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Master Thesis

Is winter cover cropping a sustainable measure to enhance earthworm populations and decrease *Sclerotinia sclerotiorum* inoculum?

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Abbreviations

BCA	biological control agent
CC	cover crop
DM	dry matter
GLMM	generalized linear mixed model
LAI	leaf area index
LMM	linear mixed model
NO₃ – N	soil nitrate N
radish HD	forage radish with high sowing density
radish LD	forage radish with low sowing density
<i>S. sclerotiorum</i>	<i>Sclerotinia sclerotiorum</i> (Lib.) De Bary
SOC	soil organic carbon
SOM	soil organic matter
WHC	water-holding capacity

Abstract

Global change threatens the well-being of humanity, with agriculture being both affected and a driving force. Cover crops (CCs) can contribute to mitigation, e.g. by reducing erosion or increasing soil humus content. Furthermore, they can promote active soil life, including earthworms, which in turn enhance nutrient cycling, water infiltration and soil structure. Earthworms can even reduce the sclerotia of the widespread pathogen *Sclerotinia sclerotiorum* that also infects soybean. CCs can affect both earthworm populations and sclerotia decomposition through changes in abiotic factors or secondary plant compounds. To test the impacts of CCs on earthworm populations, sclerotia decomposition and soybean yield, a field experiment was set up in Eastern Austria with four CC treatments: radish at two sowing densities, black oat and sudangrass under rainfed and irrigated (+100 mm) conditions with a consecutive soybean crop. CCs at the rainfed trial reduced soil moisture, which in turn reduced earthworm abundance under black oat and sudangrass. Under irrigation, however, CCs increased soil moisture compared to bare fallow by covering the soil, most prominently under radish, but earthworm populations did not increase significantly. In spring, there were no significant effects of CCs on earthworm abundance and mass, indicating rapid recovery of earthworm populations under CCs after the previous drought. Sclerotia decomposition was reduced under radish with high sowing density at the irrigated trial, probably due to lower soil temperatures. Higher rates of sclerotia decomposition under sudangrass compared to radish suggested a biological effect induced by sudangrass. Soybean was not infected with *S. sclerotiorum* and CCs did not affect yield. Cover cropping has many environmental benefits but cannot counteract *S. sclerotiorum* alone. CCs, especially radish, can improve conditions for earthworms, but vigorous CCs like sudangrass can cause extra water stress during drought.

Zusammenfassung

Der globale Wandel bedroht die Lebensgrundlagen der Menschheit, dabei ist die Landwirtschaft sowohl beeinträchtigt als auch ein Treiber. Zwischenfrüchte (ZF) können zur Mitigation beitragen, z.B. durch Erosionsminderung oder höhere Humusgehalte in Böden. Zudem können ZF ein aktives Bodenleben fördern, so auch Regenwürmer, die wiederum Nährstoffe freisetzen, die Wasserinfiltration und die Bodenstruktur verbessern können. Regenwürmer können sogar die Sklerotien des Krankheitserregers *Sclerotinia sclerotiorum* reduzieren, der neben vielen anderen Kulturen auch Soja befällt. ZF können sowohl Regenwurmpopulationen als auch die Zersetzung von Sklerotien durch Veränderungen abiotischer Faktoren oder durch sekundäre Pflanzenstoffe beeinflussen. Um die Auswirkungen von ZF auf Regenwurmpopulationen, Sklerotienabbau und Sojaertrag zu testen, wurde ein Feldversuch mit vier ZF-Varianten angelegt: Rettich mit zwei Aussaatstärken, Sandhafer und Sudangras unter bewässerten (+100 mm) und unbewässerten Bedingungen, sowie einer anschließenden Sojakultur. Die ZF im unbewässerten Versuch verringerten die Bodenfeuchtigkeit, was wiederum die Anzahl der Regenwürmer unter Sandhafer und Sudangras reduzierte. Bei Bewässerung hingegen erhöhten die ZF die Bodenfeuchtigkeit im Vergleich zur Brache durch die Bodenbedeckung, vor allem unter Rettich, jedoch ohne eine signifikante Zunahme der Regenwurmpopulationen. Im Frühjahr unterschieden sich die Regenwurmpopulationen nicht signifikant, was darauf hindeutet, dass sich die Regenwürmer unter ZF gut erholen konnten. Der Abbau von Sklerotien war unter Rettich mit hoher Aussaatstärke im bewässerten Versuch reduziert, was auf die niedrigeren Temperaturen zurückgeführt wurde. Aufgrund der höheren Zersetzungsraten von Sklerotien unter Sudangras im Vergleich zu Rettich wurde ein biologischer Effekt durch Sudangras vermutet. Der Sojabestand wurde nicht mit *S. sclerotiorum* infiziert, und die ZF hatten keinen Einfluss auf den Ertrag. Der Zwischenfruchtanbau hat viele Vorteile für die Umwelt, kann aber nicht effektiv *S. sclerotiorum* bekämpfen. ZF, insbesondere Rettich, können die Bedingungen für Regenwürmer verbessern, aber wüchsige ZF wie Sudangras können bei Trockenheit zusätzlichen Wasserstress verursachen.

1 Introduction

1.1 Cover crops

The use of cover crops (CCs) is gaining new popularity in Europe (Bert et al., 2019; Brust and Gerhards, 2012). In addition to crop rotation and animal manure, CCs, especially legumes, have a long history of maintaining soil fertility (Scholberg et al., 2010). CCs are plants sown on fields not primarily for harvest, but for providing benefits to the soil. Winter CCs are grown after the harvest of the main crop covering the soil instead of fallow (Poeplau and Don, 2015). Growing CCs is a promising technique that may help tackle some of the increasing challenges in the agricultural sector resulting from pressure from multiple sources. On the one hand, there is increasing criticism of the negative impact of agriculture on the environment, such as the influence of agriculture on insect decline in Europe (Habel et al., 2019; Basset and Lamarre, 2019). On the other hand, agriculture itself suffers from environmental degradation and is threatened by the effects of anthropogenic climate change (Malhi et al., 2021; Rosenzweig et al., 2001). At the same time, the growing human population and dietary habits require an increased agricultural production, while the area under agricultural cultivation is competing with habitat protection, and timber production (UN, 2017; Ramankutty et al., 2018; Haberl, 2015). Thus, these agricultural challenges are strongly interconnected with the social and economic processes that are driving global change - a term describing the accelerating changes in the environment on a global scale caused by human activity that is now reaching thresholds that can alter the environment to such an extent that the natural foundations for human societies are at stake (Steffen et al., 2006, 2015).

A prominent example where CCs helped to counteract such environmental degradation dates back to the first half of the 20th century. During the Dust Bowl, Poaceae CCs were seeded to minimize the heavy wind erosion and to stabilize the soil (Hurt, 1985). CCs can reduce wind but also water erosion, directly through their above- and belowground biomass and indirectly by increasing soil organic carbon (SOC), aggregate stability, and infiltration rates (Siddoway et al., 1965; Sarrantonio and Gallandt, 2003; Liu et al., 2005; De Baets et al., 2011).

One of the critical planetary boundaries proposed by Rockström et al. (2009) are the N and P cycles. Agriculture is a major contributor of reactive N and P input to ecosystems, which can lead to eutrophication, anoxia and loss of biodiversity (Steffen et al., 2006; Rockström et al., 2009; Birk et al., 2020). Here, CCs, especially from the Poaceae and Brassicaceae families, can prevent the leaching of N and P (Finney et al., 2016; Abdalla et al., 2019). Retaining soil nitrate N ($\text{NO}_3 - \text{N}$) within the field also has mitigation effects of greenhouse gas emissions, by reducing the need for synthetic N-fertilizers (~1% of the global energy demand) and by reducing indirect N_2O emissions (Cherkasov et al., 2015; Abdalla et al., 2019).

Altogether the advantages and disadvantages of growing CCs, as well as the species that are being used in agricultural practices, are numerous. For example, CCs can help control weeds through shading, allelopathy or competition (Osipitan et al., 2019). Some CCs such as lucerne, pearl millet and radish can even reduce medium soil compaction and increase the rooting of subsequent cash crops, while strong compaction can only be

loosened through woody species (Calonego and Rosolem, 2010; Bodner et al., 2015). Drawbacks may be the extra costs for seeds and machinery required by the CC establishment (Cover Crop Survey, 2020). In some circumstances, the use of CCs can lead to reduced seedling emergence or the depletion of soil water reservoirs (Crawford et al., 2018; Duval et al., 2016).

In the past, the focus lay on checking whether CCs were non-hosts of certain pathogens in order to prevent reinfections (Brust and Gerhards, 2012). Now, growing attention is put on the ability of CCs to suppress pathogens. One of these methods is called biofumigation, where CCs are chopped to release biocidal compounds to control soil borne diseases. Plants like forage radish from the Brassicaceae family are often used, as they contain glucosinolates that break down into antipathogenic isothiocyanates (Ojaghian et al., 2012; Larkin, 2013). CCs can also reduce disease incidence through the promotion of beneficial fungi or bacteria that counteract the pathogens or can strengthen the crop plant through increasing mycorrhizae populations. Oat (*Avena* ssp.) is a candidate that combines both of these properties (Patkowska et al., 2018; Patkowska, 2020; Murrell et al., 2020).

Not only are bacteria and fungi stimulated by CCs, but macrofauna can also be increased, such as earthworm populations, which can promote several beneficial soil functions (Frasier et al., 2016; Blanchart et al., 2006; Crotty and Stoate, 2019; Bertrand et al., 2015a). A study released by the European Commission found that cover crop use in Europe varies widely by region, ranging from about 10 % to nearly 100 % (Bert et al., 2019). In Austria, about 23 % of agricultural land stayed without cash crops or CCs during the winter of 2015/2016 (STATISTIK AUSTRIA, 2016). Further research on CCs is needed to exploit the full range of its possibilities and to spread its use more widely. This includes the use of CCs against pathogens or to enhance beneficial soil life.

1.2 Earthworms

Because of their strong physical and chemical impact on the soil environment, earthworms are considered ecosystem engineers (Lavelle et al., 2006). Their most prominent impacts on the soil ecosystem are bioturbation and nutrient cycling, which they perform by ingesting large amounts of litter and soil (Scheu, 1987). The mostly positive services of earthworms for plant growth can benefit the sustainability of agriculture (Bertrand et al., 2015a).

One example of such benefits is the provision of nutrients to plants and microorganisms. Earthworms assimilate only a small portion of litter, mineral soil, and soil organic matter (SOM) during the passage through their digestive tract, while the rest is mixed and broken into small pieces and then excreted (Bertrand et al., 2015a). These casts, mucus and urine deposited by earthworms contain readily available N-molecules and store considerable amounts of P and micronutrients, which then are available for plants (Blouin et al., 2013; Bityutskii et al., 2012). Because earthworm activity depends on soil moisture and soil temperature, it is suggested that earthworms are synchronizing the release of nutrients with plant uptake demands (Bertrand et al., 2015a). Earthworms may also enhance plant growth through altered water regulations, the stimulation of bacteria that can activate hormone signal pathways in plants, the increase of symbionts and

changes in soil structure (Van Groenigen et al., 2014; Blouin et al., 2013). Nonetheless, Van Groenigen et al. (2014) concluded that the increased N availability through the enhanced mineralization of plant residues and SOM is the single most important pathway by which earthworms promote biomass production. Therefore, grain crops profit the most from earthworms, followed by pasture grasses, while legumes usually do not profit significantly, as these plants can use N through atmospheric fixation (Van Groenigen et al., 2014; Bertrand et al., 2015a).

Another benefit of the presence of earthworms can be an improved soil structure. Earthworm activity affects bulk density differently, depending on the species. There are compacting and decompacting species, whereas in natural systems they usually co-occur leading to enhanced soil structure (Blanchart et al., 1999; Blouin et al., 2013). The mixing of soil with saliva and mucus seems to have a priming effect on microbial activity, leaves the casts as microbial hotspots and promotes the creation of stable macroaggregates (Derouard et al., 1997; Lavelle et al., 2006).

Earthworm populations vary greatly between land-use types and are subject to seasonal fluctuations (Hendrix et al., 1992; Schmidt and Curry, 2001; Johnston, 2019). In a global meta-analysis, Johnston (2019) found the highest earthworm abundances under temperate climates across all ecosystem types, except for natural forests. Abundances in temperate climates were reported to range from an average of 123 individuals m^{-2} in arable fields to 425 individuals m^{-2} in managed pastures. In Mediterranean climates, where drought is a common phenomenon, numbers were generally much lower. Here arable fields counted only 50 individuals m^{-2} (Johnston, 2019). Site-specific and seasonal changes are pronounced, therefore single observations under favorable conditions can easily exceed 1000 individuals m^{-2} , even in arable fields (Schmidt and Curry, 2001).

Often discussed management practices that affect earthworm abundance are tillage, crop sequence, organic inputs and pesticide use (Bertrand et al., 2015a). Increased tillage intensity and pesticide application decrease especially anecic and epigeic species (Briones and Schmidt, 2017; Bertrand et al., 2015a). According to Bouché's ecological niche group classification, these species rely on litter from the soil surface as a food source (Bouché, 1977; Bottinelli and Capowiez, 2021). While endogeic earthworms live primarily within the upper mineral soil and feed on SOM, they are more tolerant to tillage and can profit from incorporated organic matter (Smith et al., 2008; Briones and Schmidt, 2017; Bottinelli and Capowiez, 2021). Especially species like *Aporrectodea caliginosa* are less affected by disturbances and therefore often dominate arable field populations (Smith et al., 2008; Pelosi et al., 2009).

Organic materials rich in protein and readily available carbohydrates are preferred by earthworms, even though some fresh litter is avoided due to phenolic compounds and other antinutritional factors but becomes palatable after initial degradation. Earthworms also feed on dead and living roots, but there is no evidence that roots are a preferred food source, not that earthworms could pose a danger to plants (Curry, 2004; Curry and Schmidt, 2007). In a field study, Euteneuer et al. (2019) found increased earthworm populations after a radish winter CC compared to oat and bare fallow. Another field study by Roarty et al. (2017) showed the highest earthworm populations under a pea CC, while oat yielded the second-highest populations, even though not significantly higher than bare

fallow. An early study by Westernacher and Graff (1987) also found the legume species subterranean clover to support the most earthworms. Overall, the literature on the effect of CCs on earthworm populations is still very limited.

Besides food constraints, earthworms are also restricted in survival, growth and reproduction by abiotic factors such as soil temperature, soil moisture and pH. Earthworm activity is usually limited to soil temperatures between 0 °C and 25-35 °C (Curry, 2004). Eriksen-Hamel and Whalen (2006) found the highest growth rates for *Aporrectodea caliginosa* at 20 °C. At temperatures of 5 °C and below no earthworm growth was observed anymore (Eriksen-Hamel and Whalen, 2006). Generally, environment and species-specific thresholds for temperature and moisture have interacting effects on earthworm growth (Eriksen-Hamel and Whalen, 2006). The moisture optimum for earthworms is around field capacity, with seasonal mortality in temperate climates being mostly attributed to drought (Schmidt and Curry, 2001; Curry, 2004; Curry and Schmidt, 2007). The water content of the soil is affected by CCs, mainly through transpiration and evaporation. Species with a low transpiration coefficient, such as vetch, take up less water, while species with early soil coverage can save water by reducing evaporation through shading (Bodner et al., 2007). Additionally, CCs can increase the water-holding capacity (WHC) through aggregation and increased SOM, which can bridge critical phases of drought and stabilize yields of cash crops (Daryanto et al., 2018; Lotter et al., 2003; Williams et al., 2016). Bodner et al. (2015) see little concern for soil water depletion by winter-killed CCs in eastern Austria due to low temperatures during their growing phase and thus low evapotranspiration and expect enough precipitation to replenish soil water stores before the cash crop. Nevertheless, water depletion by CCs might have an adverse effect on earthworm growth (Schmidt and Curry, 2001). But how the trade-off between water depletion by different CCs versus the provision of food and shading impacts earthworm populations, remains an open question.

Furthermore, there is growing evidence that earthworms can play a vital role in controlling plant diseases. For example, earthworms might play a role in keeping plant pathogenic nematodes below a tolerable level (Bertrand et al., 2015a; Lavelle et al., 2006). It was shown that earthworms assimilate fungi into their biomass and preferentially forage on litter infested with fungi (Shan et al., 2013; Wolfarth et al., 2011). In this way, inoculum sources of fusarium head blight were reduced by earthworms (Meyer-Wolfarth et al., 2017). Other examples in which earthworms reduced crop susceptibility to fungi were eyespot disease on wheat and *Rhizoctonia solani* Kühn on various crops (Bertrand et al., 2015b; Stephens et al., 1993; Stephens and Davoren, 1997). Enhanced nutrient uptake by plants facilitated through earthworms was suggested to help plants to resist pathogen attacks (Stephens et al., 1994). Recently it was shown that earthworms may reduce the number of sclerotia in the soil from the pathogen *Sclerotinia sclerotiorum* (Lib.) De Bary (Euteneuer et al., 2019).

1.3 *Sclerotinia sclerotiorum*

The fungus *S. sclerotiorum* is a widespread parasitic ascomycete capable of infesting more than 400 plant species causing severe yield damages throughout the world, including in soybean. Symptoms of *S. sclerotiorum* infection are water-soaked lesions with

subsequent bleaching of the stem, usually accompanied by white mycelia on infected tissue which can result in wilting and lodging of soybean plants. The disease is therefore called white mold or Sclerotinia stem rot (Boland and Hall, 1994; Peltier et al., 2012).

