Monitoring the impact of soil management on plant spectral reflectance and soil-borne disease resistance

Master Thesis
Natural Resources Management

Maria de Lamares Pereira

2015
Monitoring the impact of soil management on plant spectral reflectance and soil-borne disease resistance

Thesis submitted to the University of Trás-os-Montes e Alto Douro in partial fulfilment of the requirements for the degree of Master of Science in Natural Resources Management

Maria de Lamas das Piedade e Teixeira Pereira

Under orientation:

Dra. Sabrina Carvalho
Prof. Dra. Berta Gonçalves

Jury:

Prof. Dra. Edna Cabecinha
Prof. Dr. José Moutinho Pereira
Dra. Sabrina Carvalho

Life Sciences and Environment School
VILA REAL, 2015
“It is not the most intellectual or the strongest of species that survives; but the species that survives is the one that is able to adapt to and adjust best to the changing environment in which it finds itself.”

Charles Darwin
Acknowledgement

It’s been a long road, but here I am at the end, but there are so many people to whom thanks I extend.

First and foremost I offer my sincerest gratitude to my supervisor, Dra. Sabrina Carvalho, who has supported me throughout my thesis with her patience and knowledge whilst allowing me the room to work in my own way. Great mentorship, good advices, thanks you very much for the precious time! I attribute the level of my Master’s degree to her encouragement and effort and without her this thesis, would not have been completed or written. One simply could not wish for a better or friendlier supervisor.

My utmost gratitude goes to my Portuguese supervisor Prof. Dra. Berta Gonçalves, for her moral support and helped me to maintain the focus during this year.

To Prof. Dr. Maarten Schrama, thank you very much for the big smile every day in the greenhouse, expert guidance and lab-work help, and all the knowledge shared.

To Maaike Van Agtmaal, Angela Straathof and Levi Bin thank you for the partnership and good advices.

I am grateful to Prof. Dr. Wim Van der Putten, who gave me the opportunity to work in NIOO-KNAW.

To my grandmother, you were the best example of life that I could have known. My warrior, companion and above all thank you for the support you gave me when I risked and went to live in the Netherlands.

To my parents, for all the support, love, patience, thank you for everything. What I am is because of you two.

To my brother, you have shown me how to dream big and to believe.
To my aunt Gi, thank you were a key in this process you have nurtured my learning, my enthusiasm, supporting always my dreams!

For all my colleagues in NIOO, who made my everyday a nice day.

For all, that helped me in this thesis anyhow.
Abstract

Soil and soil biodiversity are the driving force of all the terrestrial production systems and ecosystem services. The intensification of agriculture production and shifts from extensive crop rotation have, regularly, a profound negative effect on soils and their biodiversity. Biodiversity losses result in less complex soil communities. The increasing demand of soil ecosystem implies the improvement of soil and crop management, and it's a key opportunity for supporting sustainable economic development. The sustainable management of any ecosystem requires, amongst other information, a thorough understanding of plant-soil feedback attempting to describe natural patterns and relations between the plants and their environment.

Plants produce organic substances by photosynthesis. Photosynthesis depends upon the absorption of light by pigments, as chlorophyll-a among other accessory pigments, in the leaves of the plants. Therefore, leaf optical properties are influenced by the concentration of the photosynthetic pigments, metabolites, water content, leaf structure and leaf anatomy. Hyperspectral reflectance in remote sensing has gained scientific and commercial importance but still remains underdeveloped despite its potential. Vegetation remote sensing is a great tool, as it can extrapolate to synoptic scales and time sequences can be acquired. It is increasingly used for measurements of agricultural crop condition and also for plant-soil interactions. It is known that reflectance signal is sensitive to abiotic changes, but concerning biotic changes, there are still several limitations.

Therefore, was conducted a 9 weeks greenhouse bioassay with two different crops, Sugar beet (Beta vulgaris) and Corn (Zea mays), three different types of soil management and six different treatments were applied. The treatments applied were: the fungus Rhizoctonia solani, the nematode Pratylenchus penetrans, the Gamma radiation, the nutrients, the R. solani with
nutrients and a control treatment. The types of management were the Biologic, the Artificial Fertilizer and the Manure. In total were 3650 plants.

Spectral reflectance data were collected with an ASD FieldSpec 3 spectrometer with an ASD plant-probe and leaf-clip device attached. One of the objectives of the measurement was to monitor the differences between leaf reflectance over time. The spectral data was analyzed using vegetation indices. The effects of soil biota were analyzed in a multivariate ANOVA analysis with plant species, soil regime and soil treatment.

The total biomass of the pathogens increase with a more intensive agriculture and shoot biomass in both plant species increased with disposal of the nutrient supply in the Biologic soil. The application of manure compost that is rich in nitrogen may have reduced soil-borne diseases. The lowest biomass was found in the sterilized treatments suggesting that the soil biota has influenced the plant performance. The best soil management had positive effect in growth of the plants. Disease suppression can be influenced by management practices. It was demonstrated that plant spectral signatures changes due induced stress and soil type. The best soil regime overall in this study case was considered the Biologic type.

