	102 ± 14 xy	56.8 ± 6.0 x	2.76 ± 0.40 x	1.83 ± 0.40 y	162 ± 8 x	63 %	35 %	1.70 %	6.04 ± 0.43	47.0 ± 12.1	1.83 ± 0.83	0.85 ± 0.41	2.48 ± 0.54	71.5 ± 32.2	nd
	t6	103 ± 20 ab	59.8 ± 6.5 a	1.37 ± 0.41 a	1.75 ± 0.38 a	164 ± 19 a	63 %	36 %	0.84 %	6.26 ± 0.20	36.4 ± 5.3 a	0.87 ± 0.02	0.50 ± 0.11	4.54 ± 4.33	70.2 ± 19.8	21.7 ± 5.11
CM	**t5**	110 ± 17 y	52.8 ± 3.7 x	8.06 ± 2.60 y	2.09 ± 0.41 yz	171 ± 18 x	64 %	31 %	4.71 %	6.34 ± 0.37	46.8 ± 10.1	1.02 ± 0.71	0.86 ± 0.37	2.62 ± 0.46	72.4 ± 24.5	nd
	t6	114 ± 19 bc	59.3 ± 2.1 a	4.48 ± 0.48 b	1.94 ± 0.38 a	178 ± 17 ab	64 %	33 %	2.52 %	6.26 ± 0.25	37.5 ± 7.5 a	0.70 ± 0.38	0.45 ± 0.16	2.18 ± 0.27	69.7 ± 21.7	20.5 ± 5.20
PM	**t5**	112 ± 6 y	54.1 ± 2.7 x	3.54 ± 0.37 x	2.07 ± 0.19 yz	169 ± 4 x	66 %	32 %	2.09 %	6.12 ± 0.45	47.5 ± 8.9	1.24 ± 0.81	0.92 ± 0.51	2.52 ± 0.24	70.0 ± 27.5	nd
	t6	118 ± 7 bc	61.3 ± 4.5 a	1.94 ± 0.55 a	1.94 ± 0.19 a	181 ± 8 ab	65 %	34 %	1.07 %	6.18 ± 0.31	39.2 ± 6.4 ab	0.85 ± 0.31	0.49 ± 0.17	2.21 ± 0.12	73.2 ± 22.7	22.1 ± 4.90
OM	**t5**	130 ± 21 z	57.8 ± 3.9 x	3.18 ± 0.44 x	2.26 ± 0.37 z	191 ± 22 y	68 %	30 %	1.66 %	6.40 ± 0.34	44.0 ± 6.9	0.62 ± 0.26	0.70 ± 0.20	2.42 ± 0.26	63.7 ± 13.2	nd
	t6	126 ± 10 c	61.1 ± 4.0 a	1.80 ± 0.41 a	2.08 ± 0.28 a	189 ± 8 b	67	32 %	0.95 %	6.35 ± 0.16	35.9 ± 5.0 a	0.61 ± 0.38	0.41 ± 0.10	2.22 ± 0.37	63.4 ± 12.3	20.0 ± 2.58
LD	**t5**	113 ± 15 y	53.2 ± 1.7 x	2.14 ± 0.42 x	2.13 ± 0.28 yz	169 ± 15 x	67 %	31 %	1.27 %	6.30 ± 0.08	44.0 ± 4.1	0.87 ± 0.07	0.65 ± 0.75	2.41 ± 0.23	60.1 ± 10.0	nd
	t6	121 ± 11 c	60.2 ± 2.9 a	1.64 ± 0.19 a	2.00 ± 0.25 a	183 ± 10 ab	66 %	33 %	0.90 %	6.30 ± 0.06	37.3 ± 2.2 a	0.71 ± 0.17	0.42 ± 0.02	2.24 ± 0.23	61.8 ± 7.8	20.3 ± 2.01
Ref	**t5**	90 ± 8 x	66.4 ± 5.5 y	1.91 ± 0.18 x	1.36 ± 0.16 x	158 ± 11 x	57 %	42 %	1.21 %	6.20 ± 0.04	53.3 ± 5.4	1.48 ± 0.10	0.85 ± 0.12	2.10 ± 0.63	69.7 ± 9.3	nd
	t6	90 ± 8 a	70.1 ± 6.9 b	1.66 ± 0.21 a	1.30 ± 0.18 b	162 ± 11 a	56 %	43 %	1.02 %	6.17 ± 0.04	48.0 ± 5.0 b	1.16 ± 0.11	0.53 ± 0.03	1.98 ± 0.12	68.4 ± 6.1	18.3 ± 1.44

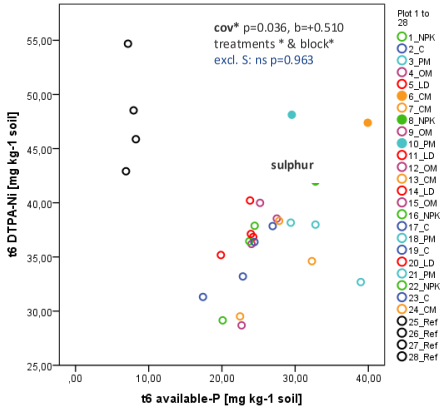
Fig. A.1: Results of covariance analyses (univariate regression analysis), with covariate (cov) on x-axes. Significant increases or decreases of dependent with correlating variables are indicated with asterisk (* for $p < 0.05$), as well as significant differences between treatments and blocks. In addition, results of covariance analyses exclusive sulphur plots (6_CM, 8_NPK, 10_PM, 15_OM) are expressed in blue (excl. S).