Sclerotia are mycelial structures approximately 2 to 35 mm large (depending on the host) that allow the fungus to survive inactive in the soil for approximately up to eight years and start germinating following internal and environmental factors to infect new hosts (Adams and Ayers, 1979; Bolton et al., 2006). After successful infection of the host plant, new sclerotia start to be formed and constitute the source of infection for future crops (Adams and Ayers, 1979).

The term carpogenic germination of sclerotia refers to the development of one or more apothecia, small mushroom-like structures that can emerge from a depth of up to 3 cm. These eject millions of ascospores into the air, of which some can travel for kilometers (Peltier et al., 2012). Ascospores need an exogenous energy source for germination and the successful invasion of healthy plant tissue (Bolton et al., 2006). Soybean plants usually get infected by ascospores that land on dying flowers which serve as an initial nutrient source for the pathogen that subsequently penetrates the stem. Soybean plants can also infect each other through direct plant to plant contact (Peltier et al., 2012). Infection can also occur through myceliogenic germination, in which sclerotia develop hyphae that directly invade host tissue, but this is usually only observed in sunflowers and some vegetables. *S. sclerotiorum* does not form any type of asexual spores i.e. conidia (Bolton et al., 2006). A severe infestation of a soybean stand by *S. sclerotiorum* is favored by moist weather conditions at cool to moderate temperatures or otherwise high relative humidity during the late flowering stage. Therefore, wider row spacing is a common cultural measure to reduce *S. sclerotiorum* incidence (Peltier et al., 2012; Derbyshire and Denton-Giles, 2016). The formation of apothecia is not easily predictable. While sclerotia need an external nutrient source for myceliogenic germination, apothecia usually form under nutrient-poor conditions. Furthermore, apothecia need light to develop successfully (Le Tourneau, 1979). Abiotic conditions stimulating apothecia formation vary with the origin of *S. sclerotiorum* isolates. Sclerotia from temperate regions may need cold conditioning, before germinating. A critical factor is soil moisture, thus irrigation and rainfall stimulate apothecia formation, while favorable temperatures lie between 10 - 25 °C (Bolton et al., 2006).

Managing the pathogen with crop rotation alone is difficult due to the wide host range and is often not a satisfactory option because mainly cereals serve as non-hosts (Boland and Hall, 1994). Spraying synthetic fungicides against *S. sclerotiorum* has variable efficacy and does not reach full control, whereas in organic agriculture these measures are not allowed at all (Mueller et al., 2002; Bradley et al., 2006). There are various cultural measures at hand, e.g. wide row spacing or choosing partially resistant cultivars, though none of these measures provide complete protection (Peltier et al., 2012; Chen and Wang, 2005). It is, therefore, necessary to look for combined methods of control.

Due to the monocyclic infection cycle of *S. sclerotiorum*, targeting the sclerotia is a promising strategy to reduce the infestation severity. The survival of sclerotia in the soil depends on biotic and abiotic factors. Especially detrimental to sclerotia is flooding with anoxic conditions (Wu et al., 2008; Smolińska and Kowalska, 2018). Soil moisture, burial

depth and soil temperature all have an interacting effect on the survival of sclerotia. Both, the combination of high soil temperatures with deep burial in the soil and the combination of high soil moisture with high soil temperature, seem to be detrimental to sclerotia (Wu and Subbarao, 2008). Many soil microorganisms have been associated with the decay of sclerotia from *S. sclerotiorum*. Especially fungi were found to play a role in the decomposition of sclerotia in the soil, while bacteria rather reduce the formation of apothecia, the germination of ascospores and mycelial growth (Derbyshire and Denton-Giles, 2016; Smolińska and Kowalska, 2018). Many of these microorganisms have been proposed as biological control agents (BCAs), lately, even viruses were drawn into consideration (Smolińska and Kowalska, 2018; Jiang et al., 2013; Hamid et al., 2018). Most successful and most widely used is the mycoparasitic fungus *Paraphaeosphaeria minitans* (W.A. Campb.) Verkley, Göker & Stielow (formerly *Coniothyrium minitans*), which has been shown to reduce sclerotia in the soil to a high degree, with a subsequent reduction of disease incidence comparable to chemical fungicides (Zeng et al., 2012a; Çolak Ateş, 2019). In soybean, no full resistance against *S. sclerotiorum* has been found so far. Nevertheless, partial resistances can help to counteract the pathogen, and selection techniques for these traits have been advancing (Chen and Wang, 2005; Sun et al., 2020; Boudhrioua et al., 2020). The dark-winged fungus gnats (*Bradysia coprophila* Lintner), which prey on sclerotia, do not seem to be a viable option in soybean cultivation, since these insects prefer highly organic soils and low pH values (Anas and Reeleder, 1988).

The effect of CCs on *S. sclerotiorum* infestation severity in subsequent cash crops has been the subject of several studies with diverse outcomes (Collange et al., 2014; Patkowska et al., 2018; Pethybridge et al., 2019; Pung et al., 2004). While Patkowska et al. (2018) attributed limited growth of *S. sclerotiorum* to the stimulation of antagonistic microorganisms by an oat CC, Pethybridge et al. (2019) attributed the reduced production of functional apothecia to shading and increased moisture under residues from a rye CC. Civardi et al. (2019) reported increased carpogenic germination of sclerotia due to a Congo grass CC in a tropical environment - the apothecia appeared in the off-season and therefore resulted in lower infestation in the subsequent soybean crop. In high tunnel experiments, Collange et al. (2014) found higher *S. sclerotiorum* infestation after sudangrass cover cropping within an intensive lettuce-melon crop rotation, possibly due to favorable conditions for the fungus to germinate. A better understanding of how CCs affect the development of *S. sclerotiorum* is needed and most likely depends on CC species and the timing of both main crop and CC.

1.4 Soybean

Soybean (*Glycine max* (L.) Merr.) ranks among the most important crops, with the bulk being used for oil production and livestock feed (Hartman et al., 2011). After a global expansion of the area cultivated with soybean, the area under cultivation in Austria has also almost doubled within the last decade (Hartman et al., 2011; AMA, 2020). Since one of the major pests in soybean cropping is *S. sclerotiorum*, further research on how to control this disease is needed (Hartman et al., 2011). The effect of CCs on soybean yield led to diverging results in different studies. Positive effects on soybean yield have been observed when preceded by forage radish that alleviated drought stress through the

creation of root channels in a compacted soil (Williams and Weil, 2004). High biomass residues of sunflower, rye and oat have led, in some studies, to negative effects on soybean emergence and yield (Reddy, 2001; Venturoso et al., 2013; Crawford et al., 2018).

1.5 Research questions

The objective of this master thesis was to investigate the influence of CCs on earthworm populations and the decomposition of sclerotia of *S. sclerotiorum* within the soil. Furthermore, a consecutive soybean crop was evaluated in regard to *S. sclerotiorum* infestation and yield. For the field trial in the Vienna Basin, the CCs black oat (*Avena strigosa* Schreb.), sudangrass (*Sorghum sudanense* (Piper) Stapf), and forage radish (*Raphanus sativus* var. *longipinnatus* Bailey) were chosen for their capacity to build up a lot of biomass (Brust and Gerhards, 2012; Al-Suhaibani, 2006). Radish was included with two different sowing densities as this may affect root diameter and biomass development (D'hooghe et al., 2018). To account for the sensitivity of CCs as well as earthworms to drought, an irrigation factor was included.

Hence the central research questions of this master thesis were the following:

- How are the chosen cover crops and their influence on soil moisture and soil temperature affecting earthworm population dynamics?
- Are the investigated cover crops influencing sclerotinia decomposition and subsequent disease incidence in a soybean crop?
- How are differences in soil moisture and soil temperature under cover crops affecting the decomposition of sclerotia?
- Are the chosen cover crops affecting soybean yield?

2 Materials and Methods

2.1 Experimental setup

The experimental site was located near Raasdorf (48°14'14.5"N, 16°35'53.9"E) and is part of the conventionally managed fields of the BOKU experimental farm Gross-Enzersdorf. The soil is a chernozem with silty loam of alluvial origin. Mean annual temperature amounts to 10.8 °C, while annual precipitation averages 536 mm (1998-2019) (Wetterstation-Raasdorf, 2020). Two experiments in randomized complete block design, one rainfed, the other with irrigation, were laid out side by side. Plots were 3 x 10 m in size with four replicates for each treatment. The four CC treatments were black oat cv PRA-TEX (*Avena strigosa* Schreb.) at 400 seeds m⁻², sudangrass cv PIPER (*Sorghum sudanense* (Piper) Stapf) at 180 seeds m⁻², forage radish cv FORZA (*Raphanus sativus* var. *longipinnatus* Bailey) at 50 seeds m⁻² (radish LD), as well as forage radish cv FORZA (*Raphanus sativus* var. *longipinnatus* Bailey) at 250 seeds m⁻² (radish HD). Bare fallow plots, which were kept free from weeds, served as control. The pre-crop was winter barley. For seedbed preparation, residues were incorporated twice with a cultivator to a

depth of 7 cm. CCs were sown with a drilling machine (Plot seeder S, Wintersteiger AG, Ried, Austria) on 17 July 2018 to a depth of 2 cm. CC residues were incorporated with a cultivator to a depth of 7 cm. Soybean was sown on 17 April 2019 with a precision planter (MS 4230, MaterMacc S.p.A., Pordenone, Italy), at a seeding rate of 65 seeds m⁻² and a row space of 50 cm. Seeds were of the variety 'Solena' and inoculated with rhizobia. During June 2019 the Herbicides Pulsar® 40 14g/ha (BASF Ag, Ludwigshafen am Rhein, Germany) and HARMONY® SX 14g/ha (FMC Agro Austria GmbH, Graz, Austria) were applied. Additional hand weeding was done with a hoe.

2.2 Irrigation

Irrigated CCs received irrigation six times from the beginning of August until the end of September 2018, each time 20 mm. CCs of the rainfed trial received 20 mm additional irrigation during August 2018, once. During the growth period of soybean, the irrigated trial received a total amount of 345 mm. At weekly intervals, 15–30 mm of water was applied from June 2019 to early August and then continued at two-week intervals until harvest (Table 1). The irrigation was carried out with a hose reel irrigation system (Rainstar Series E, BAUER GmbH, Voitsberg, Austria), which was supplied with local groundwater.

2.3 Earthworm sampling

For the determination of earthworm mass and abundance, four soil blocks of 20 cm x 20 cm width and 28 cm depth were taken from each plot with a spade. Samples were filled into plastic bags, stored at 4 °C and then hand sorted within 4 days. Earthworms belonging to different ecological groups sensu Bouché (epigeic, endogeic, anecic), as well as juveniles and adults were counted separately, rinsed in water and dried on paper towels before determining the mass (Bottinelli and Capowiez, 2021). The first sampling period ranged from 16 till 23 October 2018 and the second period from 26 March until 1 April 2019, referred to as October and April, respectively.

2.4 Sclerotia sampling

The *Sclerotinia sclerotiorum* isolate originated from a sunflower field near Gross-Enzersdorf in 2014 and was maintained in the lab on agar plates. The sclerotia for the experiment were grown on autoclaved wheat kernels (*Triticum aestivum* L.) inside polypropylene boxes at 24 °C within six weeks. The sclerotia were filled into plastic tubes with different mesh sizes, one of which allowed access for earthworms (3 × 10 mm), while the other restricted access for earthworms (1 × 1 mm). Each tube contained 15 sclerotia of which five were of size 4–8 mm and ten of size 2.5–4 mm. Within each plot, six pairs of tubes were buried in the soil at a depth of 3 cm at the beginning of August 2018. Three pairs of tubes were buried in the first half of the plot, while the other three pairs were buried in the second half of the plot and each was marked for later identification. At the beginning of November 2018, mid-December 2018 and beginning of April 2019 two pairs were excavated, respectively. The sclerotia which were collected in April 2019 were buried again in May 2019 after the sowing of soybean and excavated again in September

2019. After each excavation, the number of intact sclerotia from each mesh tube was counted, as well as the amount of germinated sclerotia and the number of stipes on each sclerotium. All plots were checked for apothecia on 28 June, 5 July and on 11 July 2019.

2.5 Plant biomass sampling

Aboveground biomass of CCs was assessed three times - at the beginning of August 2018, November 2018 and April 2019. Biomass of soybean plants was assessed on 14 June 2019. Aboveground plant parts of CCs were cut at one random location in the inner part of each plot just above ground level on an area of 0.25 m² and two random locations for soybean biomass. Afterward, biomass was dried at 105 °C for 24 hours and then weighed to determine dry matter (DM).

Belowground biomass was sampled together with aboveground biomass in November 2018 and April 2019 with a root auger on an area of 0.004 m² and a depth of 15 cm between seeding rows. The straw of the pre-crop and roots were sorted separately. First, hand sorting was done without wetting the soil samples. In a second step straw and roots were sorted while sieving the soil in water. Before mass determination, biomass was washed and then dried for 24 hours at 105 °C. Taproots of radishes were dug out separately on an area of 0.25 m², washed and dried for 24 hours at 105 °C, and then weighed for DM determination.

The leaf area index (LAI) of CCs was measured using the LAI Ceptometer AccuPAR LP-80 (Decagon Devices, Pullman, Washington, USA) on 18 September 2018. Each plot was measured twice at two different locations, following manual instructions.

The emergence rate of soybeans was assessed on 31 May 2019 by counting the emerged soybean plants on an area of 1 m². This was done two times per plot. The BBCH scale of the current crop development stage was noted according to Munger et al. (1997).

Soybeans on the rainfed plot matured earlier and therefore were harvested on August 28, while soybeans on irrigated plots were harvested on September 17. Whole soybean plants were cut just above the soil surface on an area of 3 m² per plot. Pods were threshed with a laboratory thresher (LD-180, Wintersteiger AG, Ried, Austria). Soybean grains were dried at 60 °C for 24 hours. For the thousand seed mass, 1000 soybean seeds from each plot were weighed.

The C/N ratio of CC biomass was assessed in fall 2018 and spring 2019. An amount of 50 mg dry shoot biomass (105 °C, 24 h) was ground, sieved (<1 mm) and analyzed by the Dumas combustion method (vario MACRO cube CNS; Elementar Analysensysteme GmbH, Germany) (Winkler et al., 2000).

2.6 Soil parameters

Volumetric soil moisture and soil temperature of the topsoil were measured using a moisture meter of the type HH2 with a WET-2 sensor (Delta-T Devices, Cambridge, United Kingdom) based on dielectric properties, by following the instructions. Each plot was measured twice at a random location. The depth of measurement covers approximately the first 7 cm of the soil.

The $\text{NO}_3 - \text{N}$ was analyzed from eight samples taken across the whole trial as a baseline in July 2018 and then from four pooled samples per plot in December 2018, in April 2019 and September 2019. Soil samples were taken with a gouge auger (Purckhauer type, core diameter: 30 mm) and separated by the depths of 0–30 cm, 30–60 cm and 60–90 cm. The soil samples were immediately cooled in insulated boxes and frozen (-20°C) at the experimental farm for later analysis. Soil samples were extracted with a 0.0125 M CaCl_2 solution, shaken for one hour and $\text{NO}_3 - \text{N}$ determined photometrically (FIASSTAR 5000, FOSS GmbH, Germany) according to ÖNORM L1091. Gravimetric soil water content was determined by weight difference after drying 100 – 150 g of the soil samples at 105°C for 24 hours.

Soil penetration resistance was assessed twice per plot with Penetrologger version 6 (Eijkelkamp Agrisearch Equipment, Giesbeek, Netherlands). A cone with 1 cm^2 and a 60° top angle was used. Sampling was done at the end of October 2018 and at the end of March 2019. For statistical analysis values were averaged within depth layers of 5 cm.

As a measure of soil decomposition activity, the Tea Bag Index was measured following the protocol of Keuskamp et al. (2013). Four batches of tea bags were buried in the soil at a depth of 8 cm in each plot for three months. Burial periods were from August 2018 – October 2018, December 2018 – March 2019, January 2019 – April 2019 and May 2019 – August 2019.

Table 1: Monthly air temperature, precipitation and irrigation. Monthly air temperature ($^\circ\text{C}$) with monthly minima and maxima of daily means and monthly precipitation (mm) were measured at a nearby weather station close to Raasdorf. Irrigation is given for the irrigated site. The rainfed site received 20 mm of extra irrigation in August 2018.

Month	Air temperature ($^\circ\text{C}$)			Precip. (mm)	Irrigation (mm)
	Mean	Max	Min		
Jul 2018	22.1	27.4	15.9	71.7	
Aug 2018	23.3	27.4	15.1	22.8	80
Sep 2018	17.4	21.6	8.5	78.8	40
Oct 2018	13.2	17.4	8.6	0.4	
Nov 2018	6.3	14.8	-2.9	28.0	
Dec 2018	2.5	8.1	-2.9	70.0	
Jan 2019	0.5	5.4	-6.0	29.5	
Feb 2019	4.2	11.2	-2.1	8.9	
Mar 2019	8.4	12.3	4.7	21.1	
Apr 2019	11.6	19.0	5.8	20.5	
May 2019	12.5	19.0	5.3	127.0	
Jun 2019	22.7	27.6	19.6	75.9	120
Jul 2019	20.9	26.0	15.8	92.1	90
Aug 2019	21.3	24.9	17.1	67.5	30
Sep 2019	16.6	23.7	10.9	187.2	

2.7 Meteorological data

Air temperature, soil temperature and precipitation data from a weather station (Adcon A733) located a few hundred meters from the trial were accessed online. Data from 4 July 2019 until 20 August 2019 were missing because of unknown problems with the station (Wetterstation-Raasdorf, 2020).

2.8 Statistics

All calculations and statistics were done using RStudio Version 1.2.5033 and R version 3.6.2 (RStudio Team, 2019; R Core Team, 2019).