Key words: Soil biodiversity; Sustainable management; Hyperspectral reflectance; Plant-soil interactions.
O solo e a sua biodiversidade são o motor de todos os sistemas de produção terrestres e serviços de ecossistemas. A mudança na produção agrícola de extensiva para intensiva tem um efeito negativo profundo nos solos e na sua biodiversidade. A perda de biodiversidade resulta em comunidades do solo menos complexas. Com o aumento da procura dos serviços dos ecossistemas, como o solo, existe a necessidade da melhoria da gestão dos solos e das produções agrícolas. Esta melhoria pode trazer vantagens a nível do desenvolvimento de práticas mais sustentáveis que contribuem para um desenvolvimento económico sustentável. A gestão sustentável de qualquer ecossistema requer, entre outras informações, uma compreensão completa da interação solo-planta para tentar descrever padrões naturais.

As plantas produzem substância orgânica através da fotosíntese. A fotosíntese depende da absorção de luz pelos pigmentos fotosintéticos presentes na folha. Portanto, as propriedades óticas da folha são influenciadas pela concentração dos pigmentos fotosintéticos e metabolitos, do seu teor em água e da estrutura e anatomia da folha.

A reflectância hiperspectral tem ganho importância comercial e científica, contudo, permanece ainda subdesenvolvida apesar do seu potencial. A deteção remota da vegetação é uma boa ferramenta, pois pode extrapolar escalas de tempo, e é cada vez mais utilizada para perceber interações planta-solo. Sabe-se que o sinal de reflectância é sensível a mudanças abióticas e bióticas, mas ainda há um longo caminho a percorrer.

Consequentemente, foi realizado durante nove semanas um bioensaio com duas culturas diferentes, a Beterraba (Beta vulgaris) e o Milho (Zea mays). Foram plantadas em três diferentes tipos de gestão do solo e aplicados seis tipos de tratamentos. Os tratamentos aplicados foram: o fungo Rhizoctonia solani, o nemátoide Pratylenchus penetrans, a radiação gama, os nutrientes, e
o fungo *R. solani* com nutrientes e um controlo. Os tipos de gestão que foram aplicados foram o solo Biológico, os fertilizantes artificiais e Fertilizantes orgânicos. No total foram consideradas 3650 plantas.

Os dados da refletância espectral foram obtidos com um espectrómetro de campo ASD plant-probe e clip-foliar. Um objetivo deste estudo consistiu em monitorizar a refletância espectral das folhas das duas espécies durante o período experimental. Os dados espetrais foram analisados utilizando índices de vegetação. Os efeitos do biota do solo foram analisados numa análise multivariada ANOVA com os fatores, espécie de planta, tipos de solo e tratamentos.

A biomassa total de patogénicos tende a aumentar quanto mais intensiva for a prática agrícola. No solo Biológico observou-se uma intensificação da cor verde da planta nas duas espécies, com o aumento da disponibilidade de nutrientes. A adição de fertilizante pode ter influenciado a resistência das plantas às doenças do solo. A menor biomassa foi encontrada no tratamento com radiação gama (estéril), sugerindo que o biota do solo influenciou o desempenho da planta. O melhor tipo de gestão do solo teve um efeito positivo no crescimento das plantas. As melhores práticas agrícolas permitem uma supressão das doenças inoculadas. Foi demonstrado que o espetro da planta difere quando é induzido stress e também consoante o tipo de gestão do solo. A melhor gestão agrícola foi considerada a Biológica.

**Palavras-chave:** Biodiversidade do solo; Gestão sustentável; Refletância hiperspetral; Interações planta-solo.
# Table of Contents

## CHAPTER 1 - Introduction

1.1 Introduction .............................................................................................................. 2  
1.2 Soil biodiversity ...................................................................................................... 3  
1.3 Soil-borne diseases ................................................................................................. 6  
1.4 (A) Biotic stress and disturbance in crops ............................................................... 8  
1.5 Sustainable agriculture systems ............................................................................. 10  
1.6 Spectroscopy and its potential for monitoring agriculture management ............... 12  
1.7 Research Objectives ............................................................................................... 15  
  1.7.1 General objective ............................................................................................... 15  
  1.7.2 Specific objectives ............................................................................................. 15  
1.8 Research Questions and Hypothesis ...................................................................... 16  

## CHAPTER 2 - Materials and Methods

2.1 Research workflow and steps ................................................................................ 19  
2.2 Species description ................................................................................................ 20  
  2.2.1 Plant (crop) species .......................................................................................... 20  
  2.2.2 Soil pathogens ................................................................................................ 21  
2.3 Field Sampling ...................................................................................................... 22  
2.4 Experimental design ............................................................................................... 23  
  2.4.1 Treatments setup ............................................................................................... 23  
2.5 Soil pathogen inoculation ..................................................................................... 25  
2.6 Quantitative Nematodes determination ................................................................ 26  
  2.6.1 Extraction of active nematodes from the soil, the Oostenbrink elutriator method 26  
  2.6.2 Cold Staining of root material ........................................................................ 27  
2.7 Spectral measurements ......................................................................................... 27  
2.8 Spectral data processing ....................................................................................... 28  
2.9 Spectral data analysis ............................................................................................ 28  
2.10 Data analysis ......................................................................................................... 29
CHAPTER - 3 Results

3.1 Soil treatment effects on plant biomass ................................................................. 32
3.2 Spectral Reflectance ............................................................................................... 36
3.3 Temporal Difference in Spectral Indices ................................................................. 41

CHAPTER 4 - Discussion

CHAPTER 5 - Conclusion/Recommendations

Conclusion and Recommendations .............................................................................. 52

CHAPTER 6 - Supplementary information ................................................................. 53