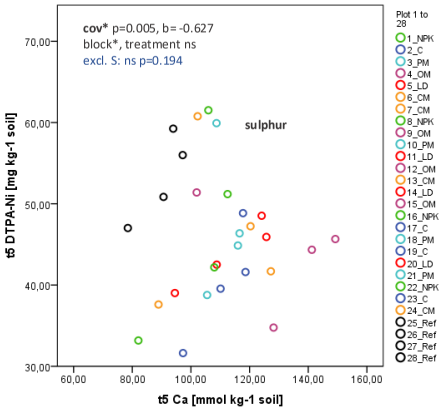
DTPA-Nickel

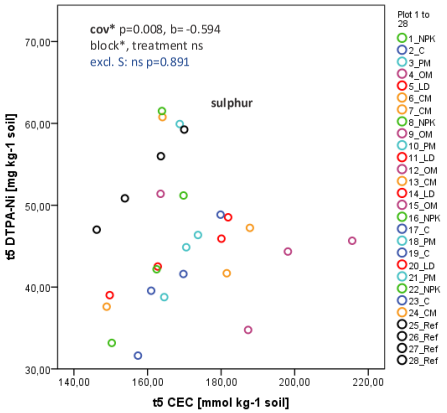




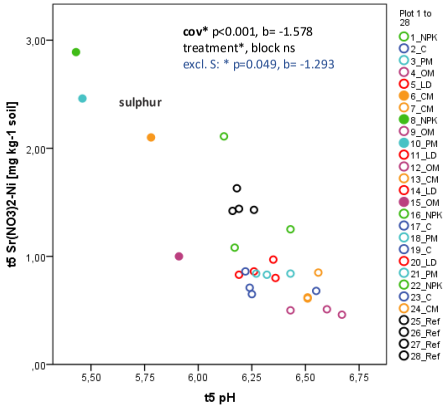


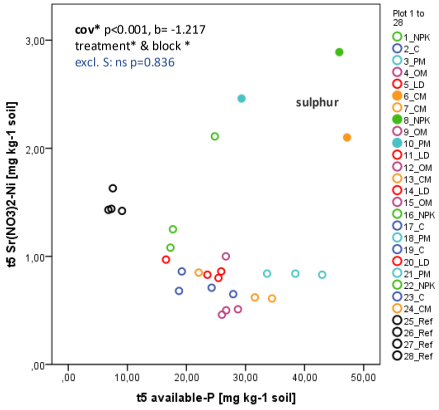


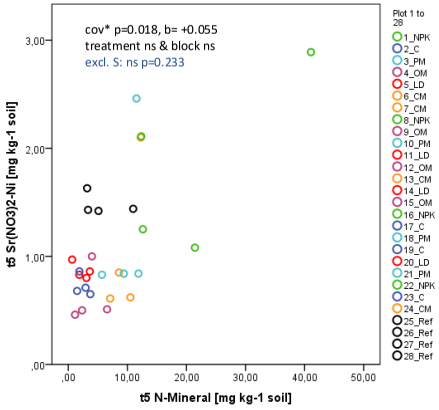


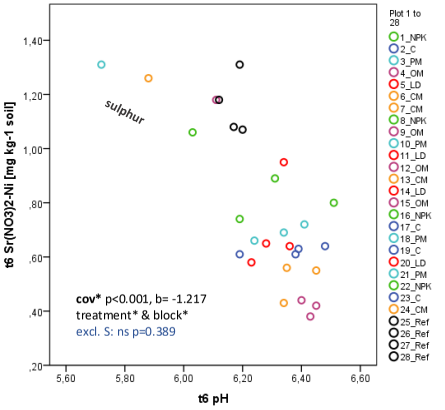


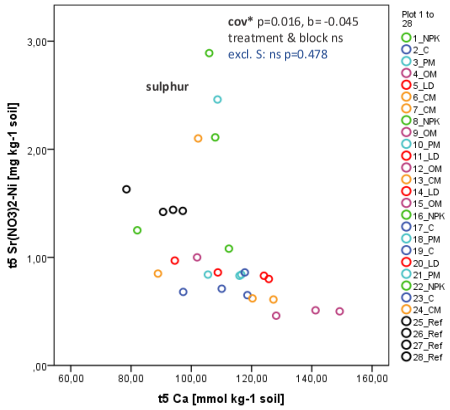
Sr(NO$_3$)^2-Nickel

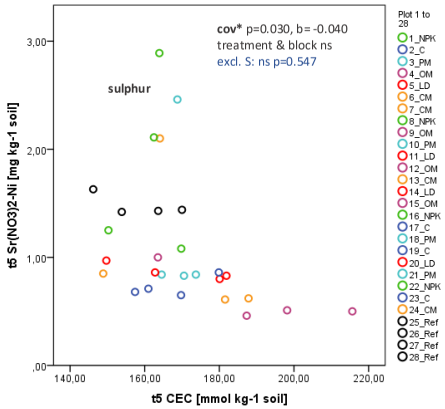




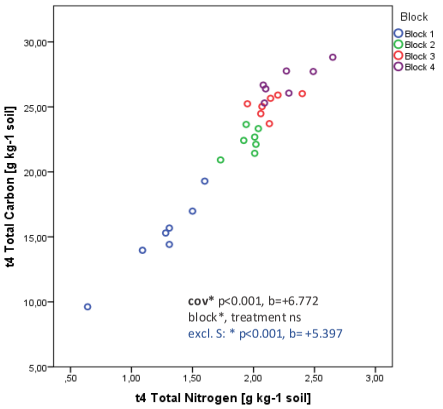


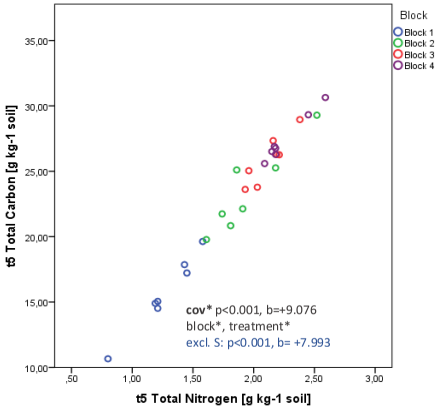


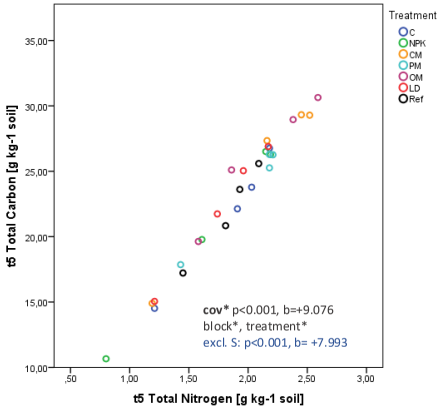


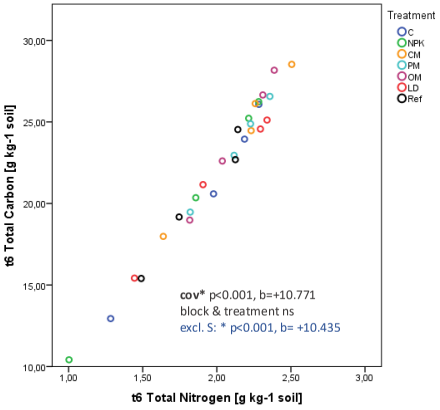


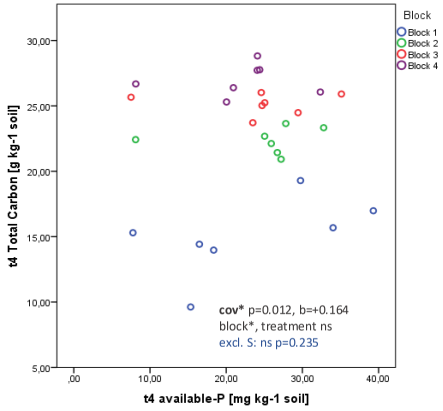
Total Carbon:

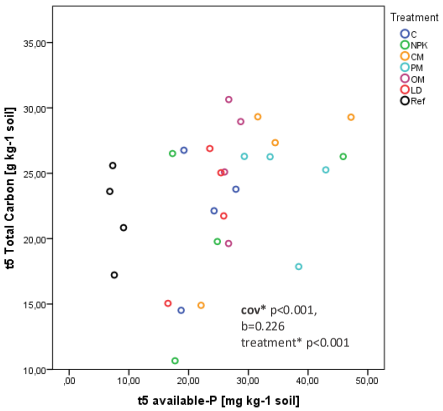


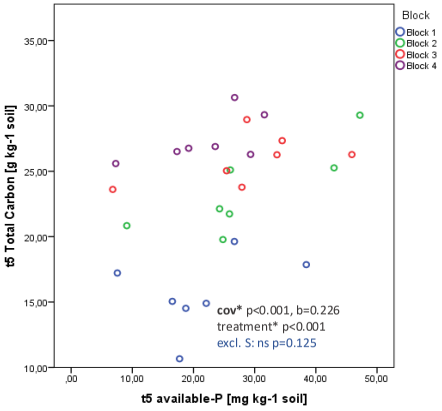


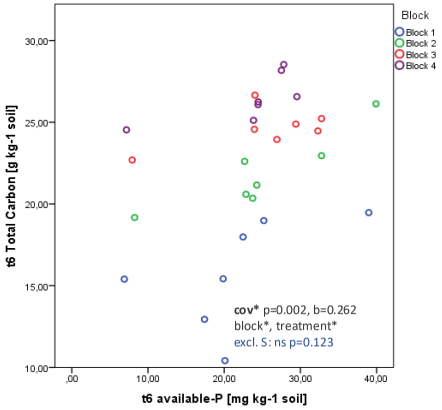


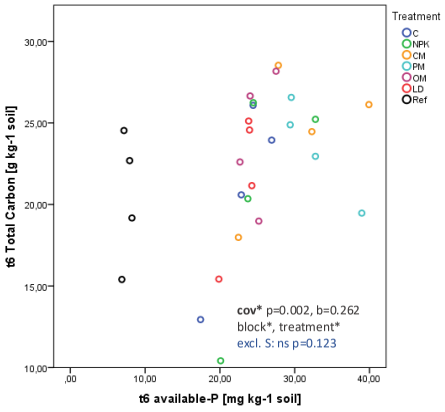


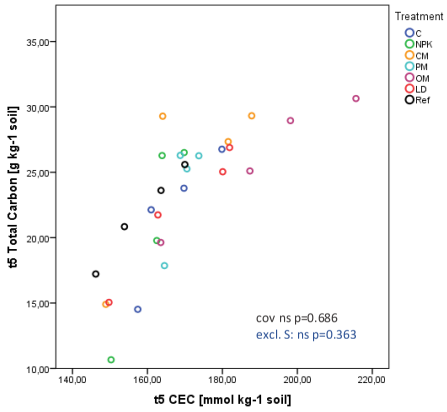


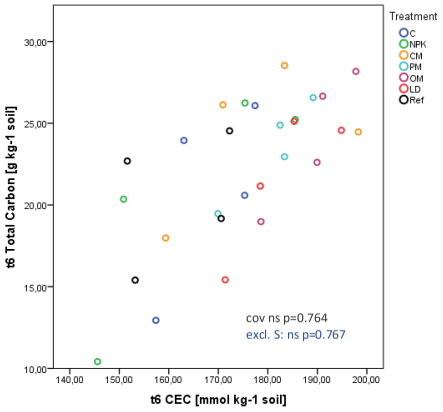


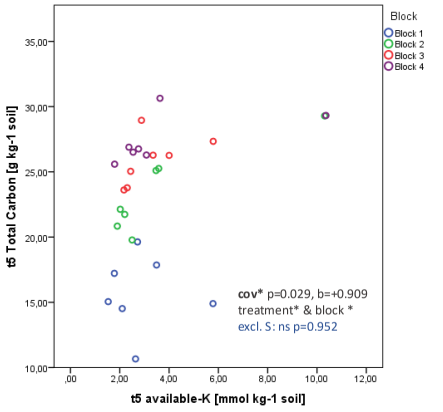


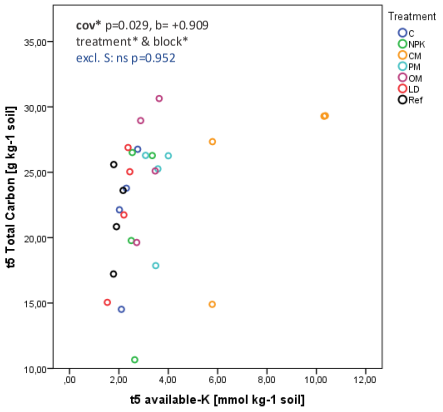


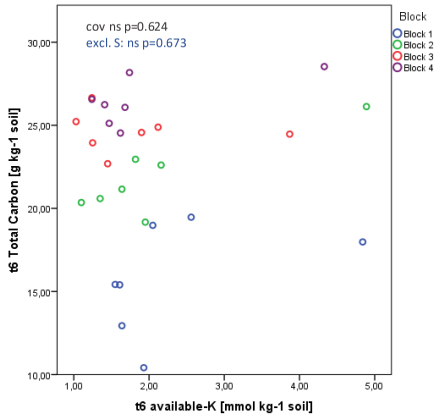

t6 Total Carbon [g kg-1 soil]
t6 available-P [mg kg-1 soil]
Treatment
C
NPK
CM
PM
OM
LD
Ref
cov* p=0.002, b=0.262
block*, treatment*
excl. S: ns p=0.123