Significant differences were tested using linear mixed models (LMMs) and generalized linear mixed models (GLMMs) with the block effect set as the random factor (eight blocks over both trials). Models were selected using the Akaike information criterion (AIC), following procedures described by Zuur et al. (2009). Model assumptions were tested using graphical analysis of residuals versus fitted and quantile-quantile plots, in addition, models were checked with the simulation-based approach of the 'DHARMA' package (Hartig, 2020). The dispersion was tested by a chi-squared test of the Pearson residuals and residual degrees of freedom, as well as with the DHARMA package. Post hoc analysis was performed using the 'emmeans' package (Lenth, 2020). ANOVA tables were calculated using the Wald test for GLMMs and the Satterthwaite approximation with restricted maximum likelihood (REML) for LMMs. Pairwise comparisons were calculated only within each irrigation regime as they were installed as individual randomized complete block designs, i.e. the factor irrigation had no true replication. P-values were corrected for multiple testing by the Tukey method. Differences were considered significant at P-values smaller than 0.05. When multiple comparisons are reported together, the P-value with the smallest confidence is given.

Earthworm data were analyzed using the glmer function of the lme4 package with the full three-way interaction between date, irrigation and CCs (Bates et al., 2015). It was assumed that serial correlation was minor due to the only two samplings with a long time gap in between them (October 2018, April 2019). Earthworm mass was analyzed using a gamma distribution (link=log), while the earthworm abundance was analyzed with a Poisson distribution (link=log).

Sclerotia decomposition was analyzed using the glmmTMB package with a beta-binomial distribution (link=logit) after the poisson and negative binomial distribution had resulted in overdispersion and non-convergence (Brooks et al., 2017). The dates November 2018, December 2018 and April 2019 were analyzed together in one model, with date as a random factor and the block effect nested within date. In consequence differences between treatments (bare fallow, radish HD, radish LD, black oat and sudangrass) were not calculated for single dates. A random intercept showed to be sufficient, therefore a random slope was discarded. The model contained an interaction between cover crop and irrigation. The September 2019 data were analyzed separately with a similar model structure, just without the factor date.

The germination rate of sclerotia was analyzed with the glmmTMB package and the beta-binomial (link=log) distribution (Brooks et al., 2017). This time all sampling dates

were analyzed together, and date was set as a fixed factor. Differences between treatments (bare fallow, radish HD, radish LD, black oat and sudangrass) were tested only within each sampling date. No major temporal dependencies were detected in the model. Three 2-way interactions were considered between cover crop, date and irrigation. Similarly, the number of stipes on the recovered sclerotia was analyzed using the glmmTMB package and a negative binomial distribution with linear parameterization (link=logit) (Brooks et al., 2017). A 3-way interaction was considered between cover crop, date and irrigation.

For soil moisture and soil temperature, average values per season were calculated. The first season ranged from August to October 2018, the second season comprised February and March 2019, while the third season contained measurements from June and July 2019. For soil moisture, in addition, the individual observations were evaluated. Soil moisture data were log-transformed before the analysis with the lmer function of the lme4 package (Bates et al., 2015). The LMMs contained three 2-way interactions between the factors: irrigation, season and CCs. Soil temperature was analyzed using the lmer function of the lme4 package, with interactions between irrigation and season and between CCs and season (Bates et al., 2015).

The $\text{NO}_3 - \text{N}$ data and the co-sampled soil moisture data were analyzed using separate LMMs for the three depth levels. Both were analyzed using the lmer function of the lme4 package, with 3-way interactions between the factors: irrigation, CCs and date (December 2018, April 2019 and September 2019) (Bates et al., 2015). The $\text{NO}_3 - \text{N}$ data were log-transformed before the analysis.

Soil penetration resistance data were square root transformed and analyzed using the lmer function of the lme4 package (Bates et al., 2015). Separate LMMs with aggregated data of different depth levels at 5 cm intervals were calculated without interactions between the factors: irrigation, CCs and date (October 2018, April 2019).

The LAI data were analyzed using the lmer function of the lme4 package with interactions between the factors: irrigation and CCs (Bates et al., 2015).

CC aboveground biomass data were square root transformed and analyzed using the lmer function of the lme4 package (Bates et al., 2015). For the dates August and November 2018, all four CCs were included, while for April 2019 the CCs radish HD and radish LD were excluded. The model contained interactions between CCs and date, as well as between irrigation and date.

Root biomass data were square root transformed and analyzed using the lmer function of the lme4 package (Bates et al., 2015). The model contained an interaction between CCs and date (November 2018, April 2019), as well as between irrigation and date. Taproot data were square root transformed and analyzed using simple linear models.

Soybean grain yield data were square root transformed and analyzed using the lmer function of the lme4 package (Bates et al., 2015). The model contained an interaction between CCs and irrigation.

Thousand-seed mass, soybean emergence rate and soybean aboveground biomass were analyzed using the lmer function of the lme4 package without interactions (Bates et al., 2015).

The C/N ratio data were log-transformed and analyzed using the lmer function of the lme4 package with the full three-way interaction between date (October 2018, April 2019), irrigation and CCs (Bates et al., 2015).

N uptake into aboveground CC biomass was calculated from N concentration within plant tissues (C/N analysis) and biomass estimation. Data from November 2018 were log-transformed and analyzed using the lmer function of the lme4 package without interaction (Bates et al., 2015).

The factor CCs usually contained five levels, i.e. radish HD, radish LD, black oat, sudangrass and the bare fallow control, whereas for the different CC biomass data, C/N and LAI data, the bare fallow control was not considered.

All data are presented as mean values, with standard errors (SE) in parenthesis.

3 Results

3.1 Weather

The course of air temperature and precipitation during the trial is shown with monthly means in Table 1 and daily precipitation in Fig. 1. Mean temperatures in September and October 2018 were above the longtime average, with 17 °C and 13 °C, respectively. Whereas precipitation was very low from mid-September until mid-November 2018. In May 2019 during soybean germination precipitation was very high and temperatures rather low. During the main flowering stage of soybean from mid-June until the beginning of July, there was very low precipitation and high temperatures. Thereafter precipitation remained modest with high temperatures until September.

3.2 Soil parameters

3.2.1 Soil moisture

Irrigation resulted in increased soil moisture especially in plots where CCs were growing as can be seen in Fig. 1. After irrigation stopped at the end of September 2018 soil moisture also dropped at irrigated plots but did not reach values below 10 %.

Averaged soil moisture did not yield significant differences between CC treatments during the period from August until October 2018 (Tukey; $P > 0.1$) (Table 2). Nevertheless, single sampling dates resulted in significant differences (Tukey; $P < 0.05$). Thus, at six dates, sudangrass had lower soil moisture than bare fallow at the rainfed site (24 August, 21 September, 5, 18, 25 and 31 October). On 18 and 31 October also black oat plots were dryer than bare fallow at the rainfed trial. Whereas at the irrigated trial on 21 and 27 September all CCs plots showed higher soil moisture than bare fallow and the radishes even on 7 and 13 September. After irrigation stopped the picture changed and soon became similar to the rainfed conditions. Now sudangrass was dryer than bare fallow (25 and 31 October) and black oat dryer than radish HD plots (18 and 31 October).

Water depletion by CCs in deeper soil layers was pronounced and remained even after the onset of rain as can be seen in Fig. A1. At a depth of 0-30 cm the soil of the rainfed plots of sudangrass (Tukey; $P = 0.0001$), black oat (Tukey; $P < 0.0001$) and

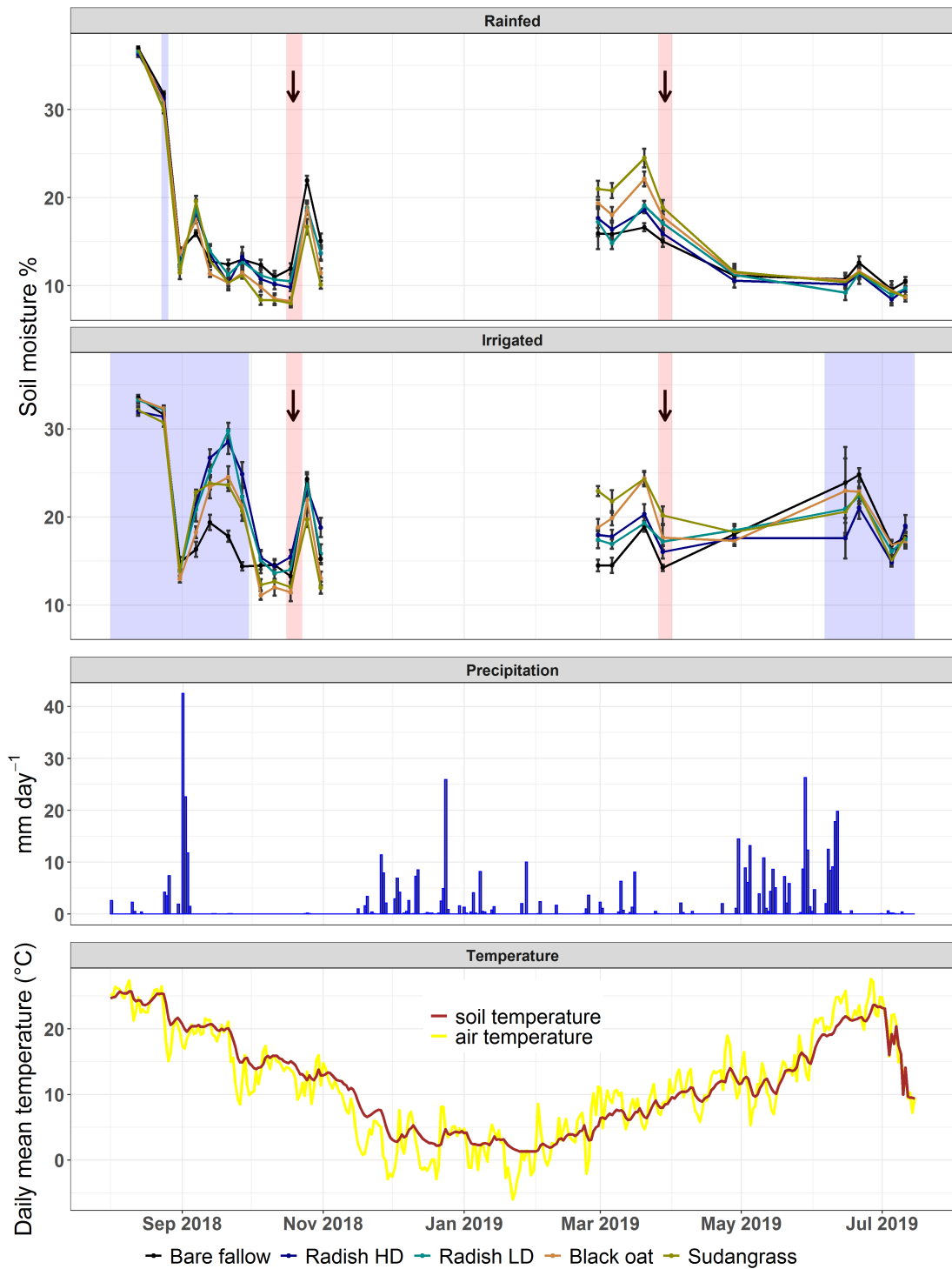


Figure 1: Soil moisture, precipitation and soil temperature. Upper panels show regular soil moisture (vol. %) measurements of the topsoil in two equivalent trials (Rainfed and Irrigated), displayed are means ($n=4$) with standard errors. Cover crop treatments were: sudangrass, black oat, forage radish with high and low sowing density (Radish HD and Radish LD) and bare fallow control. The blue background color indicates periods of irrigation. Arrows indicate periods of earthworm sampling. Within the lower two panels daily precipitation (mm), soil temperature (°C) and air temperature (°C) measured by a nearby weather station are shown.

Table 2: Soil moisture of the topsoil. Given for three sampling periods (averaged over 12, 4 and 4 sampling dates, respectively). Data came from two equivalent trials with different irrigation regimes (Rainfed and Irrigated). Cover crop treatments were: sudangrass, black oat, forage radish with high and low sowing density (Radish HD and Radish LD). Displayed are means (n=4) with standard errors (SE). Values for each period that do not share a common letter within a column are significantly different by pairwise comparisons (Tukey; $P < 0.05$).

Treatment	Soil moisture (vol. %)					
	Rainfed			Irrigated		
	Mean	SE		Mean	SE	
August - October						
Bare fallow	17.4	0.3	a	19.1	0.15	a
Radish HD	16.6	0.26	a	22.2	0.71	a
Radish LD	16.8	0.38	a	21.6	0.13	a
Black oat	15.7	0.1	a	19.6	0.51	a
Sudan grass	15.3	0.31	a	19.7	0.12	a
February - March						
Bare fallow	15.9	0.83	a	15.5	0.64	a
Radish HD	17.1	0.45	ab	18.1	0.29	bc
Radish LD	16.9	0.58	a	17.7	0.49	b
Black oat	19.3	0.44	bc	20.2	0.88	cd
Sudan grass	21.3	0.68	c	22.3	0.61	d
June - July						
Bare fallow	10.9	0.59	b	20.8	2	a
Radish HD	9.9	0.63	a	18.1	0.87	a
Radish LD	9.8	0.43	a	19.2	1.31	a
Black oat	10.1	0.4	ab	20	1.59	a
Sudan grass	10	0.32	ab	19	0.61	a

radish HD (Tukey; $P = 0.029$) was significantly dryer than bare fallow. Especially at the medium depth of 30-60 cm, differences were pronounced. At the rainfed site, the soil of black oat and sudangrass had the lowest water contents, while radishes ranged between the grasses and bare fallow. At the irrigated trial soil moisture at the black oat plots had rather similar values to the radish plots, while under sudangrass soil was the driest. Interestingly, moisture decreased only a little with depth at the irrigated trial under bare fallow, resulting in much higher water contents at a depth of 60-90 cm compared to CCs, indicating higher infiltration of irrigation water into deeper soil horizons.

During the winter months, the trend of soil moisture changed. Now sudangrass plots showed significantly higher soil moisture than radishes and bare fallow (Tukey; $P < 0.0001$). After sudangrass, black oat and radish HD ranged second and third in soil moisture, respectively (Table 2). In deeper soil layers, water storage has recharged from December 2018 to April 2019 and differences between treatments have decreased.

At the rainfed trial, during June and July 2019 bare fallow plots were significantly wetter than the radish plots by 1 % (Tukey; $P < 0.05$). Sudangrass and black oat plots had intermediate values. At the irrigated trial no differences were detected.

3.2.2 Soil temperature

Averaged measured soil temperatures are shown in Table 3. From August until October 2018 irrigation led to reduced temperatures by approximately 2 °C. Mean soil temperatures ranged from 17.5 °C at irrigated radish HD plots to 20.4 °C at rainfed bare fallow plots. The bare fallow plots were significantly hotter than all the CC plots (Tukey; $P < 0.05$). During the second measurement period in spring, temperatures hovered around 10 °C. Bare fallow plots were again hottest, followed by radish LD, radish HD and black oat were intermediate, while sudangrass showed the lowest soil temperatures. During June and July soil temperatures were ranged between 27.1 °C until 31.3 °C. Again, the irrigated plots were 2-3 °C cooler than the rainfed plots. During this period no significant differences between CC treatments were discovered (Tukey; $P > 0.2$).

3.2.3 Tea Bag Index

Tea bag index was measured to evaluate possible effects of CCs on litter decomposition and dependencies with sclerotia decomposition. The results of the tea bag index are shown in Fig. 2. The first burial period from August until the end of October 2018 revealed small values for the constant decomposition rate k within the rainfed plots. For irrigated plots, the picture is more diverse. Here the k value of sudangrass was similar to the rainfed plots with 0.01, while the value for bare fallow plots was three times as high with a k value of 0.03. The value for radish HD was about twice as high with a value of 0.02. In between the values of sudangrass and radish HD were the values for black oat and radish LD. During the first burial, the S factor of all treatments was very small (low decomposition of the labile fraction), only irrigated radishes had slightly higher values. The burial from mid-December 2018 until the middle of March 2019 gave quite different results. Now the irrigated bare fallow plots had the lowest k values, but generally, the results for the k factor were quite similar across all treatments. Results for the S

Table 3: Soil temperature of the topsoil (°C). Given for three sampling periods (averaged over 12, 4 and 4 sampling dates, respectively). Data are derived from two equivalent trials with different irrigation regimes (Rainfed and Irrigated). Cover crop treatments were: sudangrass, black oat, forage radish with high and low sowing density (Radish HD and Radish LD). Displayed are means (n=4) with standard errors (SE). Values for each period that do not share a common letter within a column are significantly different by pairwise comparisons (Tukey; $P < 0.05$).

Treatment	Soil temperature (°C)					
	Rainfed			Irrigated		
	Mean	SE		Mean	SE	
August - October 2018						
Bare fallow	20.4	0.08	b	19.1	0.15	b
Radish HD	19.2	0.09	a	17.5	0.15	a
Radish LD	19.6	0.37	a	17.9	0.28	a
Black oat	19.8	0.04	a	18.2	0.29	a
Sudangrass	19.3	0.16	a	17.6	0.08	a
February - April 2019						
Bare fallow	10.1	0.27	c	11.4	0.15	c
Radish HD	9.5	0.39	ab	10.7	0.3	ab
Radish LD	10	0.27	bc	11.1	0.32	bc
Black oat	9.5	0.42	ab	10.4	0.43	ab
Sudangrass	9	0.34	a	9.9	0.26	a
June - July 2019						
Bare fallow	31.3	0.13	a	27.5	0.25	a
Radish HD	30.7	0.53	a	27.8	0.4	a
Radish LD	31.3	0.41	a	27.7	0.43	a
Black oat	30.7	0.55	a	27.1	0.41	a
Sudangrass	30.5	0.31	a	27.8	0.23	a

factor were roughly three times as high as for the previous period. Rainfed sudangrass and irrigated radish HD had somewhat lower values but with a high standard error. The third period from mid-January until the middle of April 2019 showed similar values to the previous period. In this period rainfed sudangrass and irrigated radish HD had higher S values with smaller standard errors that were close to the values of other treatments. The last burial period from the beginning of May until August 2019 showed a large variation within the k factor, but a low variation for the S factor, which varied around 0.35. Rainfed sudangrass had by far the lowest k factor with 0.01. Some of the other treatments had quite big standard deviations but were spread around a k factor of 0.025. A small, but significant correlation was found between the k factor and soil $\text{NO}_3 - \text{N}$ of the upper soil layer (Pearson's $r=0.3$, $P < 0.001$).