6.1 Major pathogen groups .......................................................................................... 55
   6.1.1 Plant parasitic nematodes and fungal pathogens .............................................. 55
6.2 Plant spectral properties ......................................................................................... 57
   6.2.1 The visible region ............................................................................................ 58
   6.2.2 The red-edge .................................................................................................... 59
   6.2.3 Near-infrared region ....................................................................................... 59
   6.2.4 Mid-infrared region ....................................................................................... 60
   6.2.5 Vegetation indices ........................................................................................... 60
   6.2.6 Ratio indices (RI) .......................................................................................... 61
6.3 Extra Information .................................................................................................... 63

CHAPTER 7 - References
List of Figures

Figure 1 Graphical and mathematical description of resistance and resilience .................................4

Figure 2 The equilateral plant disease triangle. ........................................................................................................6

Figure 3 The relationship between planned biodiversity associated and how the two promote ecosystem function ........................................................................................................................................11

Figure 4 Research workflow and steps..........................................................................................................................19

Figure 5 Average total biomass per pot in dry leaves in six different treatments .................................................32

Figure 6 Average shoot biomass per pot in dry leaves ............................................................................................33

Figure 7 Mean per pot of leaves reflectance indices per soil treatment .................................................................40

Figure 8 Leaves temporal differences per soil treatment ........................................................................................44

Figure S9 (A) The solar radiation spectrum above and below the atmosphere, and (B) Typical reflectance spectra of crop, tree and soil surfaces ..................................................................................................57
List of Tables

Table 1 Overview of the Dissolved Organic Carbon .......................................................... 23

Table 2 ANOVA analysis of the effects of species, treatments and soils on the average total biomass. .......................................................................................................................... 35

Table 3 ANOVA analysis of the effects of species, treatments and soil on the shoot biomass. ...... 36

Table 4 ANOVA analysis of the effect of species, Soil origin, and Soil Treatment on the different vegetation indices. ............................................................................................................................ 45

Table S5 Plant pigments and their absorption maxima.................................................................... 59

Table S6 Examples of vegetation indices with the formula calculation and definition. ............... 63

Table S7 Pearson Correlation matrix between several plant vegetation indices .............................. 66

Table S8 Rhizoctonia solani spread during the experiment in 3 different soil managements ......... 67

Table S9 Overall of the experiment in pictures.............................................................................. 68
Abbreviations and acronyms

% - percent
AF - Artificial Fertilizer
ARI - Anthocyanin reflectance index
B - Biologic
C - Carbone
cm - Centimeter
Dblue - Maximum value of the first derivative in the blue edge
df - Degrees of freedom
DOC - Dissolved Organic Carbon
Dred - Maximum value of the first derivative in the red edge
DSWI - Disease-water stress index
Dyellow - Maximum value of the first derivative in the yellow edge
EU - European Union
EVI - Enhanced vegetation index
FA - Fluvic acids
G - Gamma radiation
GI - Green index
ha - Hectare
HA - Humic acids
HON - Hydrofobic neutrals
HY - Hydrophilic
ind - Individual
K - Kelvin degrees
kg - Kilogram
M - Manure
MCARI – Modified chlorophyll absorption and reflectance index
mg - Milligram
min - Minute
mM - Millimolar
mm - milimeter
Mmol – Millimol
mREP - Modified red-edge position
MSI - Moisture stress index
N - Nutrients
nm - Nanometers
NR - Nutrients + *Rhizotonia solani*
NRI - Nitrogen reflection index
NVIDd - Normalized difference vegetation index
°C - Celsius degrees
OM - Organic Matter
P - *Pratilenchus penetrans*
PDA - Potato dextrose agar
PRIa - Photochemical/physiological reflectance index
PPR - Plant pigment index
PSa - Plant stress index
Pvalue - Significance of statistical test
R - *Rhizotonia solani*
REP - Red edge position
RDVI - Re-normalized difference vegetation index
RI - Ratio indices
SDIs - Specific Spectral Indices
SR - Simple ratio
TCARI - Transformed chlorophyll absorption and reflectance index
ton - Tones
USA - United States of America
v - Volume
w - Weight
WHC - Water Holding Capacity
WI - Water index
X - Control
This chapter describes the introduction and the background of the research and these are presented by:

1.1 Introduction
1.2 Soil biodiversity
1.3 Soil-borne diseases
1.4 (A) Biotic stress and disturbance in crops
1.5 Sustainable agriculture systems
1.6 Spectroscopy and its potential for monitoring agriculture management
1.7 Research objectives
1.8 Research questions and hypothesis
1.1 Introduction

Soil and soil biodiversity are the motor of all the terrestrial production systems and ecosystem services (e.g. food, biofuel, drinking water, carbon storage and fibers). The intensification of agriculture production and shifts from extensive crop rotation have a profound negative effect on soils and their biodiversity (changing land use management) (Scherr and McNeely 2008; Hol and Hedlund 2010; Hedlund 2012). Biodiversity losses often result in a less complex soil community with frequently inhibited functions leading to a higher exposure to pathogens and natural enemies resulting in frequent disease outbreaks (Peterson et al. 1998; Folke et al. 2004; Garbeva et al. 2004; Neher 2010). Soil organisms are mostly unseen but have a huge variety both in function and taxonomy. They interact in complex belowground foodwebs and feedback into above-ground plant performance as well (e.g. due to their feeding activities belowground) (Van der Putten et al. 2009; van Dam and Heil 2011). The increasing demand of soil ecosystem services implies improving soil management as a key opportunity for supporting sustainable economic development, prevent species loses and promote soil biodiversity (Tilman et al. 2002; Zhang et al. 2007). Accurate knowledge of plant species distribution and foodweb interactions is a critical component for soil management and preservation of biodiversity (Brussaard et al. 2007; Tylianakis et al. 2008). Hence, the sustainable management of any ecosystem requires, amongst other information, a thorough understanding of plant-soil feedback to try to describe natural patterns and relations between the plants and their environment. It becomes, therefore, essential to improve our monitoring and management of soil quality and crop production.