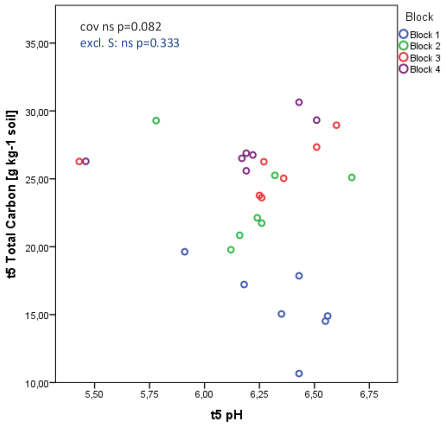

t5 Total Carbon [g kg-1 soil]
t5 CEC [mmol kg-1 soil]
Treatment
C
NPK
CM
PM
OM
LD
Ref
cov ns p=0.686
excl. S: ns p=0.363

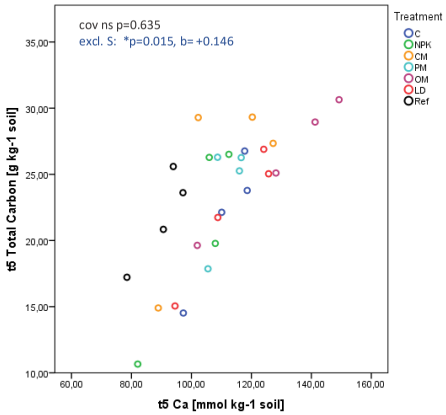

t6 Total Carbon [g kg-1 soil]
t6 CEC [mmol kg-1 soil]
Treatment
C
NPK
CM
PM
OM
LD
Ref
cov ns p=0.764
excl. S: ns p=0.767

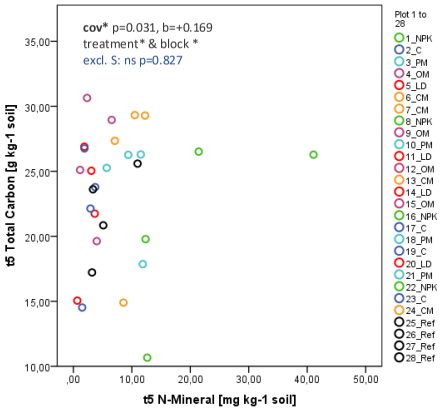

t5 Total Carbon [g kg-1 soil]
t5 available-K [mmol kg-1 soil]
Block
Block 1
Block 2
Block 3
Block 4
cov* p=0.029, b=+0.909
treatment* & block *
excl. S: ns p=0.952


t5 Total Carbon [g kg-1 soil]
t5 available-K [mmol kg-1 soil]
Treatment
C
NPK
CM
PM
OM
LD
Ref
cov* p=0.029, b= +0.909
treatment* & block*
excl. S: ns p=0.952

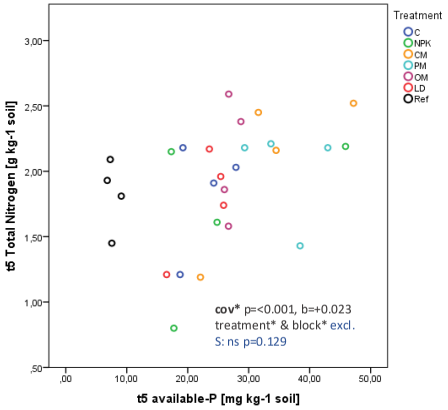

t6 Total Carbon [g kg-1 soil]
t6 available-K [mmol kg-1 soil]
Block
Block 1
Block 2
Block 3
Block 4
cov ns p=0.624
excl. S: ns p=0.673

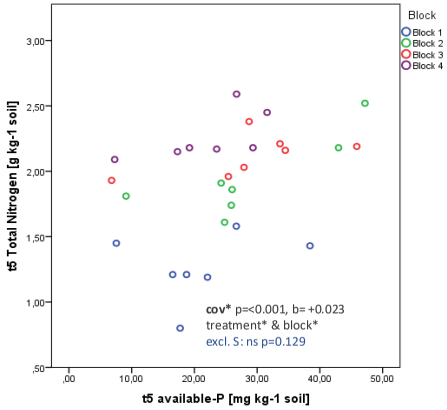

t5 Total Carbon [g kg-1 soil]
t5 pH
Block
Block 1
Block 2
Block 3
Block 4
cov ns p=0.082
excl. S: ns p=0.333

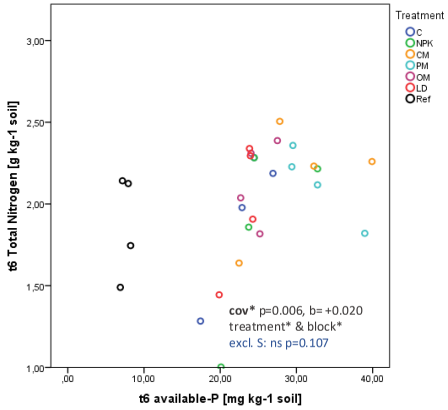

t5 Total Carbon [g kg-1 soil]
t5 Ca [mmol kg-1 soil]
Treatment
C
NPK
CM
PM
OM
LD
Ref
cov ns p=0.635
excl. S: *p=0.015, b= +0.146

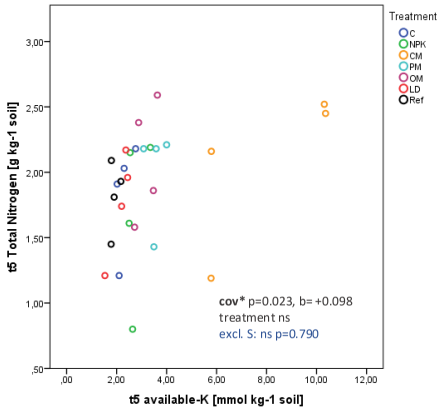

t5 Total Carbon [g kg-1 soil]
t5 N-Mineral [mg kg-1 soil]
Plot 1 to 28
1_NPK
2_C
3_PM
4_OM
5_LD
6_CM
7_CM
8_NPK
9_OM
10_PM
11_LD
12_OM
13_CM
14_LD
15_OM
16_NPK
17_C
18_PM
19_C
20_LD
21_PM
22_NPK
23_C
24_CM
25_Ref
26_Ref
27_Ref
28_Ref
cov* p=0.031, b=+0.169
treatment* & block *
excl. S: ns p=0.827

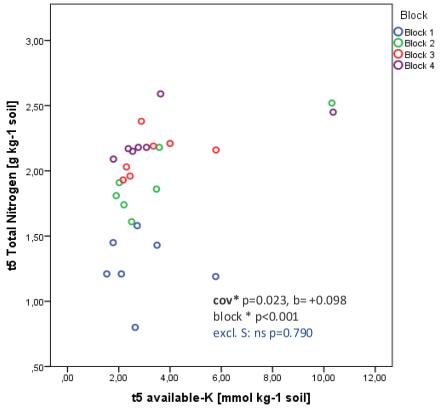
Total Nitrogen:

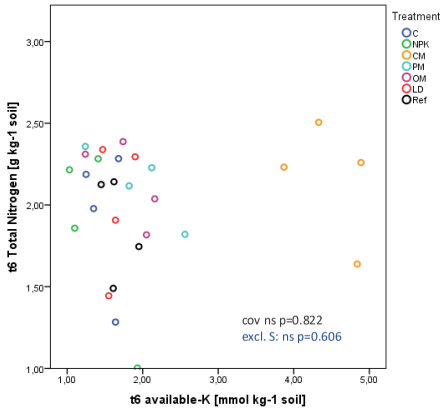


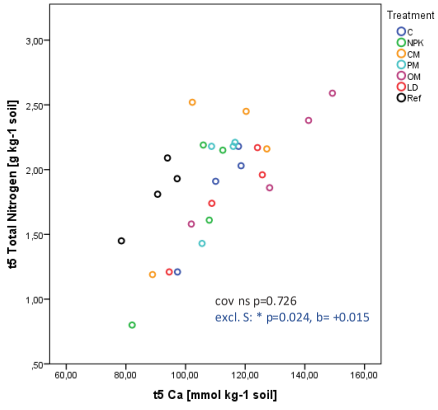


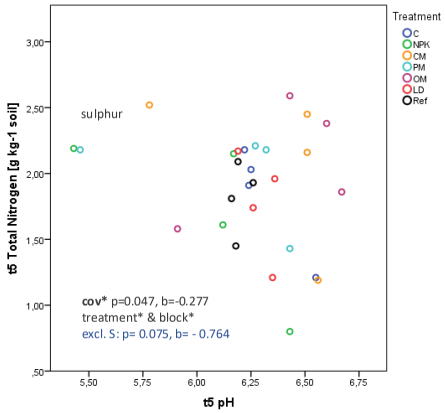


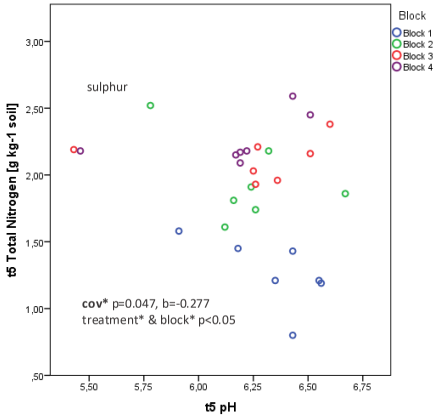


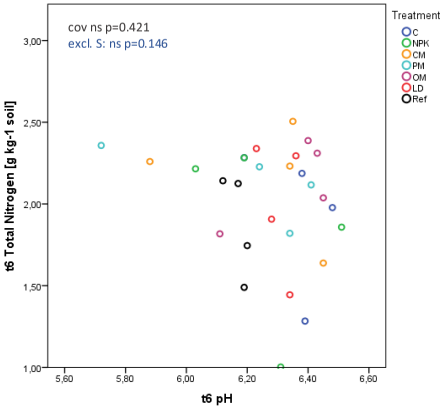


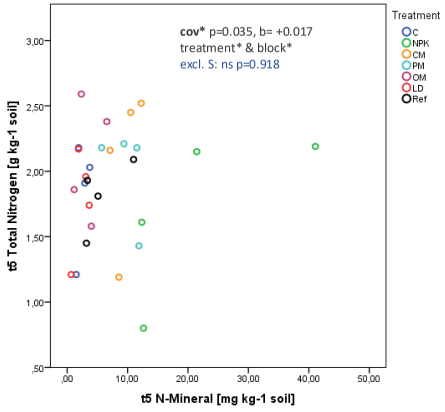


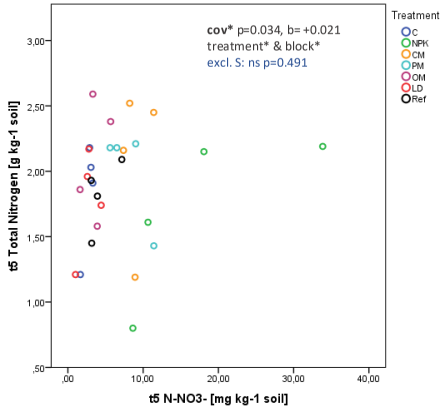




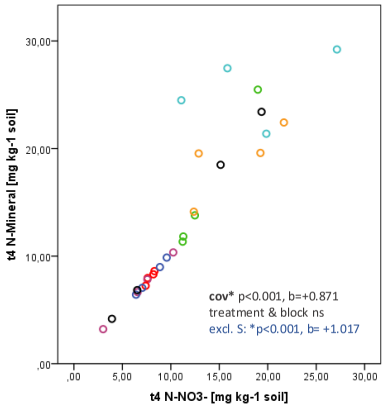


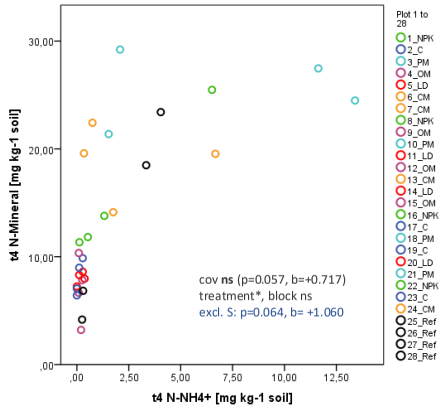


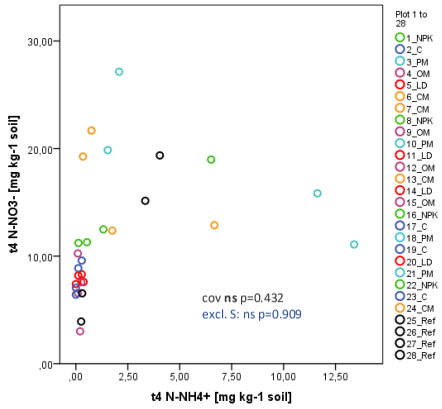


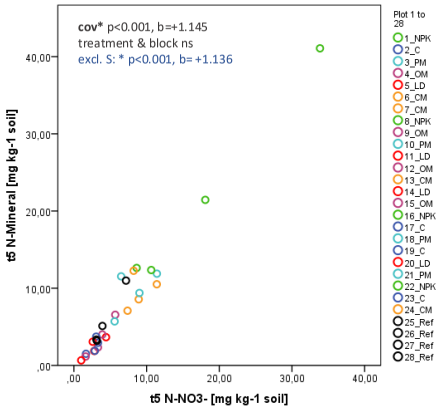


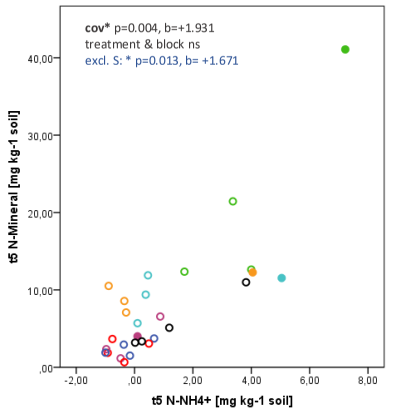
Nmin

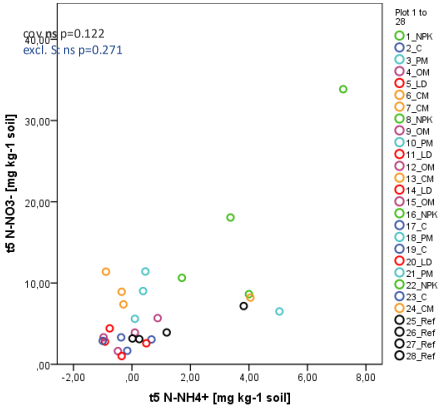

t4 N-Mineral [mg kg-1 soil]
t4 N-NO3- [mg kg-1 soil]
cov* p<0.001, b=+0.871
treatment & block ns
excl. S: *p<0.001, b= +1.017
Plot 1 to 28

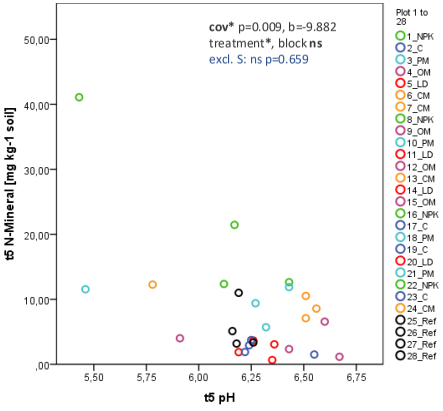

t4 N-Mineral [mg kg-1 soil]
t4 N-NH4+ [mg kg-1 soil]
cov ns (p=0.057, b=+0.717)
treatment*, block ns
excl. S: p=0.064, b= +1.060
Plot 1 to 28

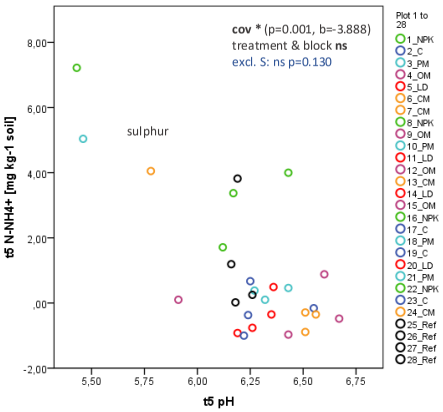

t4 N-NO3- [mg kg-1 soil]
t4 N-NH4+ [mg kg-1 soil]
cov ns p=0.432
excl. S: ns p=0.909
Plot 1 to 28