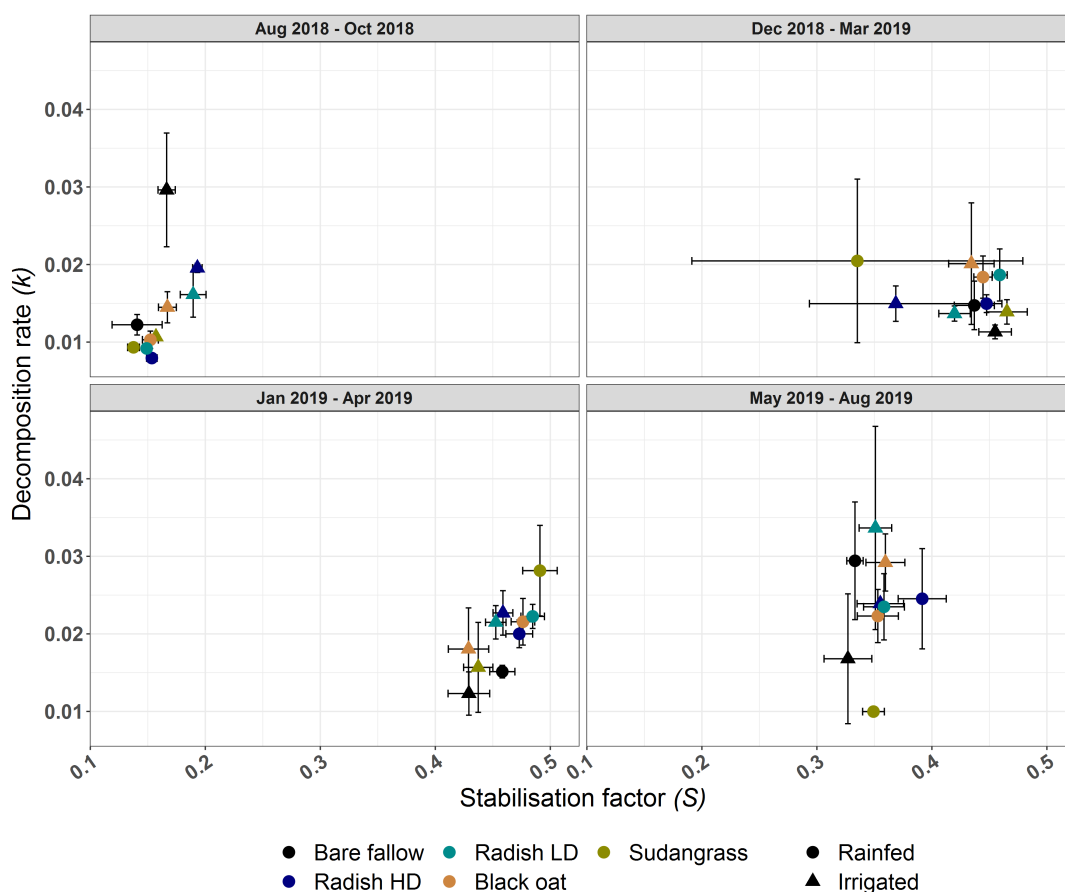


Figure 2: Tea Bag Index with decomposition rate k and stabilization factor (S). Displayed are measurements during four periods, in two trials with different irrigation regimes (Rainfed = dots and Irrigated = triangles). Cover crop treatments were: sudangrass, black oat, forage radish with high and low sowing density (Radish HD and Radish LD) and bare fallow control. Means ($n=4$) are displayed with standard errors.

3.2.4 Penetration resistance

Penetration resistance was measured in order to evaluate possible soil compacting or decompacting effects of CCs (Fig. 3). Statistical analysis yielded no significant differences between CC treatments (Tukey; $P > 0.05$). During fall penetration resistance was very high and was only measurable up to a depth of approximately 7-10 cm. Conversely

during spring 2019 soil was much softer and measurable up to more than 30 cm. In October 2018 at the rainfed trial bare fallow tended to have the lowest penetration resistance, while at the irrigated trial black oat and sudangrass tended to have the highest penetration resistance.

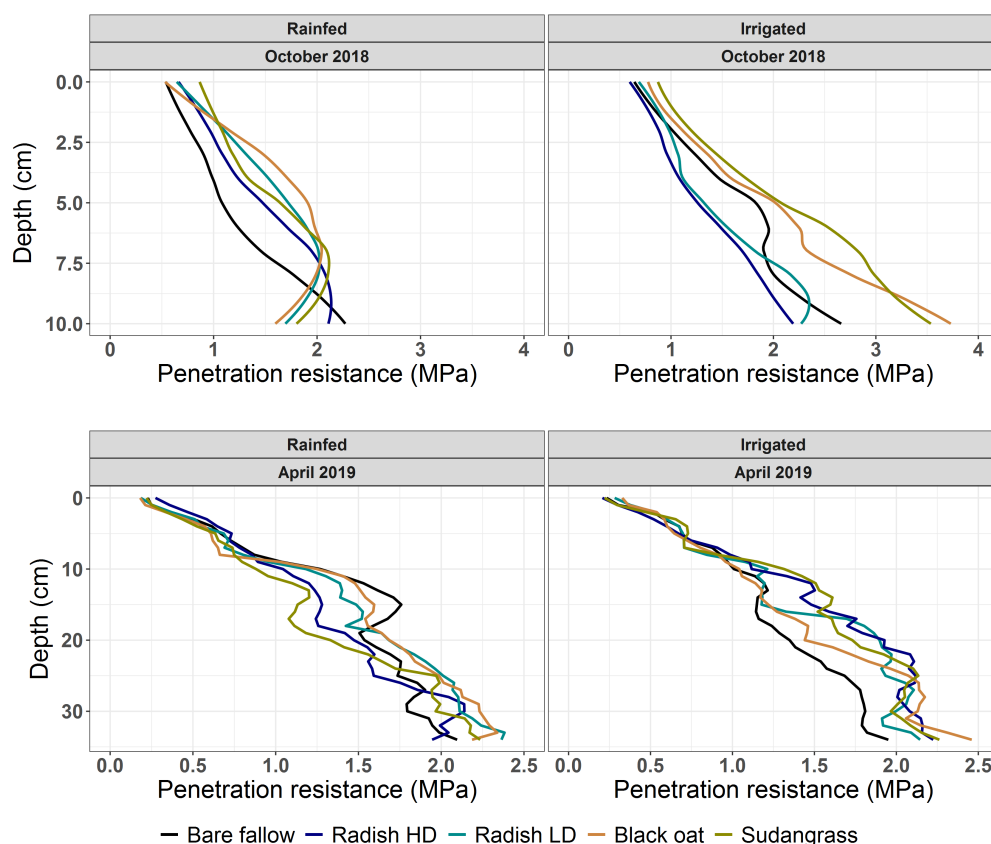


Figure 3: Penetration resistance Sampled in two trials with different irrigation regimes (Rainfed and Irrigated), on two sampling dates. Treatments were: sudangrass, black oat, forage radish with high (Radish HD) and low sowing density (Radish LD) and a bare fallow control. Displayed are means ($n=4$). No significant differences (Tukey; $P > 0.05$) were found between treatments by pairwise comparisons of 5 cm depth intervals.

3.2.5 Soil nitrate

Nitrate in the soil was measured because of its interactions with decomposition processes and CC plant growth. $\text{NO}_3\text{--N}$ in the topsoil decreased sharply from July until December 2018 (Fig. 4). This decrease was less pronounced at the rainfed bare fallow plots. Nonetheless, no significant differences were found among CC treatments throughout the trial for the soil depth until 30 cm (Tukey; $P > 0.05$). At the start of the trial $\text{NO}_3\text{--N}$ content at intermediate soil depth (30-60 cm) was roughly 60 % less than within the topsoil. At rainfed bare fallow plots, $\text{NO}_3\text{--N}$ increased at intermediate depth until December, while at lower depth (60-90 cm) $\text{NO}_3\text{--N}$ content had increased only by April 2019. Nitrate levels of bare fallow were significantly higher than the radishes in 30-60 cm depth and lower than radish HD in 60-90 cm depth in December (Tukey; $P < 0.05$). In April $\text{NO}_3\text{--N}$ in 60-90 cm depth was significantly lower than radish LD and sudangrass (Tukey; $P < 0.019$). Rainfed black oat showed slightly higher $\text{NO}_3\text{--N}$ levels than the other CCs at

mid and lower depths during sampling in December 2018 and April 2019. Black oat plots showed significantly lower $\text{NO}_3 - \text{N}$ values compared to radish LD at the depth 30-60 cm (Tukey; $P = 0.004$) and compared to radish HD at the depth 60-90 cm during December 2018 (Tukey; $P = 0.004$). At the irrigated trial all treatments had lost $\text{NO}_3 - \text{N}$ at all depths from July until December 2018. In December 2018, at intermediate depth, sudangrass had significantly less $\text{NO}_3 - \text{N}$ than bare fallow (Tukey; $P = 0.0037$). At the rainfed trial during September 2019 several values for the lower depth were missing due to very dry conditions.

Soil NO₃-N at three soil depths

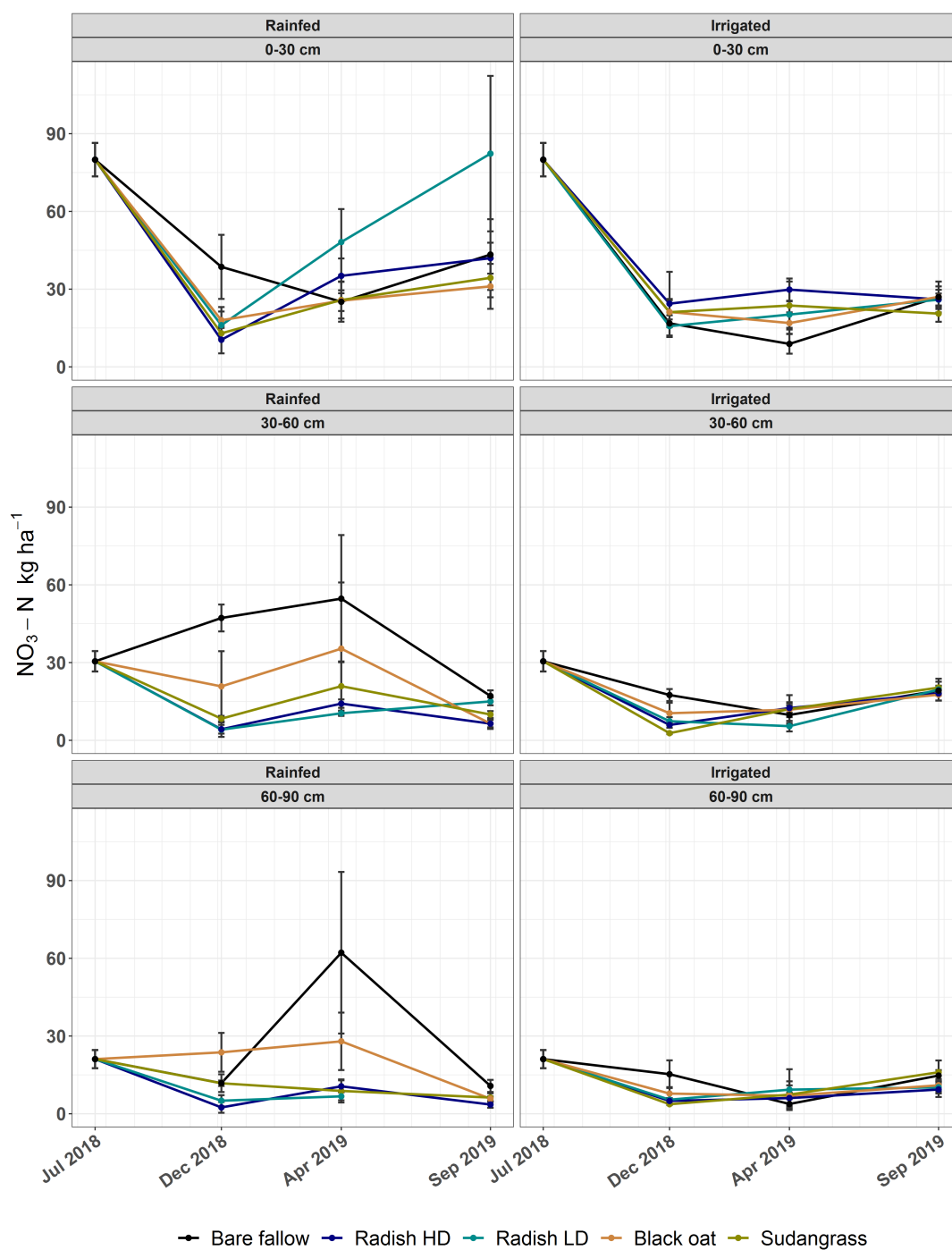


Figure 4: Soil nitrate (NO₃ – N kg ha⁻¹). Sampled with gouge auger (30 mm diameter) in two trials with different irrigation regimes (Rainfed and Irrigated), three separated soil depths and on three sampling dates. Treatments were: sudangrass, black oat, forage radish with high (Radish HD) and low sowing density (Radish LD) and a bare fallow control. Displayed are means (n=4) with standard errors.

3.3 Cover crops

3.3.1 Aboveground biomass

Aboveground biomass of CCs showed pronounced differences (Fig. 5). At the beginning of August, sudangrass and radish HD had significantly higher biomass than black oat and radish LD (Tukey; $P < 0.002$). Biomass peaked for all CCs in November, since none of them, are winter hardy. Sudangrass reached the significantly (Tukey; $P < 0.001$) highest aboveground biomass with $912 (\pm 38)$ g DM m^{-2} at the rainfed trial and $954 (\pm 39)$ g DM m^{-2} at the irrigated trial. The second highest biomass was reached by black oat with $375 (\pm 59)$ g DM m^{-2} at the rainfed trial and $484 (\pm 37)$ g DM m^{-2} at the irrigated trial. High sowing density of radish resulted in faster biomass buildup during the initial stage, but at the beginning of November, both radish variations reached similar values. Here radish HD averaged $195.4 (\pm 5)$ g DM m^{-2} and $294.9 (19 \pm)$ g DM m^{-2} for the rainfed and irrigated trial, respectively. Whereas radish LD aboveground biomass yielded $174 (\pm 43)$ g DM m^{-2} and $294.6 (59 \pm)$ g DM m^{-2} for the rainfed and irrigated trial, respectively. Hence CCs profited from irrigation in terms of biomass buildup, especially the radishes, while sudangrass had rather similarly high values at both sites. In April 2019 aboveground biomass of radishes was already in strong decay. Due to missing data, biomass for radishes was not evaluated for this date. In contrast, plenty of dead biomass remained from the graminaceous CCs, much of it in a still upright position.

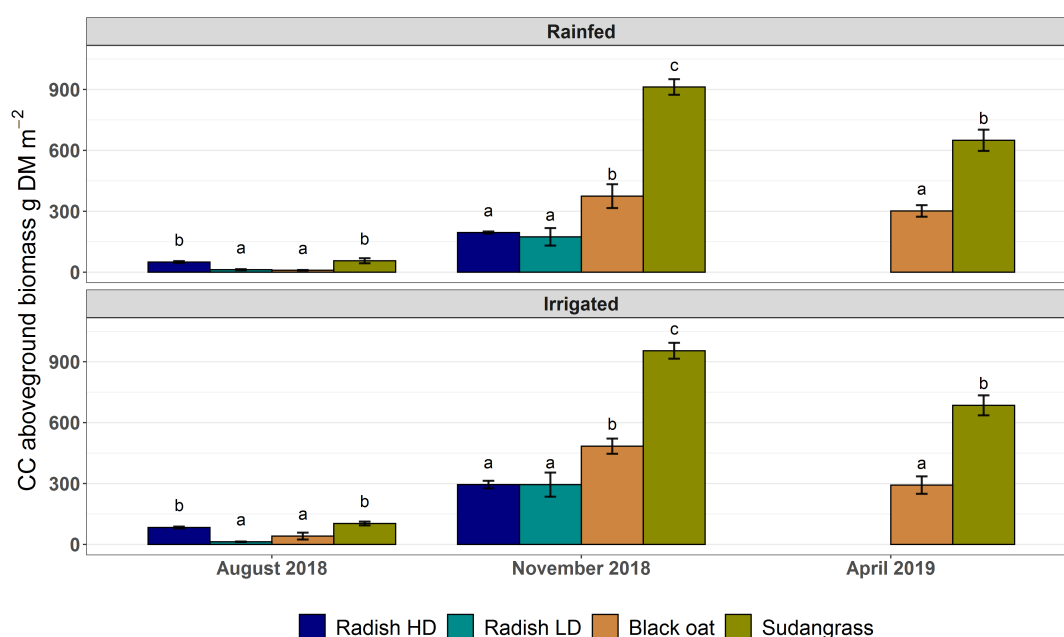


Figure 5: Cover crop aboveground biomass of four cover crops recorded on 1 August 2018, 6 November 2018 and 1 April 2019 in two trials with different irrigation regimes (Rainfed and Irrigated). Treatments were: sudangrass, black oat, forage radish with high (Radish HD) and low sowing density (Radish LD). Bars represent means ($n=4$), error bars display standard errors. Significant differences were calculated separately per irrigation regime and date, cover crops that do not share a common letter are significantly different by pairwise comparisons (Tukey; $P < 0.05$).