Remote sensing sensors are increasingly used for measurements of the agricultural crop condition or also for plant soil interactions (Schmidt and Skidmore 2003; Mutanga and Skidmore 2004; Beck et al. 2008; Knox et al. 2010;
It is known that reflectance signal is sensitive to abiotic changes (Wessman et al. 1988) but for the biotic changes there is a long way to go (Carvalho et al. 2012). In this way this technology can also provide an automatic and objective alternative to visual disease assessment of plant diseases (Hillnhütter and Mahlein 2008; Mahlein et al. 2012). However, leaf optical properties are influenced by the concentration of the photosynthetic pigments, biochemicals, water content and leaf structure (de Boer 1993), which are themselves, influence by soil biotic communities and pathogens (Bever et al. 1997; Hedlund et al. 2003; Wardle et al. 2004; de Vries and Bardgett 2012) as each stress can elicit cellular and molecular changes (Herms and Mattson 1992). Remote sensing is the major source of spatial information of the earth’s surface cover from the local region to the globe (Graetz 1990). Hyperspectral remote sensing has gained scientific and commercial importance but still remains underdeveloped (Schmidt and Skidmore 2003) despite its potential (Myers et al. 1970; Thenkabail et al. 2000; Hillnhütter et al. 2011). In this thesis we aim at the study of these changes, using spectral data in a non-destructive method that has the potential to be use in soil management and to monitor crop production in future approaches for precision agriculture.

1.2 Soil biodiversity

Soil biodiversity is defined as the variety of life found in soil, such as invertebrates and microorganisms, soil flora, mammals, birds, reptiles and amphibians. Soil communities are among the most species-rich areas in terrestrial ecosystems (Giller et al. 1997; Bardgett 2005; Brussaard et al. 2007). Most of the organisms inhabiting soil ecosystems are found within the top 10 cm of the soil profile, and there are many abiotic and biotic factors that influence this diversity (Connell 1961; Wall and Moore 1999; Wardle et al. 2004). Some known abiotic factors are the soil structure, soil texture, organic matter while
main biotic factors are the interactions between organisms, their pathogens and symbionts.

Soil texture, organic matter and structure highly influence the biodiversity of plants and animals living on the soil. Soil texture is defined by the proportion of sand, silt and clay particles bound together by organic matter (Parton et al. 1987; Bronick and Lal 2005). The percentage of organic matter found in soil has a large influence on the adjacent biodiversity as it impacts soil fertility, soil structure, workability and water holding capacity (Doran et al. 1996; Weil and Magdoff 2004). Soil biota has the function to degrade organic matter which will be fixed by primary producers and the cycle of nutrients will flow (organic matter is processed and the nutrients will be release into the environment becoming available again for the primary producers) (Witkamp 1971; Lavelle and Berhe 2005). While degrading organic matter soil microbial biota activity produces and consumes gases - carbon dioxide, methane, among others. and sequestrates carbon into the soil maintaining the physical properties of the soil (Linn and Doran 1984; Lal 2004). Soil structure affects the stability of soil and its resistance to degradation under pressure. Resistance is defined as the capacity of a system to continue to function without change through a disturbance (Pimm 1984; Peterson et al. 1998; Folke et al. 2004; Garbeva et al. 2004). Another

Figure 1 Graphical and mathematical description of resistance and resilience (Herrick and Wander 1998).
important of soil is resilience, defined as the recovery of the functional integrity of a system following a disturbance (Pimm 1984) or catastrophic disturbance (Holling and Meffe 1996) relative to its original state (Fig. 1). Structure is influenced by texture, organic matter, compaction and biological activities.

In its biotic conditions, soil communities have the invertebrates and microorganisms as the majority of their biomass (Anderson et al. 1977; Ghilarov 1977; Allen et al. 1979; Pimentel et al. 1980). These include bacteria, fungi, protozoa, nematodes, mites, collembola, oligochaetes (earthworms), myriapods (millipedes and centipedes), mollusks and insects (ants, termites, beetles). Soil biodiversity communities are more resistant to changes in surrounding biotic and abiotic conditions (Elliott and Lynch 1994) as redundancy in an ecosystem is high. High redundancy allows one species to substitute another, such that functions are continuously achieved, even with the loss of one species (Mouillot et al. 2013). With increased redundancy, soil ecosystems also have higher resistance to disturbances and are resilient to subsequent disturbances. As such, the diversity of species found in soil communities impact soil quality and functioning, providing essential services in the stability of the abiotic components of the soil.

In fact soil biodiversity is increasingly proposed as an integrative indicator of soil environmental quality, food security and economic viability, and it can be an ideal indicator of sustainable land management (Parr et al. 1992; Carter 2002; Brussaard et al. 2007). In this thesis was considered the definition of sustainable management as “The capacity of a specific kind of soil to function within natural or managed ecosystem boundaries, to sustain biological productivity, maintain environmental quality, and promote plant and animal health” as described by the Science Society of America (Karlen et al. 1997).
1.3 Soil-borne diseases

Plant disease development is a dynamic process involving living entities (the plant host and the pathogen) and the environment (biotic and abiotic components) with high impact in agriculture. The interaction between (a) biotic factors in the soil can cause a disease to break-out. This interaction can be summarized in the “disease triangle” concept (Stevenson 1960) (Fig. 2).