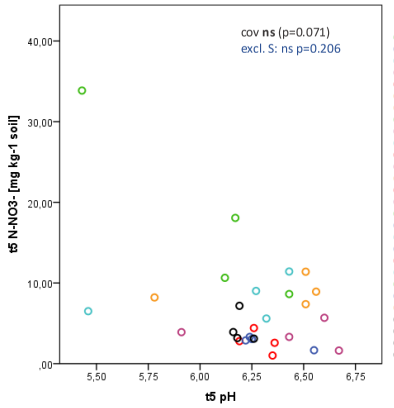

t5 N-Mineral [mg kg-1 soil]
t5 N-NO3- [mg kg-1 soil]
cov* p<0.001, b=+1.145
treatment & block ns
excl. S: * p<0.001, b= +1.136
Plot 1 to 28

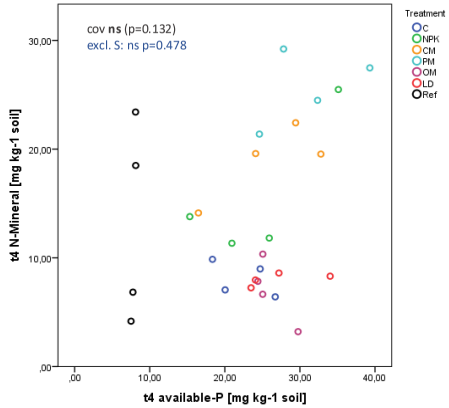

t5 N-Mineral [mg kg-1 soil]
t5 N-NH4+ [mg kg-1 soil]
cov* p=0.004, b=+1.931
treatment & block ns
excl. S: * p=0.013, b= +1.671
Plot 1 to 28

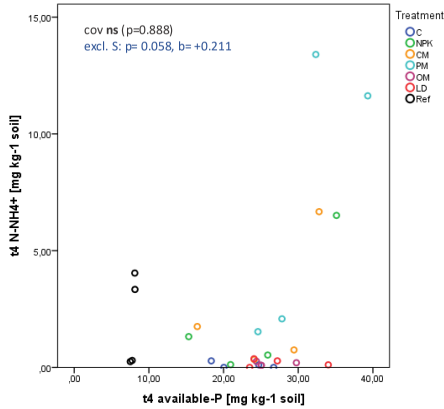

t5 N-NO3- [mg kg-1 soil]
t5 N-NH4+ [mg kg-1 soil]
cov ns p=0.122
excl. S: ns p=0.271
Plot 1 to 28

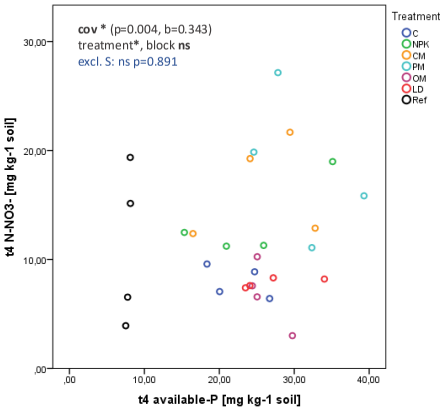

t5 N-Mineral [mg kg-1 soil]
t5 pH
cov* p=0.009, b=-9.882
treatment*, block ns
excl. S: ns p=0.659
Plot 1 to 28

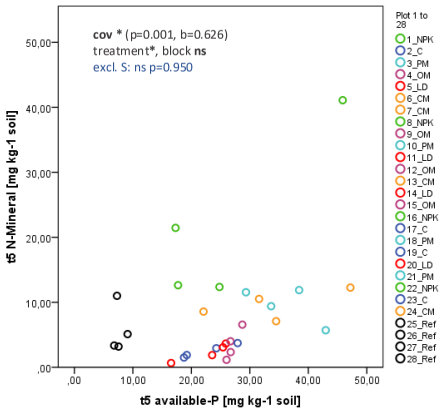

t5 N-NH4+ [mg kg-1 soil]
t5 pH
cov * (p=0.001, b=-3.888)
treatment & block ns
excl. S: ns p=0.130
sulphur
Plot 1 to 28

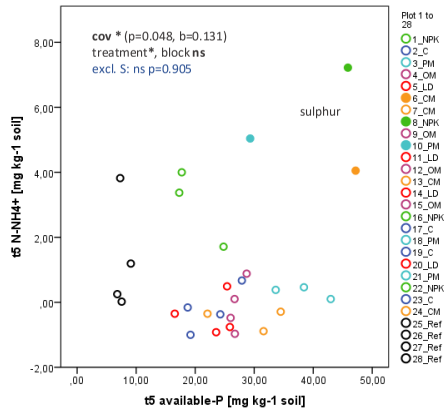

t5 N-NO3- [mg kg-1 soil]
t5 pH
cov ns (p=0.071)
excl. S: ns p=0.206
Plot 1 to 28

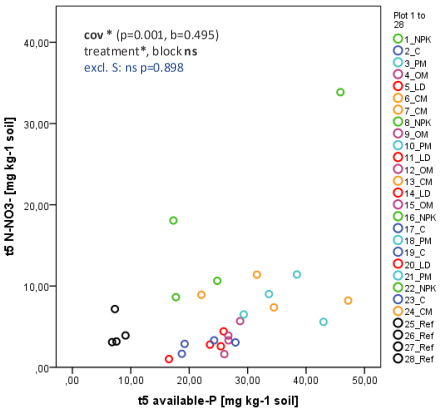

t4 N-Mineral [mg kg-1 soil]
t4 available-P [mg kg-1 soil]
cov ns (p=0.132)
excl. S: ns p=0.478
Treatment
C
NPK
CM
PM
OM
LD
Ref

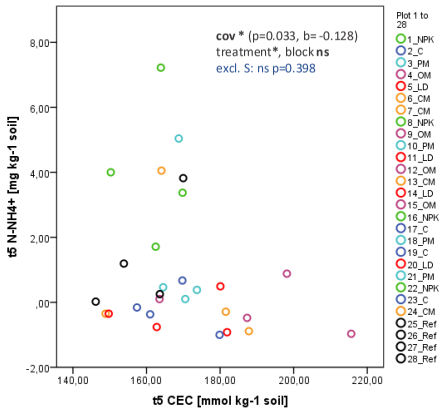

t4 NH4+ [mg kg-1 soil]
t4 available-P [mg kg-1 soil]
cov ns (p=0.888)
excl. S: p= 0.058, b= +0.211
Treatment
C
NPK
CM
PM
OM
LD
Ref

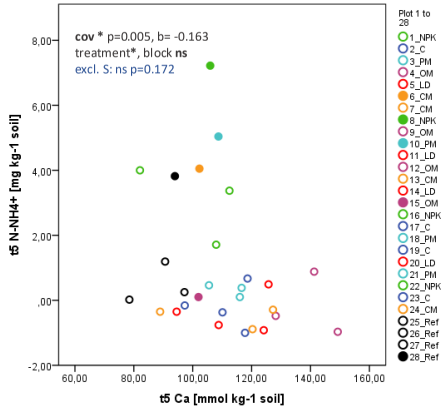

t4 N-NO3- [mg kg-1 soil]
t4 available-P [mg kg-1 soil]
cov * (p=0.004, b=0.343)
treatment*, block ns
excl. S: ns p=0.891
Treatment
C
NPK
CM
PM
OM
LD
Ref


cov * (p=0.001, b=0.626)
treatment*, block ns
excl. S: ns p=0.950
t5 N-Mineral [mg kg-1 soil]
t5 available-P [mg kg-1 soil]
Plot 1 to 28

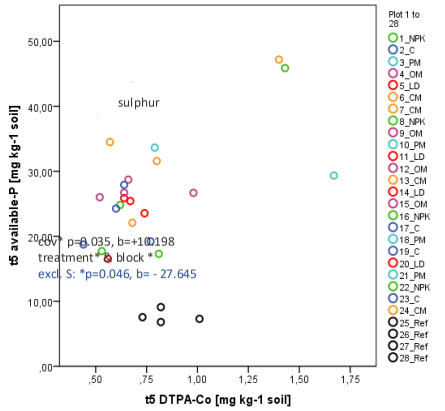

cov * (p=0.048, b=0.131)
treatment*, block ns
excl. S: ns p=0.905
sulphur
t5 N-NH4+ [mg kg-1 soil]
t5 available-P [mg kg-1 soil]
Plot 1 to 28

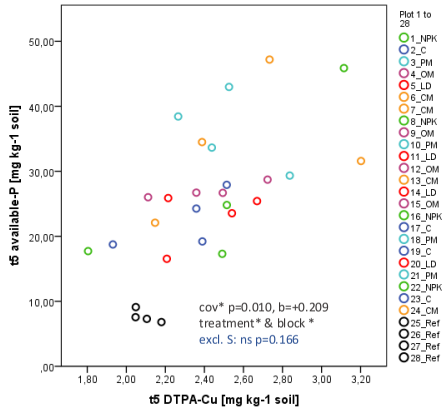

cov * (p=0.001, b=0.495)
treatment*, block ns
excl. S: ns p=0.898
t5 N-NO3- [mg kg-1 soil]
t5 available-P [mg kg-1 soil]
Plot 1 to 28