C/N ratios of the shoots were smaller for the radish CCs than for the grass CCs (Fig. 6). With progressing maturity C/N of sudangrass increased, reaching higher C/N values than black oat in April 2019.

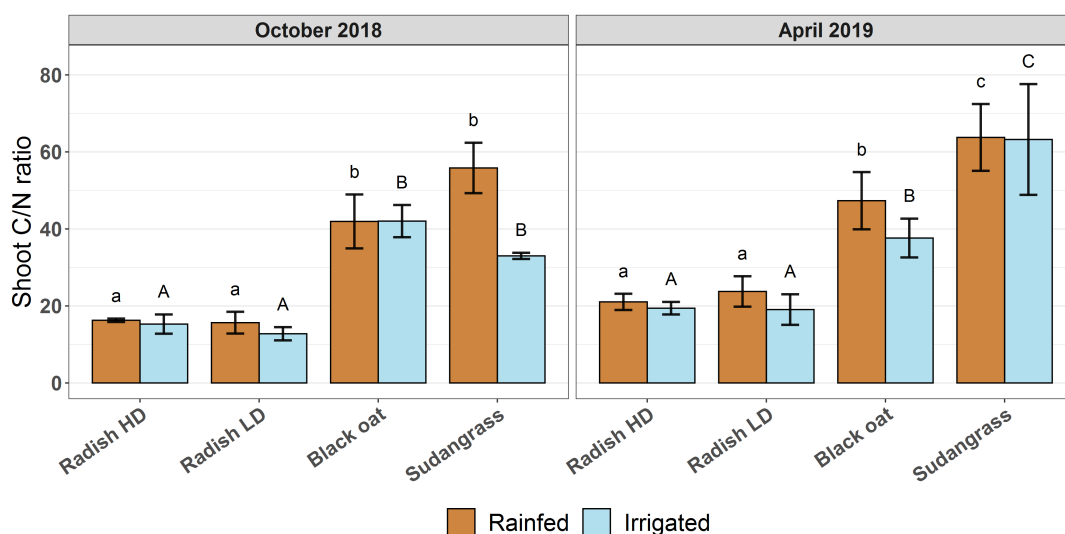


Figure 6: C/N ratio of cover crop shoots. The C/N ratios of aboveground plant tissue from four different cover crop treatments are shown: sudangrass, black oat, forage radish with high and low sowing density (Radish HD and Radish LD). Results stem from two equivalent trials with different irrigation regimes (Rainfed and Irrigated). Bars represent means (n=4), error bars the corresponding standard errors. Bars that do not share a common letter (upper and lower case separately) are significantly different by pairwise comparisons (Tukey; $P < 0.05$).

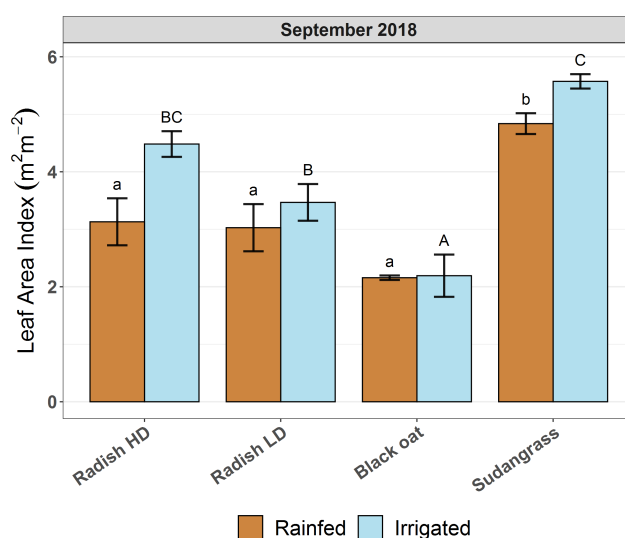


Figure 7: Leaf area index ($\text{m}^2 \text{m}^{-2}$) of four cover crops recorded on 18 September 2018 in two trials with different irrigation regimes (Rainfed and Irrigated). Treatments were: sudangrass, black oat, forage radish with high (Radish HD) and low sowing density (Radish LD). Bars represent means (n=4), error bars display standard errors. Bars that do not share a common letter (upper and lower case separately) are significantly different by pairwise comparisons (Tukey; $P < 0.05$).

Table 4: N uptake in CC aboveground biomass in November 2018. Mean values (n=4) are derived from two equal trials with different irrigation regimes (Rainfed and Irrigated). Cover crop treatments were: sudangrass, black oat, forage radish with high and low sowing density (Radish HD and Radish LD). Values within each column that do not have a letter in common are significantly different by pairwise comparisons (Tukey; $P < 0.05$).

Treatment	N uptake in CC aboveground biomass kg ha ⁻¹					
	Rainfed			Irrigated		
	Mean	SE		Mean	SE	
Radish HD	47.4	1.2	ab	79.7	14.2	ab
Radish LD	44.6	10.1	ab	100.4	28.1	ab
Black oat	47.7	12	a	54.2	3.5	a
Sudangrass	79.3	10.5	b	136.7	5.7	b

N uptake within aboveground biomass was greatest for sudangrass with 79 kg N ha⁻¹ (± 10.5) and 137 kg N ha⁻¹ (± 5.7) at the rainfed and irrigated site, respectively. Black oat took up around 50 kg N ha⁻¹ at both sites, which was significantly less than sudangrass (Tukey; $P = 0.007$) (Table 4).

The LAI in September 2018 was highest for sudangrass and lowest for black oat (Fig. 7). Values were slightly higher at the irrigated than at the rainfed trial, the greatest difference between trials was recorded for radish HD.

3.3.2 Belowground biomass

Results of belowground biomass are shown in Table 5. While sudangrass had the highest amount of regular root biomass - between two and sixfold in April 2019 - the radishes resulted in the highest belowground biomass when taproots and fine roots are considered together. Single taproots of radish LD were significantly heavier than taproots of radish LD (Tukey; $P < 0.0001$). Nevertheless, taproot weight per square meter was not significantly different and even slightly higher for radish HD due to the higher plant density (Tukey; $P > 0.48$).

3.4 Earthworms

Only endogeic earthworms were found during both sampling periods. Earthworm abundance ranged from 50 (± 9) individuals m⁻² under black oat at the rainfed trial to 142 (± 13) individuals m⁻² under irrigated radish LD. Both values were recorded during the measurements in October 2018 (Fig. 8). Earthworm mass varied between 9.7 (± 2.8) g m⁻² under black oat at the rainfed trial in October and 46.8 (± 5.1) g m⁻² under irrigated radish HD in April (Fig. 9). Generally, there was a much higher variation in earthworm mass and abundance during the sampling period in October 2018 than in April 2019. An ANOVA table from the statistical analysis of earthworm abundance and mass is shown in Table 6. At the rainfed trial in October, the bare fallow plots showed the highest earthworm mass and abundance. Differences in abundance were significant compared to black oat and sudangrass (Tukey; $P < 0.0005$). Earthworm mass at the bare fallow plots was significantly higher than at radish LD and black oat (Tukey; $P <$

Table 5: Cover crop belowground biomass. Mean values (n=4) of radish taproot DM are given for November 2018, while root biomass DM is given for November 2018 and April 2019. Data are derived from two equal trials with different irrigation regimes (Rainfed and Irrigated). Cover crop treatments were: sudangrass, black oat, forage radish with high and low sowing density (Radish HD and Radish LD). Values within each column and period that do not have a letter in common are significantly different by pairwise comparisons (Tukey; $P < 0.05$).

Treatment	Rainfed		Irrigated			
	Mean	SE	Mean	SE		
Root biomass g m^{-2} November 18						
Radish HD	12.3	4.5	a	25.9	6.4	a
Radish LD	24.1	17.0	a	19.8	4.1	a
Black oat	16.4	7.3	a	30.0	8.4	a
Sudangrass	30.1	12.7	a	48.3	10.4	a
Root biomass g m^{-2} April 19						
Radish HD	46	15.4	a	20	3.2	a
Radish LD	61	26.1	a	29	17.1	a
Black oat	21	5.6	a	19	3.6	a
Sudangrass	126	38.0	b	112	28.6	b
Radish taproot g taproot^{-1} November 18						
Radish HD	1.6	0.4	a	2.3	0.3	a
Radish LD	6.6	0.9	b	10.1	1.6	b
Radish taproot g m^{-2} November 18						
Radish HD	205	32.3	a	374	54.6	a
Radish LD	250	54.2	a	397	59.4	a

Table 6: ANOVA table for earthworm populations with the Type II Wald χ^2 test results for the effects irrigation (I) with the levels rainfed and irrigated, cover crops (CC) with the levels sudangrass, black oat, forage radish with high and low sowing density and bare fallow control and date (D) with two levels (fall, spring) on the response variables earthworm abundance and mass. With 58 residual degrees of freedom.

Earthworm abundance				Earthworm mass			
Treatment	Df	χ^2	$P(> \chi^2)$	Treatment	Df	χ^2	$P(> \chi^2)$
I	1	2.4	0.122	I	1	7.1	0.008
D	1	6.3	0.012	D	1	24.3	< 0.001
CC	4	13.6	0.009	CC	4	9.3	0.053
I x D	1	3.5	0.062	I x CC	1	2.1	0.149
I x CC	4	7.4	0.115	I x D	4	5.0	0.284
D x CC	4	17.9	0.001	D x CC	4	9.6	0.047
I x D x CC	4	12.8	0.012	I x D x CC	4	10.6	0.031

0.049). The irrigated trial presented a different picture with radish LD showing the highest and sudangrass the lowest numbers for both mass and abundance. Nevertheless, differences were not big enough to be significant (Tukey; $P > 0.07$). While earthworm abundance generally decreased from October until April the earthworm mass increased in most cases. The earthworm abundance at the rainfed bare fallow plots decreased significantly (Tukey; $P = 0.0118$) from 130 (± 35) to 65 (± 9) individuals m^{-2} . In the irrigated experiment, however, there was a significant decrease in earthworm abundance in the radish LD plots (Tukey; $P = 0.0123$). Conversely, earthworm mass increased in all CCs especially within the radish HD plots in the irrigated experiment, but not significantly (Tukey; $P > 0.06$). Whereas earthworm mass at bare fallow plots decreased slightly from October until April. At the irrigated trial there were small increases in earthworm mass for all CCs including bare fallow. Only radish LD had similar values with about 40 g m^{-2} earthworm mass in October and April. During the sampling period in April, there were no significant differences between treatments neither for earthworm mass nor abundance (Tukey; $P > 0.4$). Within the irrigated trial, radish HD showed the highest numbers for mass and abundance. Sudangrass also showed high numbers, even higher in the rainfed trial than in the irrigated trial.

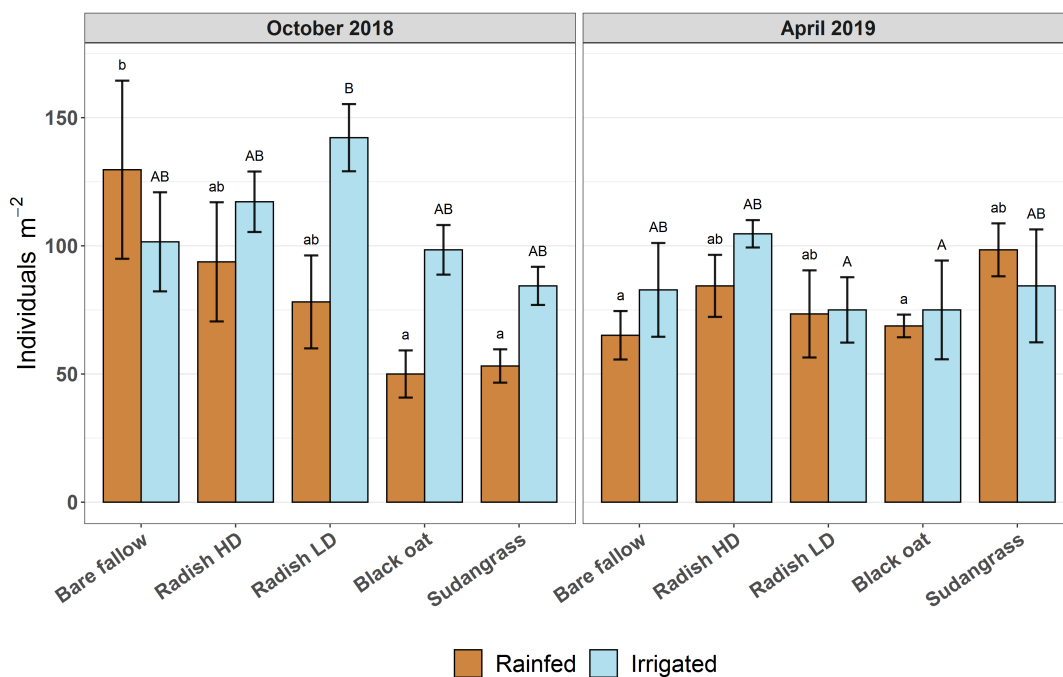


Figure 8: Earthworm abundance. Individuals m^{-2} were recorded during mid-October 2018 and during the end of March until the beginning of April 2019 in two trials with different irrigation regimes (Rainfed and Irrigated). Treatments were: sudangrass, black oat, forage radish with high and low sowing density (Radish HD and Radish LD) and bare fallow control. Bars represent means ($n=4$), error bars the corresponding standard errors. Bars that do not share a common letter (upper and lower case separately) are significantly different by pairwise comparisons (Tukey; $P < 0.05$).

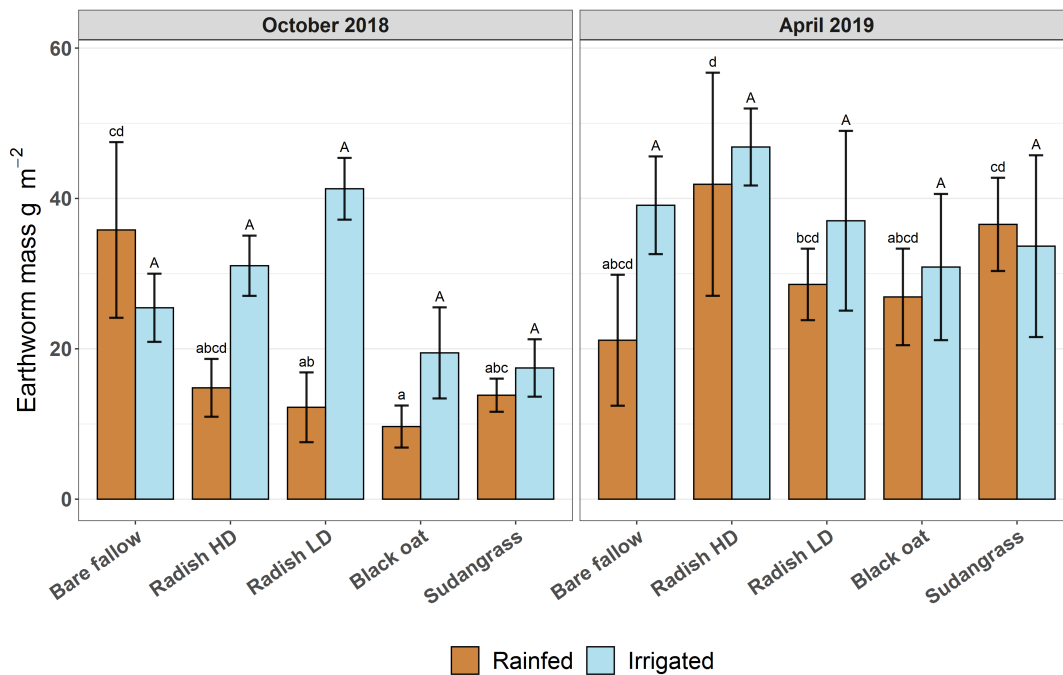


Figure 9: Earthworm mass was recorded during mid-October 2018 and during the end of March until the beginning of April 2019 in two trials with different irrigation regimes (Rainfed and Irrigated). Treatments were: sudangrass, black oat, forage radish with high and low sowing density (Radish HD and Radish LD) and bare fallow control. Bars represent means ($n=4$), error bars the corresponding standard errors. Bars that do not share a common letter (upper and lower case separately) are significantly different by pairwise comparisons (Tukey; $P < 0.05$).

3.4.1 Correlations: earthworms, cover crops, and soil properties.

A correlation matrix of the earthworm data with different variables is shown in Fig. 10. During sampling of October 2018 (fall), there was a pronounced correlation between earthworm abundance and mass with soil moisture (Pearson's $r=0.6$, $P < 0.001$), while this was not the case in spring 2019. In fall 2018, C/N was negatively correlated with earthworm mass (Pearson's $r=-0.4$) and abundance (Pearson's $r=-0.5$). However, C/N was even more strongly correlated with soil moisture (Pearson's $r=-0.6$) and CC biomass (Pearson's $r=0.6$). Furthermore, soil moisture was correlated positively with CC biomass; in October 2018 (Pearson's $r=0.6$, $P = 0.006$) and in spring 2019 slightly negatively (Pearson's $r=-0.4$, $P < 0.001$). In spring soil moisture was also correlated with C/N (Pearson's $r=0.5$, $P < 0.001$).