Pathogens are the biological agents causing disease or illness in the host. The soil borne pathogens can be defined as the pathogens that cause diseases via inoculum (biological object, spore, mycelium, cells) that comes to the plant via the soil (Koike et al. 2003). The most common symptom of soil borne pathogens in plants is root rots that affect belowground tissues and vascular wilts initiated through root infection (Grau et al. 2004). Sometimes presence of a pathogen can be visible (mycelial growth, nematode cysts), or while, in other situations, there are just symptoms visible but not the pathogen itself (root decay, crowns rot, wilting foliage). So the manifestation of the symptoms caused by the pathogen is the disease (Campbell and Madden 1990; Blok et al. 2000; Bailey and Lazarovits 2003). Soil biodiversity performs a number of vital functions that regulate the soil ecosystem, including decomposition of litter and cycling of nutrients such as nitrogen, carbon, among others (Coleman et al. 1993). These renewal processes and ecosystem services are mainly biological, thus, their persistence depends upon maintenance of biological diversity (Altieri 1999). The same biodiversity soil may generate “pathogen suppressive” conditions and greater resistance to invasion. Pathogen control is another key ecosystem service underpinned by biodiversity (Cardinale et al. 2012); it is greatly
determined by the abundance of natural enemies of the pathogen species involved. Improved pest control is dependent on a diversity of natural enemies of pests, and non-crop habitats are often fundamental for the presence and survival of these biological control agents (predators, parasitoids) (Zhang et al. 2007).

There is evidence that soil microbial diversity confers protection against soil-borne diseases, despite crop, soil type and management also playing a role (Brussaard et al. 2007). Therefore, natural pest regulation is considered an important service of biodiversity (Altieri 1999; Schläpfer and Schmid 1999; Wilby and Thomas 2002b, a; Fiedler et al. 2008). Soil borne plant pathogens can significantly reduce yield and quality in vegetable crops (Abawi and Widmer 2000). These pathogens are particularly challenging because they often survive in soil for many years and each crop may be susceptible to several species. Infections occurring at the same time from multiple soil-borne pathogens can result in a complex disease that can further damage the crop. Almost all soil-borne pathogens are difficult to predict, to detect and to diagnose (Koike et al. 2003). The soil environment is extremely complex, making it more difficult to understand the interaction between soil and diseases caused by this soil-borne pathogens (Garbeva et al. 2004). They can broadly be divided into soil inhabitants, the one able to survive in soil for a relatively long time and soil invaders those who are able to survive in the soil but for a short time. Many of these pathogens can also live in the soil as non-pathogenic under certain conditions (Chapter 6 – 6.1- Major soil pathogens).
1.4 (A) Biotic stress and disturbance in crops

Crop stress can be related to living factors (biotic) as diseases, predators, competition, insects’ outbreaks, fungi, bacteria, and nematodes. Or they can be related to nonliving factors (abiotic) that including changes in pH, \( O_2 \) availability, rainfall, light intensity, soil properties, among others. Disturbances often break the normal function of the system causing dynamic growth patterns and different patches on habitat level (Hooper et al. 2000; Van der Putten et al. 2001). In natural systems the plant community reorganization after stress or disturbance can take a long time to stabilize and is dependent of spatial heterogeneity or dispersal rates. Usually we refer to “disturbance” as a short term event (Tobor-Kapłon et al. 2006) and the equilibrium state can be reached similar to the one before. When a factor of disturbance persists is called “stress”. If the plant populations are capable to handle this kind of stress, the equilibrium reached might be different from the original one, because of the presence of the stress factor, that continuously affect the energy budget of the organisms. In agriculture, the soil biodiversity is reduced, most likely due to frequent disturbance, and stability may depend on management regime. Organically managed soil usually is more stable than the intensively managed soil (Grayston et al. 2001; Griffiths et al. 2001) and also depends on the type of stress, disturbance or the combination of these factors. Therefore, and even though resistance against outbreaks or stress of pests, diseases and resilience from disturbance is of particular importance in agriculture, still a lot of these processes are unknown (Brussaard et al. 2007). A soil is considered suppressive when, in spite of other favorable condition for the disease, it does not establish or persist with little to no damage to crop yield (Baker and Cook 1974; Janvier et al. 2007). In contrast, conductive (non-suppressive) soils are prone to disease. Soil supressiveness is related to fertility level and nature of the soil itself, as well as its microbiological community activity. The better the abiotic conditions and more
biodiversity a soil is the more suppressive it can be (Cook and Baker 1983; Garbeva et al. 2004). Supressiveness has been further defined into general suppressiveness and specific suppressiveness. General suppression is the result of high total microbial biomass and biodiversity which creates unfavorable conditions to the development of plant diseases. Specific suppression, on the other hand, is due to the effects of individual or selected groups of microorganisms during particular stages of the pathogen life cycle (Cook and Baker 1983).