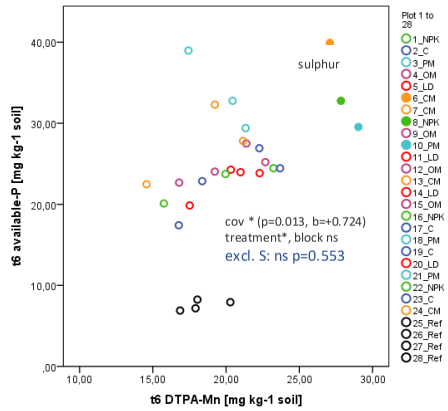

cov * (p=0.033, b= -0.128)
treatment*, block ns
excl. S: ns p=0.398
t5 N-NH4+ [mg kg-1 soil]
t5 CEC [mmol kg-1 soil]
Plot 1 to 28

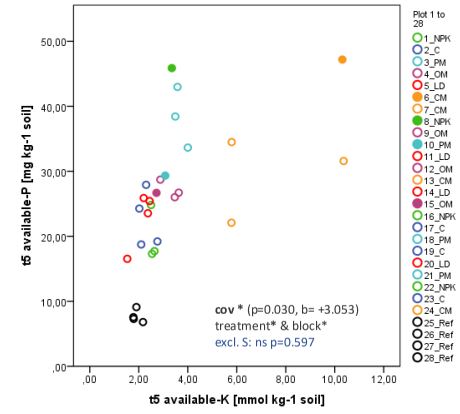

cov * p=0.005, b= -0.163
treatment*, block ns
excl. S: ns p=0.172
t5 N-NH4+ [mg kg-1 soil]
t5 Ca [mmol kg-1 soil]
Plot 1 to 28

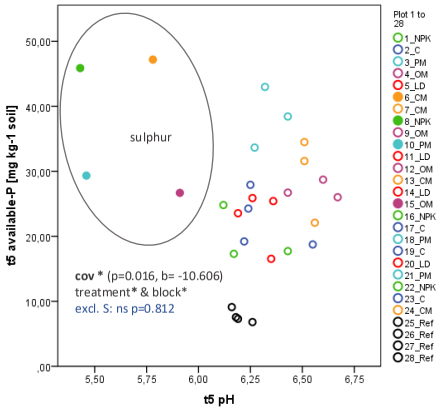
Available-P

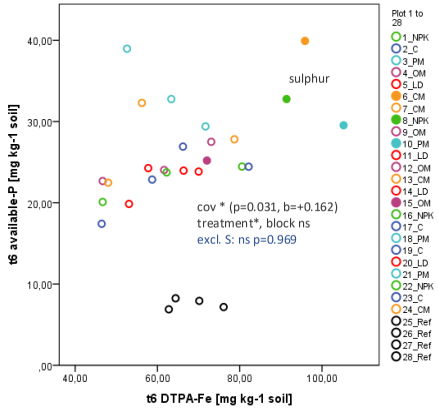

sulphur
cov * p=0.035, b=+0.198
treatment* & block *
excl. S: *p=0.046, b= - 27.645
t5 available-P [mg kg-1 soil]
t5 DTPA-Co [mg kg-1 soil]
Plot 1 to 28


cov* p=0.010, b=+0.209
treatment* & block *
excl. S: ns p=0.166
t5 available-P [mg kg-1 soil]
t5 DTPA-Cu [mg kg-1 soil]
Plot 1 to 28

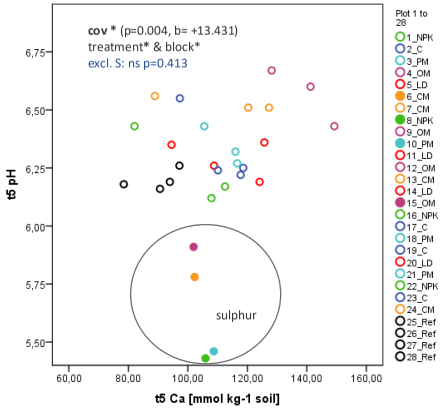

sulphur
cov * (p=0.013, b=+0.724)
treatment*, block ns
excl. S: ns p=0.553
t6 available-P [mg kg-1 soil]
t6 DTPA-Mn [mg kg-1 soil]
Plot 1 to 28

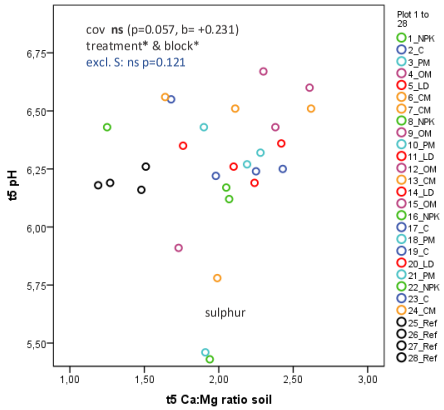

cov * (p=0.030, b= +3.053)
treatment* & block*
excl. S: ns p=0.597
t5 available-P [mg kg-1 soil]
t5 available-K [mmol kg-1 soil]
Plot 1 to 28