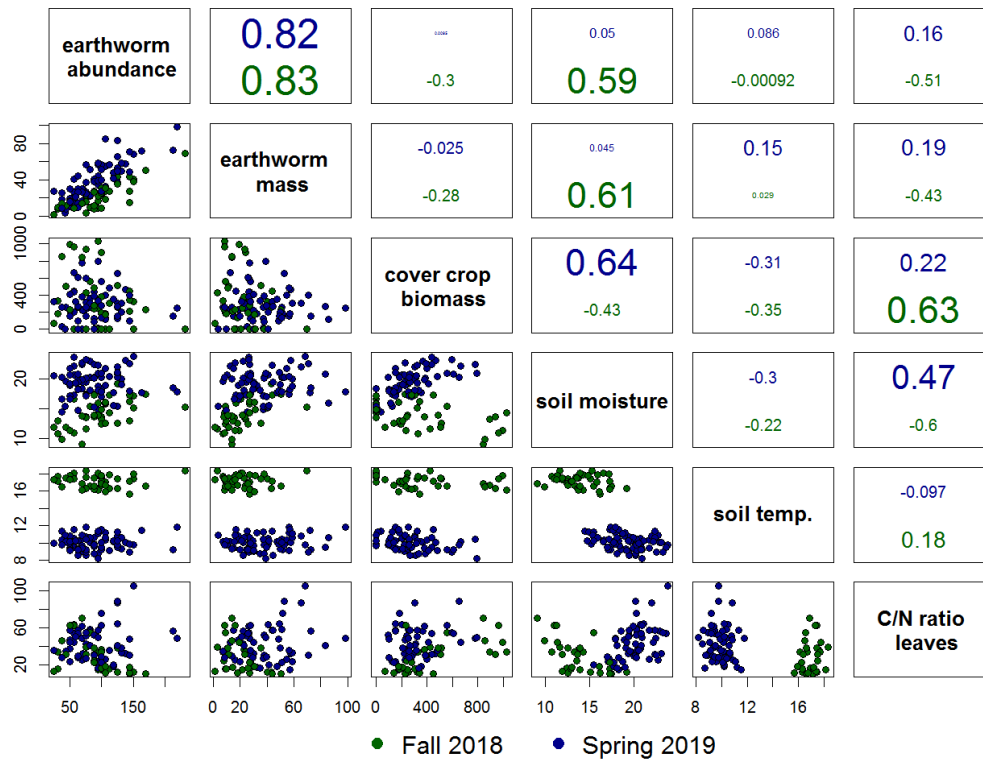


Figure 10: Correlation matrix of earthworm data. Scatter plots in the lower triangle, Pearson's r in the upper triangle, and names of variables on the diagonal: earthworm abundance, earthworm mass, cover crop biomass, soil moisture, soil temperature and C/N ratio of aboveground biomass. Data was collected at a field trial with four cover crop treatments, with and without irrigation. The data for green and blue dots/numbers were sampled during fall 2018 and spring 2019, respectively.

3.5 Sclerotinia

3.5.1 Sclerotia decomposition

Within the data of sclerotia decomposition, there could be seen a pronounced variability (Fig. 11). In some instances, the numbers of remaining sclerotia were higher in a later sampling date than in the preceding one, but not significantly so. Four data points from the sudangrass plots in April 2019 were excluded as it seemed there was some uncontrollable interference with the dense rooting. In the GLMM with all sclerotia samples during cover cropping, there was a significant interaction between cover crop and irrigation (Wald; $\text{Chi}=16.3$, $P = 0.003$), while mesh size had no significant effect (Wald; $\text{Chi}=1.4$, $P = 0.232$) (Fig. 12). When leveled over mesh size, the pairwise comparisons showed significantly lower decomposition rates at irrigated radish HD plots compared to bare fallow and sudangrass (Tukey; $P = 0.001$ and $P=0.04$, respectively). At the rainfed site, radish LD and bare fallow had significantly lower decomposition rates than sudangrass (Tukey; $P<0.024$). Sclerotia decomposition at the irrigated site tended to be slightly lower, except for bare fallow. The decomposition data from the September 2019 sampling yielded no significant differences (Tukey; $P>0.224$).

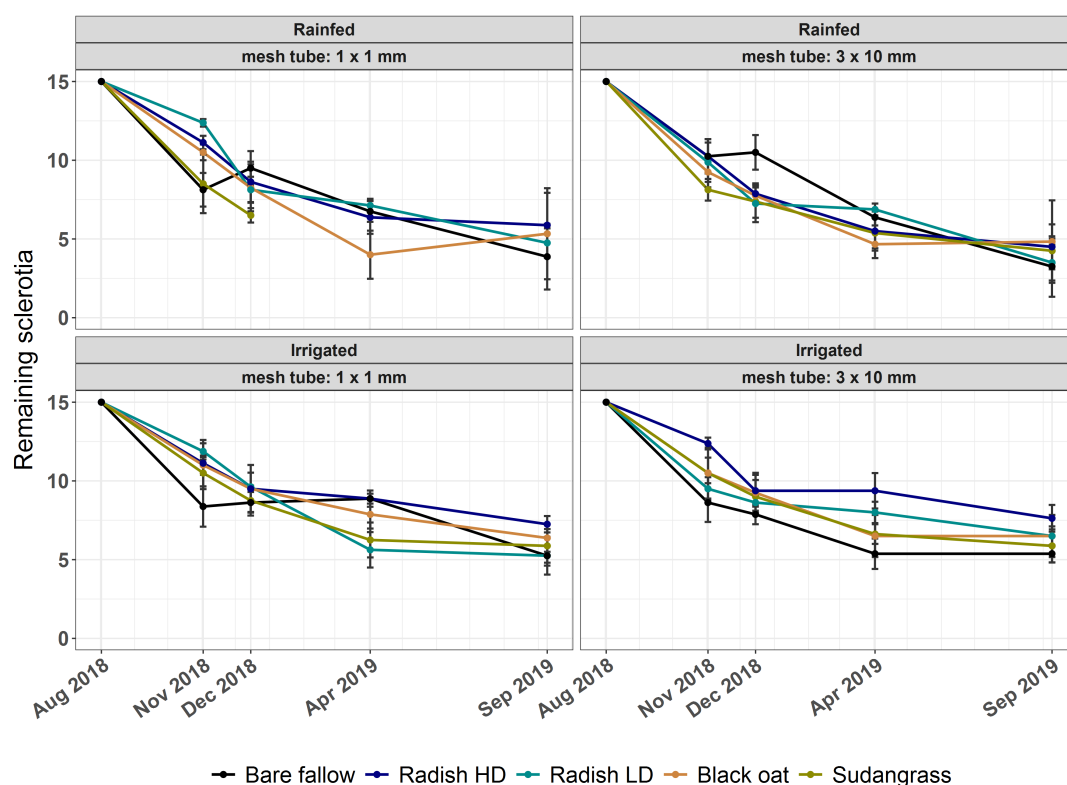


Figure 11: Sclerotia decomposition. The number of recovered intact sclerotia of initially 15 per mesh tube. Four recovery dates in two trials with different irrigation regimes (Rainfed and Irrigated). Treatments were: sudangrass, black oat, forage radish with high and low sowing density (Radish HD and Radish LD) and bare fallow control. Sclerotia were buried within plastic tubes of two different mesh sizes (1x1 mm and 3x10 mm). Means (n=4) are displayed with standard errors.

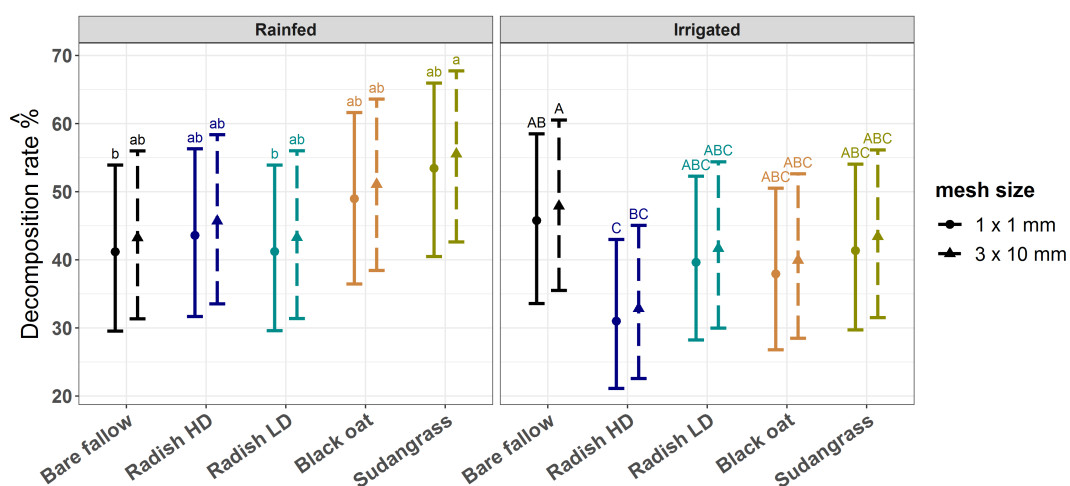


Figure 12: Modelled sclerotia decomposition rate. Means and 95% confidence intervals with Šidák correction estimated with a beta-binomial generalized linear mixed model. Data of recovered sclerotia after 92, 135 and 243 days were combined. Results from two equivalent trials with different irrigation regimes (Rainfed and Irrigated) are displayed. Treatments were: sudangrass, black oat, forage radish with high and low sowing density (Radish HD and Radish LD) and bare fallow control with four replications, respectively. Sclerotia were buried within plastic tubes of two different mesh sizes (1x1 mm and 3x10 mm). Treatments that do not share a common letter (upper and lower case separately) are significantly different by pairwise comparisons (Tukey; P < 0.05).

3.5.2 Sclerotia germination rate

The percentage of recovered sclerotia with stipes (carpogenic germination) relative to the original number of buried sclerotia is shown in Fig. 13 and Table A1. At the rainfed trial, most germinated sclerotia were found at bare fallow plots. In December 2018 and April 2019 these differences were significant (Tukey; $P < 0.03$) except for comparison with radish LD (Tukey; $P > 0.05$). At the irrigated trial sudangrass showed the lowest amount of germinated sclerotia at all sampling dates. Leveled over mesh size these were significantly lower than in plots with radishes in November 2018 (Tukey; $P < 0.005$) and significantly different from all other treatments in December 2018 and April 2019 (Tukey; $P < 0.0001$). A peak of germinated sclerotia could be observed during the sampling in April 2019. In September 2019 differences among treatments were generally small. Overall, the size of mesh tubes had a significant effect on the amount of germinated sclerotia (Wald; $\chi^2=6.3$, $P = 0.012$). Within the tubes of 3×10 mm mesh size there were more germinated sclerotia, while at the same time there was a trend of faster decomposition for this mesh size.

The number of stipes on recovered sclerotia is shown in Fig. 14. These showed a similar pattern to the percentage of germinated sclerotia. Significant differences were discovered during December 2018 and April 2019 (Tukey; $P < 0.05$). At the rainfed trial, bare fallow had significantly more stipes than all CC treatments, while at the irrigated trial sudangrass had significantly fewer stipes than all other treatments.

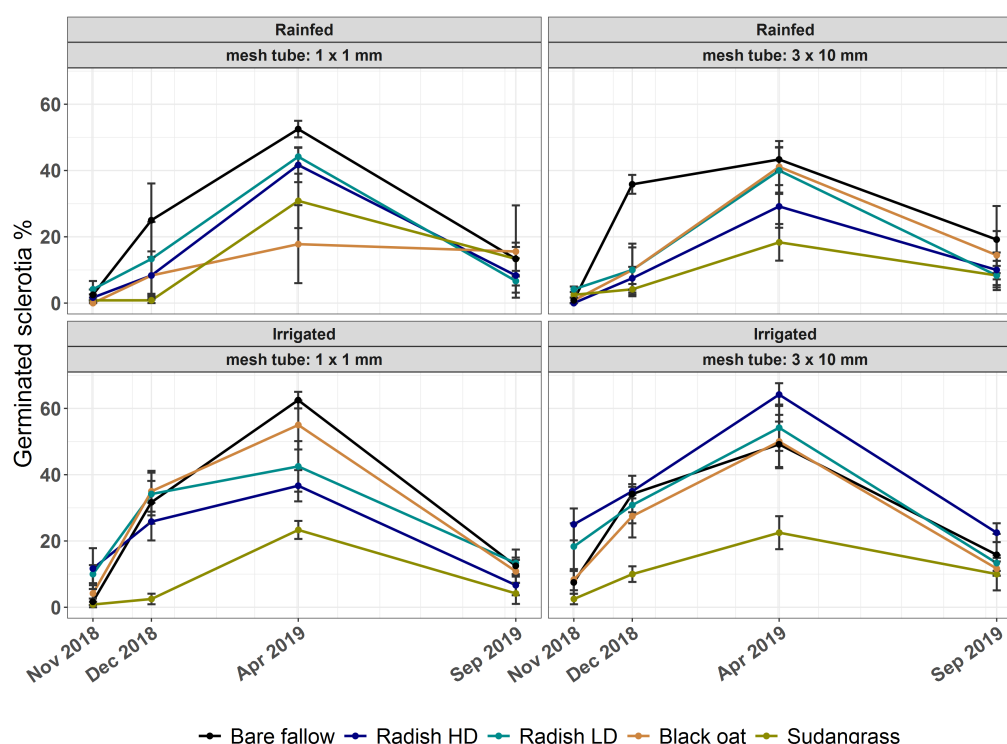


Figure 13: Germinated sclerotia. Percentage of initially buried sclerotia (15 per mesh tube) with one or more stipes. Four recovery dates in two trials with different irrigation regimes (Rainfed and Irrigated). Treatments were: sudangrass, black oat, forage radish with high and low sowing density (Radish HD and Radish LD) and bare fallow control. Sclerotia were buried within plastic tubes of two different mesh sizes (1x1 mm and 3x10 mm). Means ($n=4$) are displayed with standard errors.

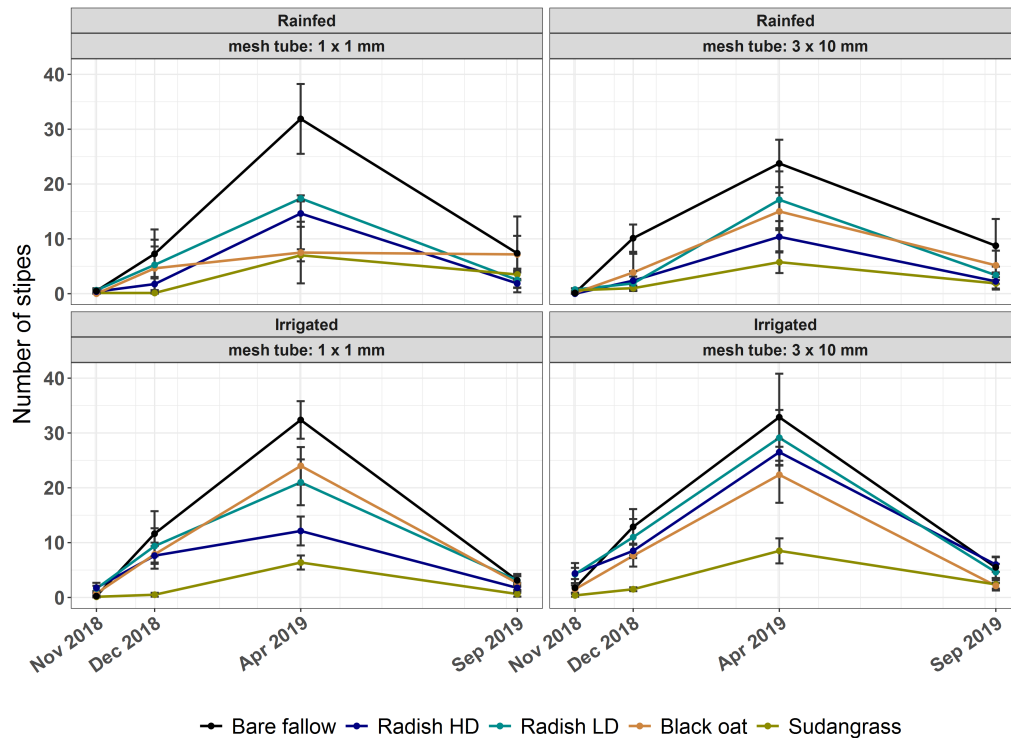


Figure 14: Number of stipes on recovered sclerotia. Four recovery dates in two trials with different irrigation regimes (Rainfed and Irrigated). Cover crop treatments were: sudangrass, black oat, forage radish with high and low sowing density (Radish HD and Radish LD) and bare fallow control. Initially, 15 sclerotia were buried within plastic tubes of two different mesh sizes (1x1 mm and 3x10 mm). Means (n=4) are displayed with standard errors.

3.6 Soybean

3.6.1 Development of the soybean stand

Soybean emergence was slow due to the moist and rather cool temperatures during May 2019. At the end of May 2019, significantly fewer soybean plants had emerged at the sudangrass plots compared to the bare fallow plots (Tukey; $P < 0.021$). Noteworthy is that at the sudangrass plots some of the incorporated residues were still visible. The later biomass assessment of soybean stands, in June, did not yield any significant differences (ANOVA; $F=2.18$, $P = 0.094$). See also Fig. 15. Soybeans were infested with spider mites (*Tetranychus* spp.), especially at the rainfed site.

3.6.2 Soybean infection

During the whole trial, there were no apothecia found emerging from the soil, nor any signs of *S. sclerotiorum* infection on soybean plants.