In agriculture, different type of management can have different effects on disease rate, as mentioned before. These effects can be positive or negative depending on the mechanisms (Litterick et al. 2004; Bonanomi et al. 2007; Bonanomi et al. 2010) which can involve toxic substances, promoting mutualism, plant chemistry defences, among others (Van der Putten et al. 2001). The importance of soil biodiversity in crop systems is still little understood, but a study in semi-natural systems has shown that biodiversity promote healthier crops (Van der Putten and Van der Stoel 1998). But threats to soil biodiversity can alter soil community structure and internal food web interactions, resulting in damaging impacts on the ecosystem’s self-regulation properties and favours pests and diseases. Biological pest control promotes primary production, what will bring more efficiency to the system and healthier crops (Altieri 2002; Turbé et al. 2010).

Particular types of weeds, for example, can harbour and support beneficial arthropods species capable to fight pest populations (Boatman 1994). In general, the more diverse and stable the agro-system, the more stable the insect community. A diverse agro-ecosystem has a balance that hinders the development of pests and invasive species, as the species present cover all the available ecological niches and the resources available in an optimal way. Thus, keeping a high bio-diverse ecosystem is an important part for a good defensive strategy of agricultural management at least for generalist pest species.
Integrated management of soil biota, biodiversity and agricultural ecosystems is a holistic process that relies largely on locally available resources, climate, and socioeconomic conditions. Above all, the direct involvement of farmers and stakeholders is a key to identify and adapt management practices to their specific context. So, the conservation of the biodiversity, the development of farming systems with greater reliance on ecosystem services should increase the sustainability of agro-ecosystems (McLaughlin and Mineau 1995; Pretty 1995; Altieri 1999; Robertson and Swinton 2005; Young et al. 2005; Klein et al. 2007).

1.5 Sustainable agriculture systems

During the last decades the intensification and specialization of crop production has been a major trend in agricultural systems. To this is associated an increased use of fossil fuel energy due to the intensive use of agrochemicals (fertilizers and pesticides), mechanization and irrigation. As a consequence of intensification and specialization, there has been an increase in soil erosion rates, a decrease in soil organic matter content and associated losses of soil structure and water holding capacity (Matson et al. 1997; Cassman 1999). The pest and crop disease problems have increased, while soil biodiversity reduces and an increase in vulnerability to climate change and related extreme disturbances is expected (Dogliotti 2003; Altieri and Toledo 2011; Reich et al. 2012). According to Millennium Ecosystem Assessment many farming systems have a high dependence on fossil energy and suffer from the deterioration of their ecosystem services (Provisioning services, Regulating services, Cultural services, Supporting services). So it is urgent to improve systems by optimizing the use of water and energy including the internal ecological cycles to achieve long term sustainability goals (Altieri et al. 2012).
Sustainable agricultural systems depend on the maintenance of the soil health (a soil to sustain biological productivity, maintain environmental quality and promote plant and animal health) (Doran and Zeiss 2000). A healthy soil has the capacity to provide resistance to external stresses, for example, the suppression of disease spread can be seen as a manifestation of soil health, (Van Bruggen and Semenov 2000; Berkelmans et al. 2003; Schmidt and Skidmore 2003). However, it should be taken into account that for different types of soils different kinds of agricultural management systems are probably needed in order to improve or maintain soil health (Abawi and Widmer 2000)(fig. 3). A general measure of soil quality and health is organic matter content. The soil organic matter is generally known to reduce the incidence and severity of soil borne disease in crop systems by inducing its suppression (Baker and Cook 1974).

Numerous cultural practices like cropping sequences, green manures, cover crops, mulches, composts and animal manures affect soil organic matter and consequently pathogens. The incorporation of compost (Lewis et al., 1992; Hoitink and Boehm 1999; Darby et al. 2006), crop residues and biological soil disinfestation (Blok et al. 2000; Gamliel et al. 2000) is commonly reported to
red to soil-borne diseases. Yet monitoring large scale intensified crop systems is challenging. For this reason, remote sensing is a great tool to understand this dynamic above-below ground interaction. It has been showed that when plants are exposed to different stress or disturbance factors have intra-specific plant chemical variation. These differences can be analysed by the plant canopy when plant is expose, and also can be linked with soil fertility (Wessman et al. 1988; Ferwerda et al. 2005; Knox et al. 2010; Skidmore et al. 2010; Asner and Martin 2011; Carvalho 2013). Certain chemicals compounds can be associated with certain stresses and by hyperspectral reflectance this can be measured (Curran 1989). It means that soil organism can influence plant responses aboveground through chemicals changes (Kostenko et al. 2012; Carvalho 2013) and this changes can been seen through spectral reflectance patterns. Furthermore, it is known that abiotic changes can also be recognized by spectral measurements (Wessman et al. 1988; Asner and Martin 2011).

1.6 Spectroscopy and its potential for monitoring agriculture management

The spectral properties can be defined as a response of the material to a sinusoidal component (waves) which can vary in frequency (Gates et al. 1965). Spectroscopy is the part of physics concerned with the production, transmission, measurement and interpretation of electromagnetic spectra. All types of materials have a specific radiation that can be reflected, transmitted or absorbed. This interaction is dependent on the materials' constitution and its physical properties (Suits 1983). Reflectance is a common measurement in spectral sensors. Reflectance is defined as the ratio of the amount of electromagnetic radiation reflected from a surface to the amount originally striking the surface. Any illuminated surface reflects and the most important characteristic is its independence from the amount of the radiation reaching the surface of the material (Monteith and Unsworth 2013). The reflectance
spectra is however sensitive to specific chemical bonds present in the materials (Colwell 1983). It has the potential to detect the presence or absence of certain compounds, such as nitrogen, chlorophyll, among others (Ollinger et al. 2008). Many spectral vegetation indices (SVI’s), have the potential of monitoring biomass, phenology and physiological conditions of the plants (Peñuelas and Filella 1998).