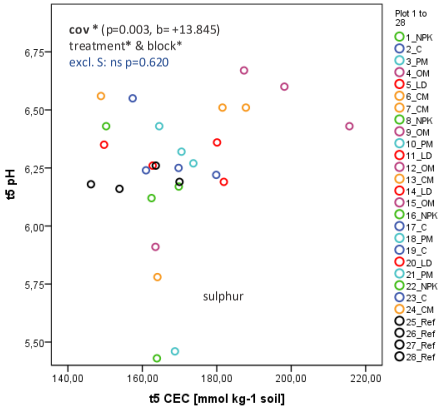


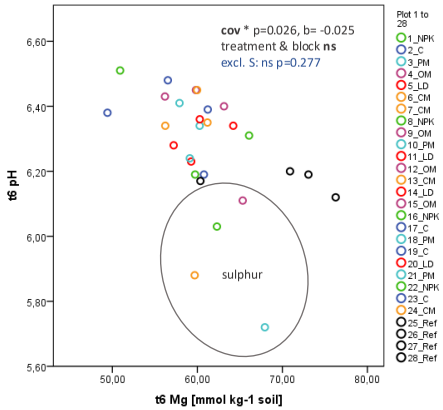


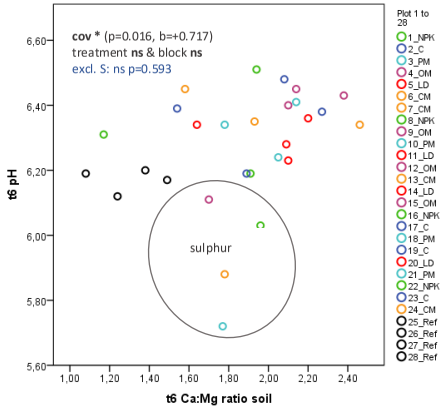
pH & exchangeable cations

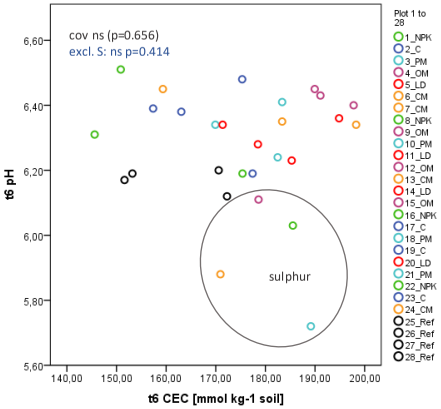


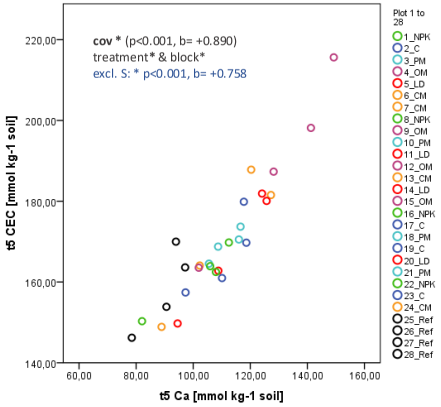


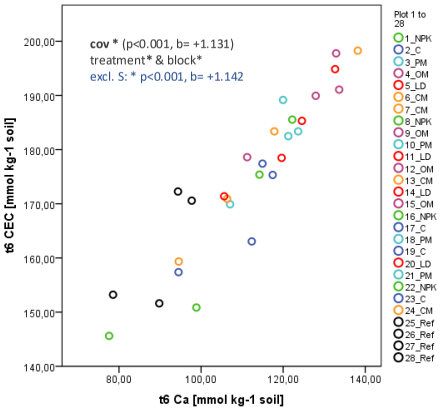


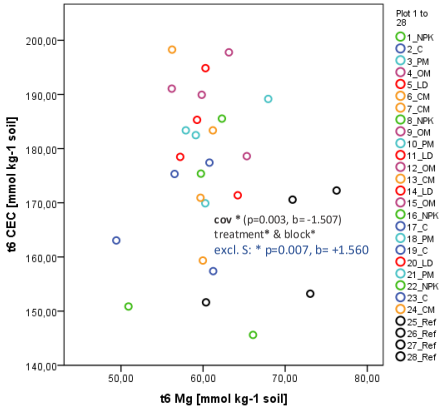




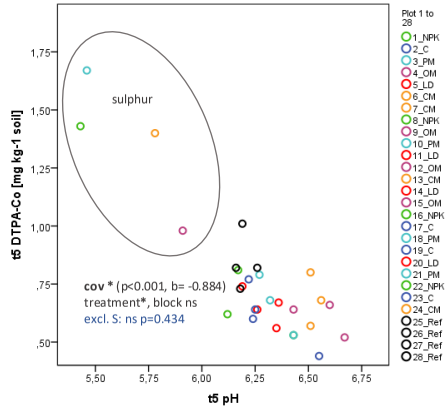


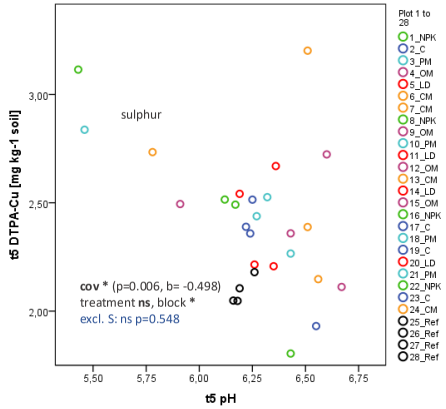


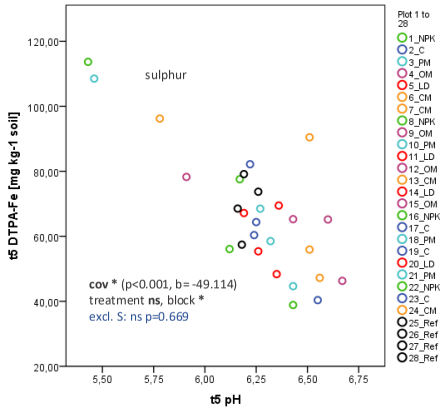


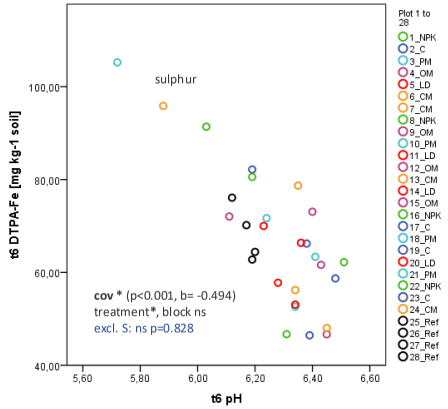


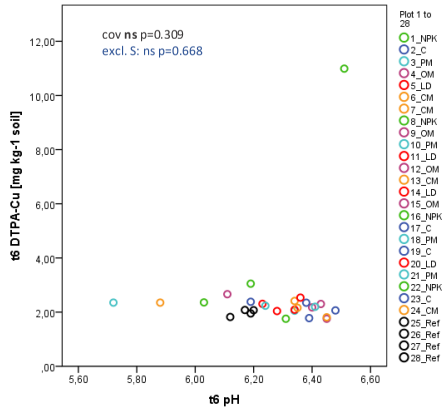
DTPA-heavy metals

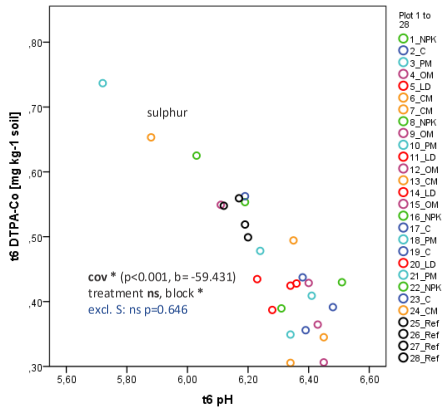


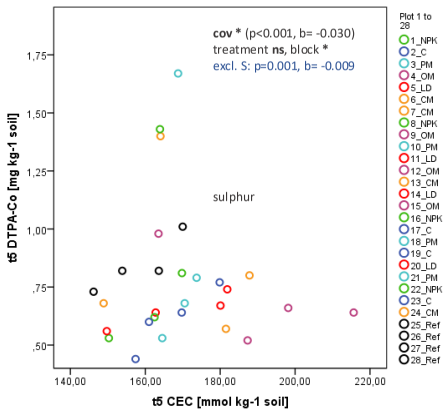


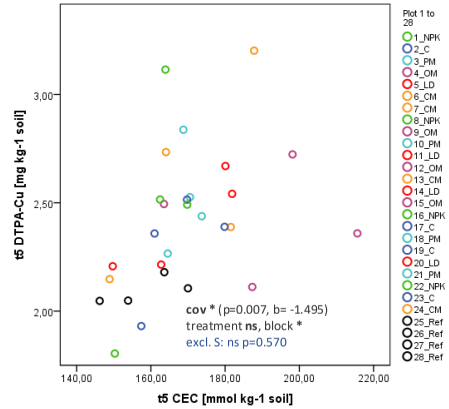


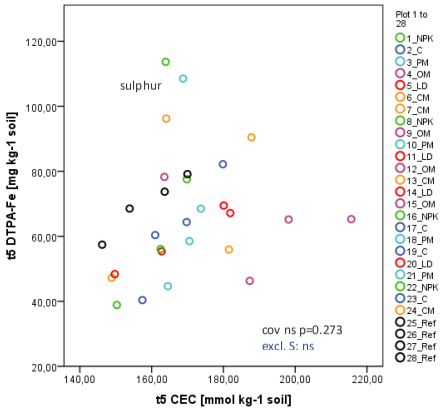




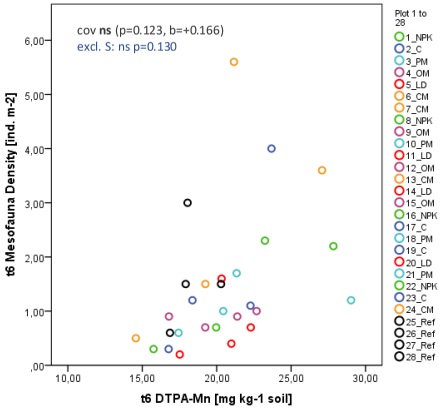


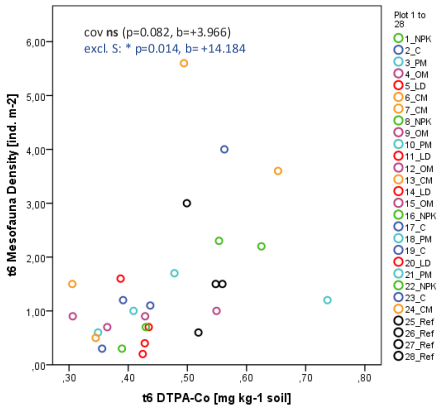


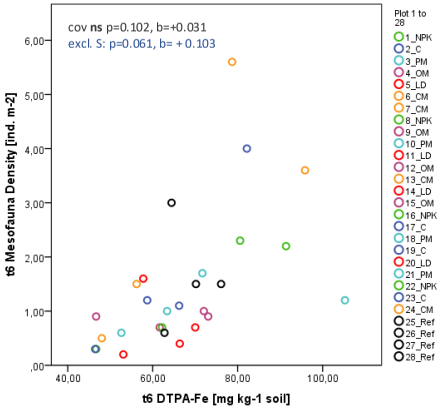


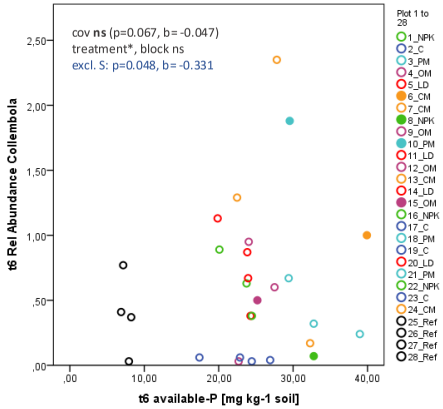


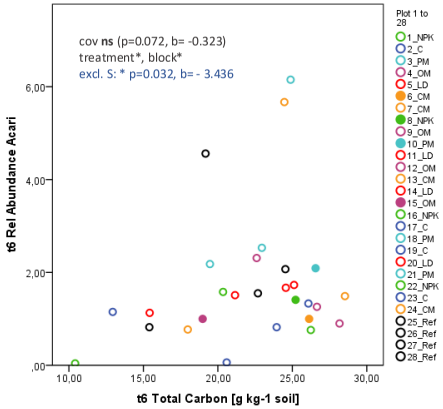
Mesofauna indices

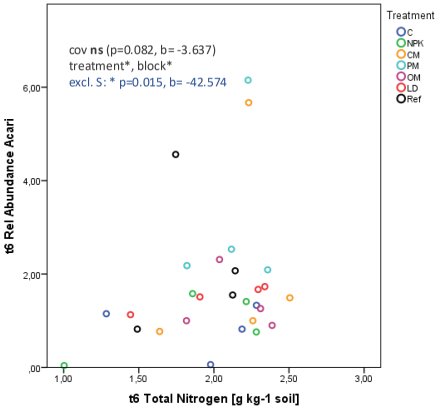




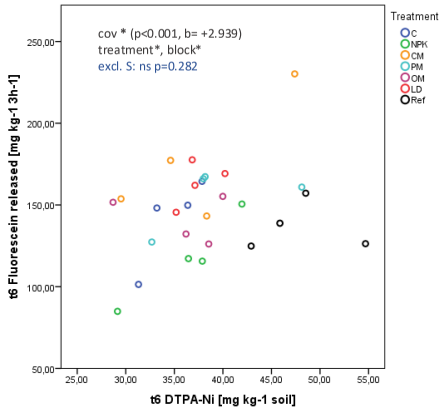


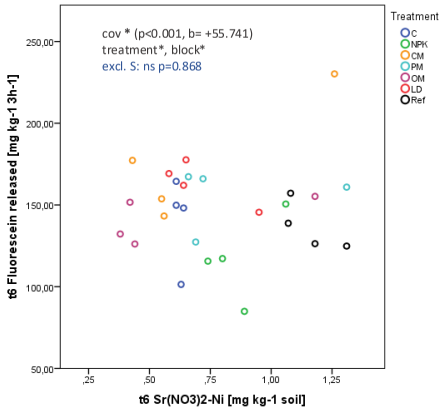


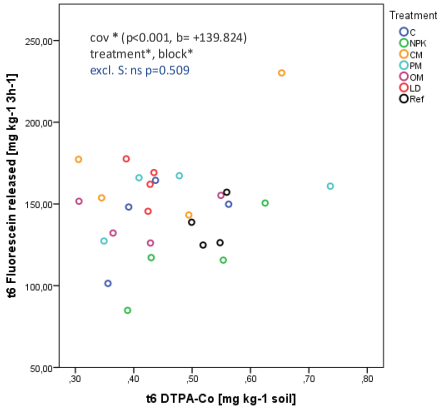


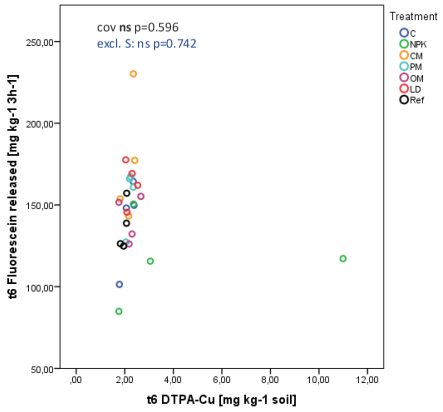


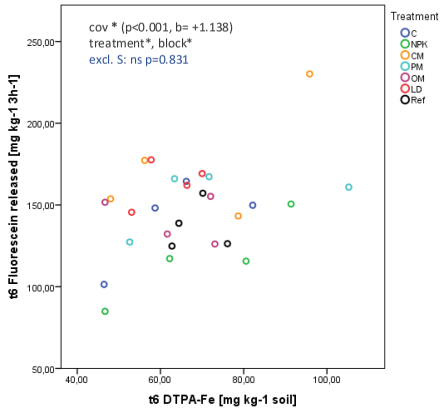
FDA

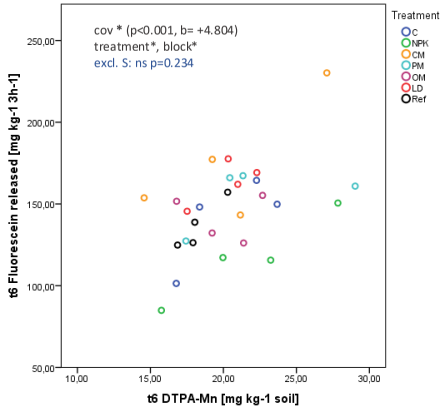

cov * (p<0.001, b= +2.939)
treatment*, block*
excl. S: ns p=0.282
t6 Fluorescein released [mg kg-1 3h-1]
t6 DTPA-Ni [mg kg-1 soil]
Treatment
C
NPK
CM
PM
OM
LD
Ref

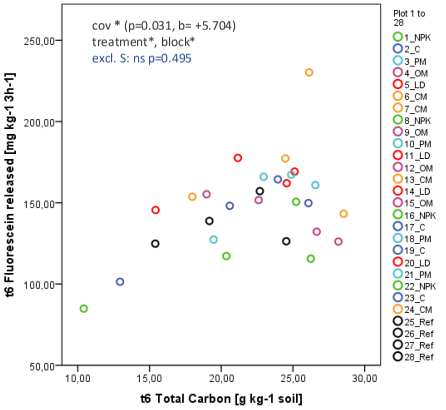

cov * (p<0.001, b= +55.741)
treatment*, block*
excl. S: ns p=0.868
t6 Fluorescein released [mg kg-1 3h-1]
t6 Sr(NO3)2-Ni [mg kg-1 soil]
Treatment
C
NPK
CM
PM
OM
LD
Ref