3.6.3 Soybean grain yield

The soybean yield is shown in Fig. 16. The different CC treatments showed very similar results for grain yield and 1000-seed mass, respectively. Mayor differences occurred only between the rainfed and the irrigated trial. Grain yield averaged for all

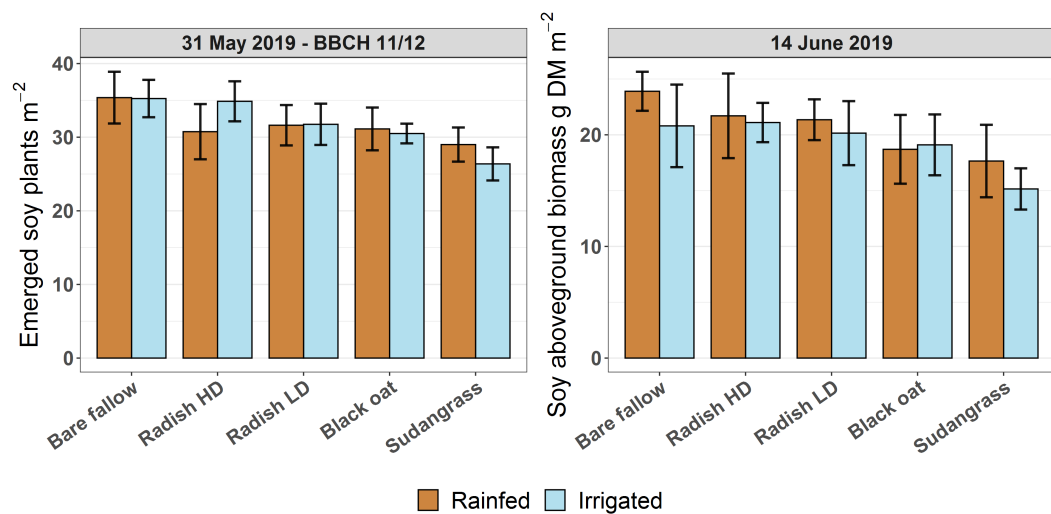


Figure 15: Development of the soybean stand. The left panel shows the emergence of soybean plants assessed at development stage 11 or 12 (BBCH scale) on 31 May 2019. The right panel shows the soybean aboveground biomass on 14 June 2019. Soybean was sown after cover crop treatments: sudangrass, black oat, forage radish with high and low sowing density (Radish HD and Radish LD) and bare fallow control. Results stem from two equivalent trials with different irrigation regimes (Rainfed and Irrigated). Bars represent means ($n=4$) with corresponding standard errors.

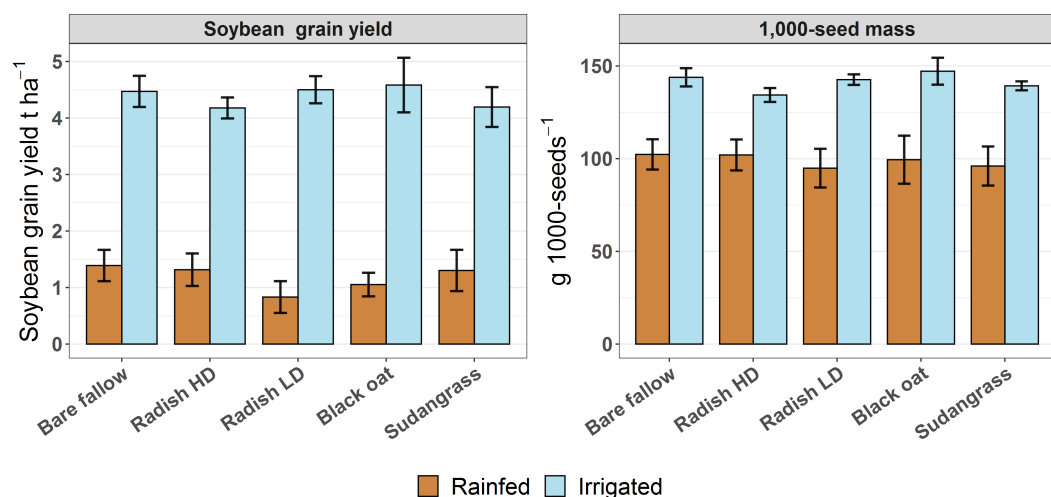


Figure 16: Soybean yield parameters. The left panel shows the soybean grain yield, the right panel shows the 1000-seed mass. Soybean was sown after the following cover crop treatments: sudangrass, black oat, forage radish with high and low sowing density (Radish HD and Radish LD) and bare fallow control. Results stem from two equivalent trials with different irrigation regimes (Rainfed and Irrigated). Bars represent means ($n=4$), error bars the corresponding standard errors. No significant differences were found by pairwise comparisons (Tukey; $P > 0.05$).

CCs 1179 (± 124) kg ha⁻¹ and 4385 (± 134) kg ha⁻¹ at the rainfed and irrigated trial, respectively. Mean 1000-seed mass resulted in 98.9 (± 4.1) g 1000 – seeds⁻¹ and 141.5 (± 2.1) g 1000 – seeds⁻¹ at the rainfed and irrigated trial, respectively.

4 Discussion

This master's thesis analyzes the effect of CCs on earthworm populations and the decomposition of sclerotia in one experiment – two topics that are usually considered separately. Anthropogenic climate change, with rising temperatures and more pronounced extreme weather events, poses serious challenges to agriculture (Mbow et al., 2019). In addition to the threat of climate change, there are many other problems associated with global change, which is why international sustainable management practices have been called for (Smith et al., 2016). Cover cropping is considered a sustainable practice because it reduces the need for chemical nitrogen fertilizers, reduces groundwater pollution, protects soil fertility, mitigates greenhouse gases, and stabilizes yields, among other benefits (Kaye and Quemada, 2017; Abdalla et al., 2019). Thus, the results presented here can help fill in knowledge gaps of this promising agricultural practice. The fall 2018 drought provided insight into the challenges earthworm populations face during drought conditions under CCs.

4.1 Cover crops

Biomass of radish HD was higher than radish LD in August 2018 but equalized until November. Analogous the LAI showed no significant differences between radish HD and radish LD in September 2018. This result can be explained by better utilization of resources, such as increased light interception, at higher plant densities, up to the point where plant growth is limited by inter-plant competition (Loss et al., 1998; D'hooghe et al., 2018). Similarly, while radish HD showed lower weights of single taproots, the mass of taproots per area was not significantly different, thus demonstrating the high plasticity of radishes (D'hooghe et al., 2018).

At the irrigated trial during September 2018, all CCs exceeded the soil moisture levels of the bare fallow control, with radish showing the wettest soil. Similarly, Bodner et al. (2007) observed relevant reductions of evaporation by CCs through ground cover, counteracting their transpirational losses. Whereas at the rainfed trial during the long period of scarce precipitation soil moisture of CC treatments was dropping faster than under bare fallow, with the grass treatments reaching values below 10 %. This can be explained, by transpiration being the dominating cause for soil water depletion, while evaporation at the dry topsoil minimized. The same effect can explain the fast drop in soil moisture under CCs at the irrigated trial after irrigation had stopped (Bodner et al., 2007). The exceptional high biomass production by sudangrass, even at the rainfed site can be attributed to the C₄ photosynthetic pathway resulting in a greater water use efficiency next to the known drought resistance (Kaplan et al., 2019). The adaptation of sudangrass to drought was also underlined by the high root biomass.

Penetration resistance was much higher in October 2018 than in April 2019, due to

differences in soil moisture (Medvedev, 2009). In October 2018, differences between treatments appeared to increase with depth, but no significant differences were found within the limited depth at which measurements could be made under such dry conditions. No significant differences were found in April 2019 either. Thus, there were no significant differences in soil moisture within the topsoil at the dates of measurement (Medvedev, 2009).

During spring the remnants of radish leaves, with their low C/N ratio and therefore fast decomposition, could provide only partial soil cover, nonetheless lead to higher soil moisture compared to bare fallow (Berg and Staaf, 1981; Odhiambo and Bomke, 2007). The grasses according to their biomass could provide more shading and increase dew formation with their lignified, still partially upright stems (Odhiambo and Bomke, 2007; Xiao et al., 2009).

Analysis of soil moisture to a depth of 90 cm in December 2018 and April 2019 revealed that CCs at the rainfed trial had depleted water resources of the lower soil profile during the fall period, but the deeper water reserves could be replenished by April. A similar trend, though less pronounced, could be observed at the irrigated trial. This is in line with Bodner et al. (2015), who stated that under Pannonian climate winter rainfall is normally able to refill water storage.

At the irrigated site at all treatments $\text{NO}_3 - \text{N}$ content of the topsoil was reduced by roughly 60 kg ha^{-1} from August until December 2018. At the same time, CCs had taken up between approximately 50 (black oat) to 140 kg N ha^{-1} (sudangrass) into their above-ground biomass, while N content in root biomass was not calculated. Thus, it can be expected that CCs reduced leaching from the topsoil to a great extent and may have recovered $\text{NO}_3 - \text{N}$ from deeper soil layers, even though leaching was not measured directly (Thorup-Kristensen, 2001; Kaye et al., 2019).

At the rainfed trial, water constraints seem to have limited nitrate transport at bare fallow plots to the medium depth of 30-60 cm until December but proceeded to the depth of 60-90 cm until April 2019, thus indicating leaching loss (Thorup-Kristensen et al., 2003). Black oat had slightly higher $\text{NO}_3 - \text{N}$ levels than the other CCs below a depth of 30 cm, therefore seemed to be less able to scavenge for nitrate at deep soil depths. This is in accordance with the slower development of biomass by black oat, especially in terms of root biomass. Also, Kaye et al. (2019) found higher leaching under an oat CC compared to radish grown after wheat. Thus, black oat compared to radish and sudangrass appears to be less suitable in preventing $\text{NO}_3 - \text{N}$ leaching loss under drought conditions. Radish and sudangrass are CCs that are known for their good potential in preventing leaching (Long, 1981; Thorup-Kristensen et al., 2003). Especially radish taproots are thought to be suited to take up N from deep soil layers (Daryanto et al., 2018). Thus, both CCs can reduce the need for N-fertilizers, mitigate eutrophication and greenhouse gas emissions (Camargo et al., 2013; Daryanto et al., 2018). Furthermore, the deep rooting of radish can also provide C-sequestration in the lower soil profile (Schmidt et al., 2011).

The steep increase of $\text{NO}_3 - \text{N}$ at rainfed radish LD plots from December 2018 until September 2019 can speculatively be attributed to random samples with nitrate hotspots from decaying rests of taproots.

4.2 Earthworms

Measured earthworm abundances were low, especially for rainfed black oat and sudangrass in October 2018 with around 50 individuals m^{-2} , whereas the long-term average of arable fields in temperate climates is given by Johnston (2019) with 123 individuals m^{-2} . Earthworm abundance measured by Euteneuer et al. (2019) at a nearby site, two years before, were three to four times higher with approximately 300 to 360 individuals m^{-2} . Regression analysis showed that CCs affected earthworm populations strongest under rainfed conditions during fall 2018. The significantly lower earthworm abundances under rainfed black oat and sudangrass compared to bare fallow coincided with the lowest soil moisture. Correspondingly, the correlation of soil moisture with earthworm abundance and earthworm mass was significant in October 2018. Hence, the low amount of precipitation starting from mid-September and lasting throughout October 2018 with a consequent drought seems to have been responsible for the high earthworm mortality and the water extraction especially by the Poaceae CCs increased the drought-stress additionally (Curry, 2004; Bodner et al., 2007). In turn at the irrigated trial, no significant differences in earthworm abundance were found between treatments, indicating less water stress through CC transpiration until sampling in October. The elevated soil moisture under radishes indicates improved conditions for earthworms when regular precipitation occurs (Curry, 2004; Bodner et al., 2007). Considerable rainfall only reappeared in the second half of November 2018, when soil temperature was already below 10 °C and soon dropping below 5 °C until the end of February 2019. Given that earthworms lose weight under dry conditions and at low temperatures there was hardly any time for them to recover before winter and most likely further earthworms had died before the next sampling (Eriksen-Hamel and Whalen, 2006). This may also explain the modest numbers in spring 2019 contrasting the results of Euteneuer et al. (2019), who found an increased earthworm abundance under radish after winter. The highly significant effect of date and irrigation from the regression analysis of earthworm mass shows an opposite trend in spring 2019 compared to fall 2018 for the rainfed site, where the increasing earthworm mass in spring under CCs was most pronounced for sudangrass. The correlation analysis showed no significant results between the recorded parameters and earthworm populations in spring 2019, indicating that there were no longer serious moisture constraints. Still, earthworms may have profited from the extra moisture under the CCs, especially the grasses, during spring (Eriksen-Hamel and Whalen, 2006). Thus, this experiment showed that earthworm populations are resilient to intermediate water scarcity under winter-killed CCs, as long as water reservoirs can be filled up later (Bodner et al., 2007). This is in line with the results of Schmidt and Curry (2001) who found strong drops in earthworm populations during drought, but fast recoveries afterward, especially in terms of earthworm mass.

No stimulation of significantly higher earthworm populations by any of the selected CCs was found during the study period. Schmidt and Curry (2001) found earthworm populations stabilized at greatly increased levels only after two years of permanent clover cover and reduced tillage, while Abail and Whalen (2018) found a significant increase in earthworm populations due to increased corn crop residues after about eleven months, presumably when decomposition left the residues more palatable to earthworms.

Whereas, Roarty et al. (2017) found significantly increased earthworm populations only under a three-year pea CC compared with several other winter CCs. Thus, the observed initial increases in earthworm biomass indicate that under more favorable moisture conditions earthworms may profit from CC residues, especially in the long-term, since particularly endogeic species tend to consume older biomass (Bertrand et al., 2015a; Abail and Whalen, 2018; Curry and Schmidt, 2007).

The expected increase in drought intensities due to climate change may not allow earthworm populations to recover as quickly as they could under current climatic conditions (Manici et al., 2014; Sillmann and Roeckner, 2008). Therefore, more CC alternatives need to be investigated, such as the water-saving hairy vetch and increased drought intensities should be examined via rainout shelters (Hoover et al., 2018).

4.3 *Sclerotinia sclerotiorum*

4.3.1 Sclerotia decomposition

In the rainfed trial, sclerotia decomposition from August 2018 to April 2018 was greater than in the irrigated trial, especially under radish HD, despite higher soil moisture. Since the measured soil temperatures from August to October 2018 were more or less consistent with the sclerotia decomposition rates, even if the differences were only 1-2°C, a dominant influence of soil temperature can be assumed (Matheron and Porchas, 2005; Wu et al., 2008). Moreover, the low sclerotia decomposition rates at irrigated radish HD coincide with a high LAI, which can have a cooling effect through shading and evapotranspiration (Monteith et al., 1991). Also, the low S values from the tea bag index from August until October 2018 indicate that moisture constraints were not too strong. However, an exception was sudangrass, where soil temperature was low and sclerotia decomposition was high compared to the other treatments. Sudangrass showed high biomass with a high LAI at both trials, similar soil temperatures as radish HD, and soil moisture was even lower than radish HD, thus increased survival of sclerotia would be expected (Matheron and Porchas, 2005; Wu et al., 2008). Nonetheless, sudangrass had the second-highest decomposition rates after bare fallow at the irrigated trial and the highest decomposition rates at the rainfed trial. Therefore, biotic factors can be suspected to have played a role here. The tea bag index did not give hints, as the decomposition rate under sudangrass was very low indicating limited decomposition, probably through low N-availability and the stabilization factor was similarly small among all treatments during the first three months of the trial. A possible explanation for the additional sclerotia decay could be either the production of cyanogenic glucosides by the sudangrass, which convert to the toxic hydrogen cyanide or, the release of other secondary plant compounds (Larkin, 2013; Nicollier et al., 1983; Weston et al., 2013). The dense rooting of sudangrass would have facilitated the contact between sclerotia and the chemical compounds. Conversely, Collange et al. (2014) found increased *S. sclerotiorum* incidence after a sudangrass CC (the only CC in the experiment) in high tunnel lettuce production but they did not record sclerotia decomposition in their experiment. The reason for their result could be a favorable microclimate for the formation of apothecia induced by sudangrass (Collange et al., 2014).

Even though oat and radish produce relevant secondary plant compounds, no signifi-

cant increases in sclerotia decomposition were recorded. Avenacin contained in black oat has anti-fungal properties, while common oat in other experiments showed suppressive results towards *S. sclerotiorum* through the stimulation of antagonistic fungi and bacteria (Bednarek and Schulze-Lefert, 2018; Patkowska et al., 2018). Possibly due to the drier climate, no such effects were seen in this experiment (Harvey et al., 1995). Radishes, like other Brassicaceae, contain glucosinolates that break down to produce isothiocyanates (Larkin, 2013). These compounds may have been released after frost, which damaged the radish tissue. The usual procedure of chopping Brassicaceae CCs before incorporation for biofumigation was not done in this experiment (Larkin, 2013).