The potential yield of agricultural and horticultural crops worldwide is affected by a variety of biotic and abiotic stress factors. These factors can play a role in plant chemical dynamics through time. Spectroscopy can measure these chemical or physical properties in a non-destructive method (West et al. 2010). The plant interaction with biotic and abiotic factors has also been reported in spectral measurements (Jackson 1986; Graetz 1990), since the biophysical to the biochemical plants change (Mahlein et al. 2012). The spectroscopy and other new technology are a great tool to develop new techniques in sustainable agriculture management. The new technology can prevent losses between 18 to 32% caused by pests, excluding also the losses because of the fungi, bacteria and viruses (Oerke and Dehne 2004).

The occurrence of diseases in plant growth (even more in early stages) in the field is heterogeneous in time and space (Waggoner and Aylor 2000). However, traditional agricultural management considers the crop field as a homogeneous site, so most of the times the input of nutrients or pesticides don’t fit the dynamic demand (Steiner et al. 2008). This will have an impact on soil health and in the economic value of crops. Therefore remote sensing techniques could be a reliable technique for accurate estimate of diseases, predict yield loss, monitor and forecast epidemics and understand biological processes.

The use of such innovative technologies in agriculture has been summarized as “precision agriculture” where the main focus is to optimize the agricultural production of crops, reducing damage and waste into the
environment. This concept takes spatial and temporal variability within the field into account, instead of the traditional management based on a hypothetical average (Pierce and Nowak 1999). The site specific management is expected to change the yield and the quality of the production, lead to a better use of the resources, while preserving the quality and the quantity of the agricultural soils and products (Gebbers and Adamchuk 2010). A recent example is reported by (Mahlein et al. 2013) as they develop specific spectral indices (SDIs) for the detection of diseases in crops in order to improve disease detection, identification and monitoring in agricultural systems. The potential of the remote sensing techniques for agricultural purpose has been showed several times, especially in fungal diseases (Thenkabail et al. 2000; Steddom et al. 2005; Hillnhütter and Mahlein 2008; Galvão et al. 2009; Oppelt, 2004; Hillnhütter et al. 2011). The precise disease control is a demanding challenge within precision agriculture but can offer a good prospective to decrease the costs of fungicide use and its environmental impact (Steddom et al. 2005; Hillnhütter and Mahlein 2008; Bock et al. 2010; Nutter Jr et al. 2010). In this I will study the difference between the biotic and abiotic factors influencing disease attack on crops using spectral data.
1.7 Research Objectives

1.7.1 General objective

Abiotic stress factors such as heat, cold, drought, salinity, and nutrient stress have a big impact on world agriculture, and it has been suggested that they reduce average yields by more than 50% for most major crop plants (Hammond-Kosack and Jones 2000). Furthermore, plants must defend themselves from attack by a vast range of pests and pathogens, including fungi, bacteria, viruses, nematodes, and herbivorous insects (Herms and Mattson 1992). Since each stress can elicit plant cellular and molecular changes (de Boer 1993), leaf optical properties are influenced by the concentration of the biochemicals, water content and leaf structure (Gueymard 2004).

In this thesis we will try to study above-below ground interactions through plant spectral reflectance and this potential to be applied in different agricultural regimes, aiming to improve crop monitorization and management in future approaches for precision agriculture.

1.7.2 Specific objectives

⇒ Determine the influence of 3 different soil regimes in the plant growth and their spectral patterns;
⇒ Test greenhouse spectroscopy to estimate plant and soil properties via the plant spectral signatures;
⇒ Study the temporal evolution of crops using spectral pattern changes.
1.8 Research Questions and Hypothesis

**Q1.** How can the 3 types of soil management influence plant performance and its spectral signature variation?

   **H1:** The best practice in soil management will have a positive effect in the growth of the plants which will lead to lower reflectance peak in the visible region from 400nm to 700nm spectral data.

**Q2.** How do the 3 types of soil management influence the speed of spread of plant pathogens? Can it be detected by spectral data?

   **H2a:** High nutrient treatment will increase the leaf reflectance spectra in the NIR and the pathogen spread will increase the reflectance in the visible region.

   **H2b:** Disturbance of normal root function leads to an increment in the leaf reflectance on visible and near infrared spectral regions.

**Q3.** How can soil biota influence plants and its spectral signature?

   **H3:** Variation in soil biotic conditions will show different plant reflectance pattern
CHAPTER 2

Materials and Methods
This chapter describes the materials and methods of the research and these are presented by:

2.1 Research flow and steps
2.2 Species description
   2.2.1 Plant (crop) species
   2.2.2 Soil pathogens
2.3 Field sampling
2.4 Experimental design
   2.4.1 Treatments setup
2.5 Soil pathogen inoculation
2.6 Quantitative Nematodes determination
   2.6.1 Extraction of active nematodes from the soil, the Oostenbrink elutriator method
   2.6.2 Cold staining of root material
2.7 Leaf spectral measurements
2.8 Spectral data processing and data analysis
2.1 Research workflow and steps

The workflow presented (Fig.4) has as the main objective the help of constructing the work method. The research was divided in three main activities: Pre-laboratory work; Laboratory and Post-laboratory work. Each of these main activities includes many other types of small activities, that some are in the workflow some of them were not included.