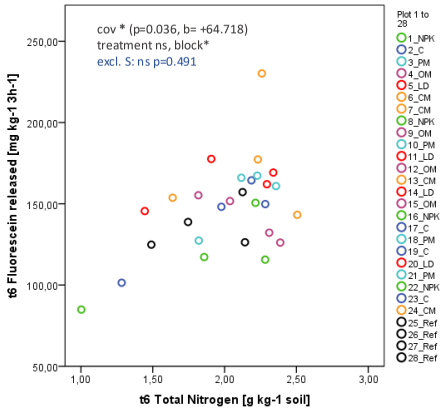

cov * (p<0.001, b= +139.824)
treatment*, block*
excl. S: ns p=0.509
t6 Fluorescein released [mg kg-1 3h-1]
t6 DTPA-Co [mg kg-1 soil]
Treatment
C
NPK
CM
PM
OM
LD
Ref

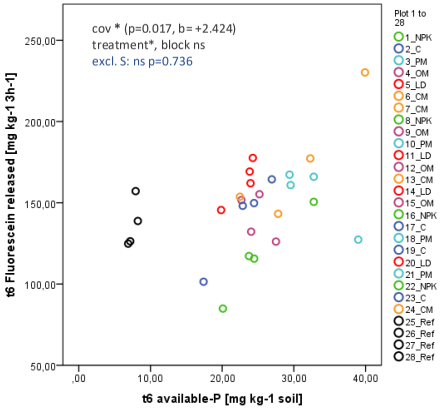

cov ns p=0.596
excl. S: ns p=0.742
t6 Fluorescein released [mg kg-1 3h-1]
t6 DTPA-Cu [mg kg-1 soil]
Treatment
C
NPK
CM
PM
OM
LD
Ref


cov * (p<0.001, b= +1.138)
treatment*, block*
excl. S: ns p=0.831
t6 Fluorescein released [mg kg-1 3h-1]
t6 DTPA-Fe [mg kg-1 soil]
Treatment
C
NPK
CM
PM
OM
LD
Ref


cov * (p<0.001, b= +4.804)
treatment*, block*
excl. S: ns p=0.234
t6 Fluorescein released [mg kg-1 3h-1]
t6 DTPA-Mn [mg kg-1 soil]
Treatment
C
NPK
CM
PM
OM
LD
Ref


cov * (p=0.031, b= +5.704)
treatment*, block*
excl. S: ns p=0.495
t6 Fluorescein released [mg kg-1 3h-1]
t6 Total Carbon [g kg-1 soil]
Plot 1 to 28
1_NPK
2_C
3_PM
4_OM
5_LD
6_CM
7_CM
8_NPK
9_OM
10_PM
11_LD
12_OM
13_CM
14_LD
15_OM
16_NPK
17_C
18_PM
19_C
20_LD
21_PM
22_NPK
23_C
24_CM
25_Ref
26_Ref
27_Ref
28_Ref


cov * (p=0.036, b= +64.718)
treatment ns, block*
excl. S: ns p=0.491
t6 Fluorescein released [mg kg-1 3h-1]
t6 Total Nitrogen [g kg-1 soil]
Plot 1 to 28
1_NPK
2_C
3_PM
4_OM
5_LD
6_CM
7_CM
8_NPK
9_OM
10_PM
11_LD
12_OM
13_CM
14_LD
15_OM
16_NPK
17_C
18_PM
19_C
20_LD
21_PM
22_NPK
23_C
24_CM
25_Ref
26_Ref
27_Ref
28_Ref


cov * (p=0.017, b= +2.424)
treatment*, block ns
excl. S: ns p=0.736
t6 Fluorescein released [mg kg-1 3h-1]
t6 available-P [mg kg-1 soil]
Plot 1 to 28
1_NPK
2_C
3_PM
4_OM
5_LD
6_CM
7_CM
8_NPK
9_OM
10_PM
11_LD
12_OM
13_CM
14_LD
15_OM
16_NPK
17_C
18_PM
19_C
20_LD
21_PM
22_NPK
23_C
24_CM
25_Ref
26_Ref
27_Ref
28_Ref