In both trials, only a small decrease in sclerotia was observed during soybean growth from May 2019 to September 2019, even though soil temperatures during summer 2019 were higher than when sclerotia were buried in summer 2018. It is questionable whether low soil moisture was responsible for the soil temperature not resulting in higher rates of sclerotia decomposition, as no such trend was observed at the irrigated site either. Furthermore, increased carpogenic germination during spring, which some authors hypothesize leads to later decomposition, did not result in increased sclerotia decomposition rates (Maloney and Grau, 2001; Zeng et al., 2012b; Pethybridge et al., 2019). One aspect of this discrepancy could be the consequence of analyzing sclerotia only by visible traits and not testing viability on culture medium. Sclerotia might have died but not yet been visibly degraded. Another explanation for the generally lower decomposition in the second half of the experiment may be differences between individual sclerotia, meaning that a certain percentage of sclerotia are short-lived while another is more persistent (Abawi and Grogan, 1979; Zeng et al., 2012b). Also, bigger sclerotia tend to be more resistant to adverse conditions (Hao et al., 2003). The decomposition rate measured by the tea bag index did not prove to be a reliable estimator for sclerotia decomposition. The lowest S values from the tea bag index were recorded in the late summer of 2018 when the highest decomposition rates were found. A low stabilization factor S indicates that the decomposition of the labile fraction, i.e. simple sugars and cellulose, is not inhibited by environmental conditions, nor compensated through the build-up of secondary metabolites (Duddigan et al., 2020). Future works may evaluate whether S values prove to be a useful predictor for sclerotia decomposition.

Mesh size did not yield significant differences in terms of the decomposition rates of sclerotia. The marginal differences could also be a result of altered abiotic conditions. Thus, no effect of earthworms on sclerotia decomposition can be expected under the investigated conditions. The differences that Euteneuer et al. (2019) found for the factor mesh size, stemmed from a site with much higher earthworm densities. Furthermore, in the same study, the ingestion of sclerotia was tested with *Lumbricus terrestris*, a large-bodied anecic earthworm, while in this study only endogeic earthworms were present, which are thought to prefer food of small particle sizes (Lowe and Butt, 2003; Euteneuer et al., 2019). Similarly, Wolfarth et al. (2011) observed significant ingestion of straw infected with *Fusarium* spp. by *Lumbricus terrestris*, while the role of the endogeic species *Aporrectodea caliginosa* was considered minor.

4.3.2 Carpogenic germination of sclerotia

Carpogenic germination during its peak in April 2019 corresponded to the differences in soil temperature, but not to differences in soil moisture between treatments. Usually, high soil moisture is considered critical for carpogenic germination, but low or very high temperatures can also limit the formation of stipes (Abawi and Grogan, 1979; Bolton et al., 2006). Thus, moisture seemed to have been sufficiently available, while higher soil temperatures were favoring carpogenic germination. Similarly, Harvey et al. (1995) observed higher rates of carpogenic germination at sun-exposed sites and therefore presumably warmer, but also brighter sites. The high carpogenic germination of bare fallow at the rainfed site and the low carpogenic germination of sudangrass at the irrigated trial may furthermore be a result of the difference in light exposure due to the difference in plant biomass (Le Tourneau, 1979). Moreover, a small difference can also be attributed to the lower survival of sclerotia under sudangrass. If the above assumed biological impact of sudangrass on sclerotia also influenced germination rate or if just abiotic factors were predominant, cannot be disentangled.

Sclerotia within tubes of larger mesh size showed small, but significant increases in germination rates, indicating that mesh size had affected the immediate environment of the sclerotia.

A flush out through CCs, i.e. a reduction of sclerotia through early carpogenic germination, as described by Maloney and Grau (2001), Civardi et al. (2019) and Pethybridge et al. (2019) was not suggested by the presented data.

Apothecia production and a subsequent infestation of the soybean stand with *S. sclerotiorum* have not been observed. An infection was presumably prevented by the high temperatures and low precipitation during the critical phase of soybean flowering (Peltier et al., 2012; Derbyshire and Denton-Giles, 2016). Similarly, Wu and Subbarao (2008) observed a mismatch between ascospore production by *S. sclerotiorum* during the moist period in early spring and a susceptible crop under drier conditions in summer.

Further research could be concentrated on other regions in northern Europe that appear to be more susceptible to *S. sclerotiorum* infestation, even under future climatic projections (Siebold and von Tiedemann, 2012; Sillmann and Roeckner, 2008). As CCs in this experiment could not effectively reduce sclerotia in the ground or even reduced its decomposition, this should not lead to the recommendation not to use CCs. CCs may positively influence control approaches of *S. sclerotiorum* such as the BCA *Paraphaeosphaeria minitans* Campbell. On the one hand, the substrate provided by CCs may improve the survival and reproduction of *P. minitans* (Ooijkaas et al., 1999; Huang et al., 2005; Bennett et al., 2006). On the other hand, increased earthworm populations may facilitate the dispersal of conidia from *P. minitans* (Bennett et al., 2006).

4.4 Soybean grain yield

Lower soybean germination rates at sudangrass plots did not result in significant differences in grain yield. Crawford et al. (2018) and Reddy (2001) attributed reduced emergence of soybean to the high biomass of rye and oat CCs. Similarly, the presented data showed high residues of sudangrass with more than 6000 kg ha⁻¹, thus probably leading

to a reduced seed to soil contact (Liebert et al., 2017; Crawford et al., 2018). The high plasticity of soybean plants and a generally high variability through spider mite infestation might have masked some differences in yield.

Other studies also found no effect on soybean yields by CCs, while yields of other crops, such as corn, were severely affected. This is most likely due to the ability of legumes to fix nitrogen from the atmosphere (Liebert et al., 2017; Duval et al., 2016; Alvarez et al., 2017). In contrast, Lotter et al. (2003) found increased soybean yields during drought years in long-term organic crop rotations that used CCs compared to conventional crop rotations without CCs. Thus, the buffering of weather extremes by CCs can only be expected in the long-term through an increased WHC induced through increases in SOC and soil aggregation (Oldfield et al., 2019). In this regard, graminaceous CC species with high biomass were most promising (Duval et al., 2016).

Irrigation resulted in increased grain yields, approximately three to fivefold, while thousand-seed mass increased 1.3 to 1.5-fold. Irrigation can alleviate drought stress, especially on soils that contain fine-textured material (Arora et al., 2011). The presented values are even more pronounced than those found by Pandey et al. (1984), indicating there had been severe moisture stress at the rainfed site. Yield reduction through spider mites might have had some influences on these differences as well, but probably were not the crucial factor (Haile and Higley, 2003; Klubertanz et al., 1990).

5 Conclusion

This master's thesis provides another contribution to the scarce literature on how CCs affect earthworm populations and how CCs can affect the decomposition of sclerotia of *S. sclerotiorum*.

It was shown that CCs can restrict earthworm survival through enhanced water depletion during periods of drought. However, under current climatic conditions in Eastern Austria earthworm populations seem to be able to recover during winter and profit from CCs. It should be verified whether this is also true during even more prolonged periods of drought, which can be expected as climate change progresses. Under irrigated conditions, the forage radish treatments were the most favorable candidates for earthworm growth due to higher soil moisture from reduced evaporation caused by rapid soil cover. No stimulation of significantly higher earthworm populations by any of the selected CCs was found during the study period, as abiotic conditions dominated earthworm development. Nevertheless, the high belowground biomass of radish with low C/N and the high aboveground biomass of sudangrass showed promising initial increases in earthworm mass after the drought.

CCs affected sclerotia decomposition in late summer presumably through changes in soil temperature caused by a cooling effect due to shading and transpiration at the irrigated site. These lower decomposition rates were most evident in the radish HD treatment. Differences in soil moisture did not seem to be responsible for varying sclerotia decomposition rates. Based on the comparison of measured soil temperatures between treatments, sudangrass had higher decomposition rates than expected. Therefore, I hypothesized that the decomposition of sclerotia under sudangrass was enhanced by the

release of hydrogen cyanide. This hypothesis should be tested in future studies and how this effect could be amplified. Since none of the CC treatments resulted in a strong reduction of sclerotia, the combination of cover cropping with other measures, such as the BCA *Paraphaeosphaeria minitans*, should be tested for synergistic effects.

No strong effect of CCs on Soybean yield could be observed within this study. Only sudangrass affected early soybean development by causing poor seed to soil contact.

Overall, forage radish and sudangrass appeared to be two promising species for use as winter CCs in sustainable soybean production in terms of biomass formation and leaching reduction.

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A Appendices

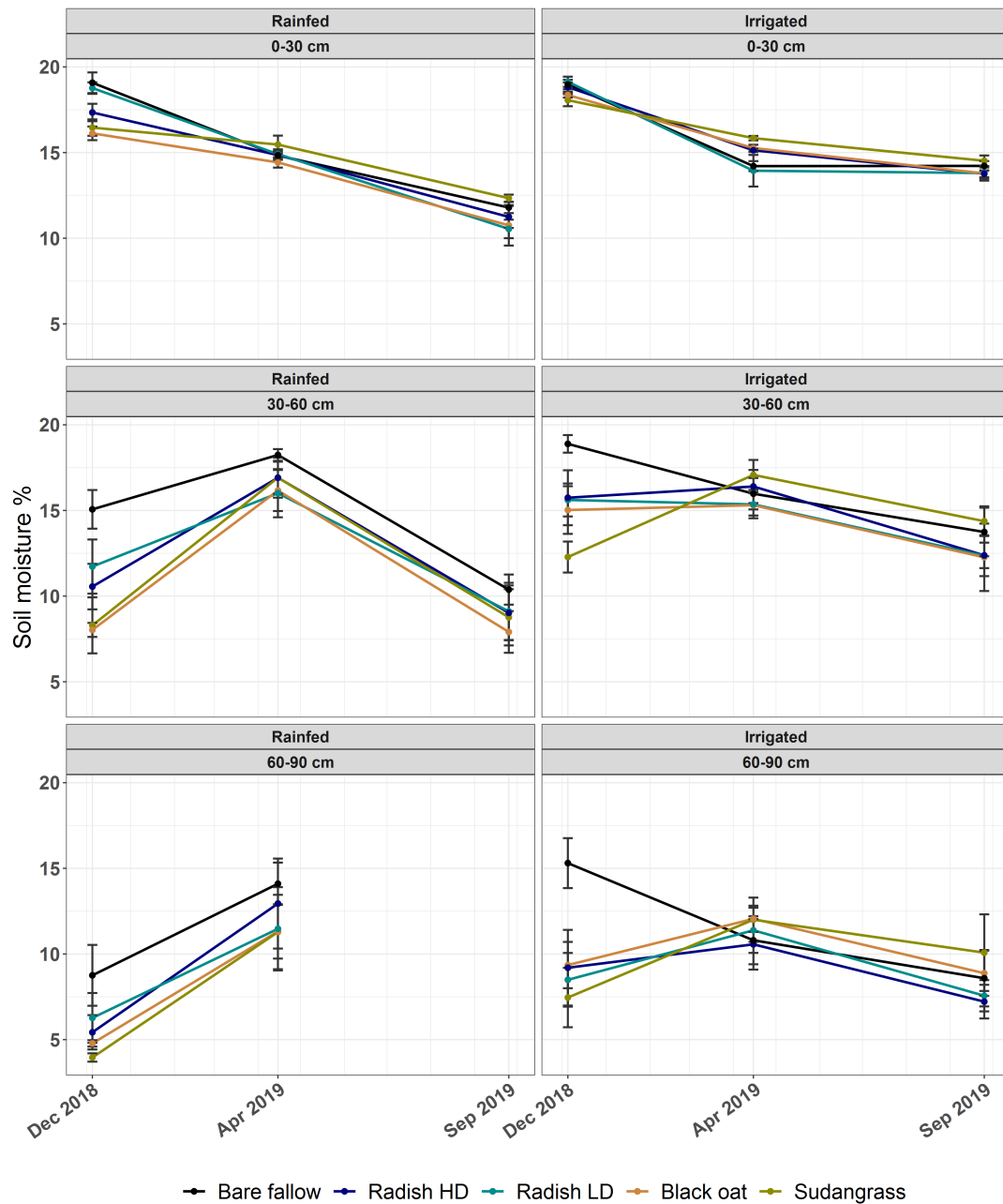


Figure A1: Soil moisture (gravimetric %) at three soil depths. Sampled with gouge auger (30 mm) in two trials with different irrigation regimes (Rainfed and Irrigated), three separated soil depths and on three sampling dates. Cover crop treatments were: sudangrass, black oat, forage radish with high (Radish HD) and low sowing density (Radish LD) and a bare fallow control. Displayed are means (n=4) with standard errors.

Table A1: Carpogenic germination of sclerotia. Mean percentage of initially buried sclerotia that germinated carpogenically on four recovery dates. Data from two equal trials with different irrigation regimes (Rainfed and Irrigated). Cover crop treatments were: sudangrass, black oat, forage radish with high and low sowing density (Radish HD and Radish LD) and bare fallow control. Sclerotia were buried within plastic tubes of two different mesh sizes (1x1 mm and 3x10 mm). Mean values (n=4) within each column that do not have a letter in common are significantly different by pairwise comparisons (Tukey; $P < 0.05$).

Treatment	mesh size	Germinated sclerotia % (I)							
		November 2018				December 2018			
		Irrigated		Rainfed		Irrigated		Rainfed	
Bare fallow	1x1 mm	1.7	ab	2.5	ab	31.7	b	25.0	de
Bare fallow	3x10 mm	7.5	abc	0.8	ab	34.2	b	35.8	e
Black oat	1x1 mm	4.2	abc	0.0	a	35.0	b	8.3	abc
Black oat	3x10 mm	8.3	abc	0.8	ab	27.5	b	10.0	abc
Radish HD	1x1 mm	11.7	bc	1.7	ab	25.8	b	8.3	abc
Radish HD	3x10 mm	25.0	c	0.0	ab	35.0	b	7.5	abc
Radish LD	1x1 mm	10.0	bc	4.2	ab	34.2	b	13.3	bcd
Radish LD	3x10 mm	18.3	c	4.2	b	30.8	b	10.0	cde
Sudangrass	1x1 mm	0.8	a	0.8	ab	2.5	a	0.8	a
Sudangrass	3x10 mm	2.5	ab	2.5	ab	10.0	a	4.2	ab

Treatment	mesh size	Germinated sclerotia % (II)							
		April 2019				September 2019			
		Irrigated		Rainfed		Irrigated		Rainfed	
Bare fallow	1x1 mm	62.5	b	52.5	cd	12.5	a	13.3	a
Bare fallow	3x10 mm	49.2	b	43.3	d	15.8	a	19.2	a
Black oat	1x1 mm	55.0	b	17.8	ab	10.8	a	15.6	a
Black oat	3x10 mm	50.0	b	41.1	abc	11.7	a	14.4	a
Radish HD	1x1 mm	36.7	b	41.7	ab	6.7	a	8.3	a
Radish HD	3x10 mm	64.2	b	29.2	abc	22.5	a	10.0	a
Radish LD	1x1 mm	42.5	b	44.2	abcd	13.3	a	6.7	a
Radish LD	3x10 mm	54.2	b	40.0	bcd	13.3	a	8.3	a
Sudangrass	1x1 mm	23.3	a	30.8	a	4.2	a	13.3	a
Sudangrass	3x10 mm	22.5	a	18.3	ab	10.0	a	8.3	a

Table A2: Earthworm abundance was recorded separately for adults and juveniles during mid-October 2018 and during the end of March until the beginning of April 2019 in two trials with different irrigation regimes (Rainfed and Irrigated). Cover crop treatments were: sudangrass, black oat, forage radish with high and low sowing density (Radish HD and Radish LD) and bare fallow control. Mean values (n=4) with corresponding standard errors (SE).

Treatment	Earthworm abundance (Individuals m ⁻²)							
	Rainfed				Irrigated			
	Adults		Juveniles		Adults		Juveniles	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
October 2018								
Bare fallow	32.8	4.7	96.9	31.2	29.7	3.9	71.9	15.6
Radish HD	17.2	6.9	76.6	24.8	32.8	4.7	84.4	12.1
Radish LD	18.8	6.8	59.4	12.1	56.3	5.7	85.9	9.3
Black oat	14.1	4.7	35.9	10.9	25.0	6.8	73.4	3.9
Sudangrass	20.3	7.8	32.8	13.4	21.9	9.0	62.5	13.7
April 2019								
Bare fallow	15.63	8.27	49.48	2.99	29.69	4.69	53.13	17.40
Radish HD	28.13	9.72	56.25	9.88	25.00	6.75	79.69	11.23
Radish LD	12.50	2.55	60.94	16.41	18.75	7.22	56.25	5.71
Black oat	10.94	2.99	57.81	6.44	17.19	9.67	57.81	12.60
Sudangrass	23.44	2.99	75.00	7.65	15.63	5.98	68.75	17.12

Table A3: Earthworm mass was recorded separately for adults and juveniles during mid-October 2018 and during the end of March until the beginning of April 2019 in two trials with different irrigation regimes (Rainfed and Irrigated). Cover crop treatments were: sudangrass, black oat, forage radish with high and low sowing density (Radish HD and Radish LD) and bare fallow control. Mean values (n=4) with corresponding standard errors (SE).

Treatment	Earthworm mass (g m ⁻²)							
	Rainfed				Irrigated			
	Adults		Juveniles		Adults		Juveniles	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
October 2018								
Bare fallow	24.2	6.9	11.6	4.9	15.3	2.4	10.2	2.2
Radish HD	7.3	3.8	7.5	2.1	19.4	3.2	11.6	2.7
Radish LD	7.9	4.0	4.4	1.0	32.9	3.8	8.4	1.2
Black oat	7.0	3.0	2.7	0.5	13.3	6.0	6.1	0.8
Sudangrass	9.7	3.9	4.2	3.3	10.9	4.5	6.6	1.6
April 2019								
Bare fallow	14.5	8.3	6.7	1.1	28.0	5.9	11.1	3.0
Radish HD	32.8	15.3	9.1	2.0	25.4	6.4	21.4	1.7
Radish LD	17.2	3.1	11.4	2.9	25.3	10.5	11.7	1.4
Black oat	14.0	5.1	12.9	1.4	15.6	8.2	15.3	2.2
Sudangrass	20.8	4.8	15.7	2.7	15.7	7.2	17.9	5.9