![Research workflow diagram](image-url)
2.2 Species description

2.2.1 Plant (crop) species

The Sugar beet (Beta vulgaris) and Corn (Zea mays)

Sugar beet (Beta vulgaris L. ssp. vulgaris var. altissima Döll) belongs to the family Chenopodiaceae (OECD) and it is a typically biannual plant. The tuber is the storage organ of the sugar beet plant, and the tuber is as large as 90% of the plant’s biomass, while the crown represents the rest of its biomass (Elliot and Weston 1993; Gonzalez Garcia et al. 2006).

The tuber contains high concentration of sucrose and is mainly used for the sugar extraction, as well as for bio-ethanol and bio-gas production (Řezbová et al. 2013).

Since the Napoleonic wars the sugar beet crop has had an upsurge in production in Europe and in the USA. Breeding increased the total content of sugar from 1.6 to 20 %, increasing the value of the sugar beet crop (FAO 2009a). However, the production and the price of sugar beet have recently decreased in the EU due to political decisions related to agricultural subsidies and due to strong competition with sugar from sugar cane. Today the EU, the USA and the Russian Federation are the biggest sugar beet producers with an overall harvested area of 1.5 million ha (FAO 2011), being an important crop in the Netherlands. Thus, 73000 ha were grown in 2013 in the Netherlands (Statistiek 2014).

Corn (Zea mays L.) derives its name from the Arawak mahizi which literally means ‘that which sustains life’ (MacCann 2001). Zea mays is an annual grass of the Poaceae family.

One main difference between corn and other cereals is that it tolerates seed heads, ears that are larger than any other grass. Corn has a higher yield of food per unit than any other grain, being this productivity is one of the main
contributing factors of corn’s appeal to farmers. Corn is generally cultivated throughout the world, occupying 159 million ha, with a yield of over 5 ton/ha (FAO 2009b), but the US produces 40% of the total world’s harvest that in 2009 was 817 million tones.

Sugar beet and maize are frequently grown in the same crop rotation. The proportion of cultivated maize in several European sugar beet growing areas is expected to rise due to a projected increase in demand for renewable resources over the next few years (Kluth and Varrelmann 2010).

### 2.2.2 Soil pathogens

As soil pathogen attack treatment we will use two of the most common soil pathogens that affect crops: the fungus Rhizoctonia solani (Kühn, 1858) and the nematode Pratylenchus penetrans (Cobb, 1917).

*Rhizoctonia solani* is a very common, soil-borne fungal pathogen of crops grown throughout the world and it has a great diversity of host plants. It’s a complex fungus composed of genetically isolated populations called anastomosis groups (Ogoshi 1987). In this experiment we will use AG 2-2I1IB which causes root and crown root in sugar beet and root rot in Corn (Sumner and Bell 1982).

It is estimated that *Pratylenchus* causes 77 billion dollars of damage worldwide each year as they are migratory endoparasites responsible for root lesion. The usual sign of disease is plant root necrosis (Abawi and Widmer 2000), but they can also introduce more pathogens as secondary infections (Perry and Moens 2006). Both diseases are difficult to monitor by farmer due to their main effects being belowground.
2.3 Field Sampling

Vredepeel is located in the south-eastern part of the Netherlands (51°32‘N, 5°52‘E) (van der Linden and van der Pas 1998). It is part of the Applied Plant Research facilities as a field station for long-term experimental farming. Long-term agricultural experiments are essential to provide empirical data necessary to evaluate sustainability of agricultural systems (Geng et al. 1990; Barnett et al. 1995). This field station is used to conduct research into arable farming, multifunctional agriculture and field production of vegetables.

The soil has been classified as a mesic Typic Haplaquod (De Bakker 1979) and it consists of 30 cm dark colored sandy topsoil overlying non-calcareous Aeolian sand. The organic matter content of the top of the soil is approximately 4.8%, and the underlying sand around 0.2%. The clay content of the soil is less than 3% (Ritsema et al. 1998).

This location had been chosen because it is a long term experiment with different kinds of soil management (Biologic treatment, artificial fertilizer treatment, and manure treatment) (Tab. 1). The soil has been part of a 25-year organic amendment trial. The annual amendment treatments were either 25 ton/ha manure, 50 ton/ha composted municipal organic waste, or a control which received mineral fertilizer (300 kg ha⁻¹ KAS) and no organic matter (OM), to see more about the field site see (Korthals et al. 2014). The Biologic, Artificial Fertilize and Manure type of soil had respectively 4.56%, 4.05% and 3.98% of OM in the beginning of the experiment (Tab. 1) (Bin 2014).

Soil was collected on 25th September 2013. In each soil treatment we made two parallel transects with one meter of distance from each other. From each treatment approximately 190 kg of soil were collected in 12 kg sterile plastic bags.
Each sampled bag was collected at a distance of 20 steps between each sample to approximately 15 cm depth. All soils were sieved with a 5mm mesh size to homogenize any spatial variation without obliteration of the soils ‘biotic characteristics’ (FAO 2011; Carvalho et al. 2012). Afterwards each soil was divided into bags of 2.75 kg to standardize the amount of soil to put in each pot and stored at 4°C.

**Table 1** Overview of the Dissolved Organic Carbon (DOC, is an important part of the naturally occurring organic matter of soils, and a key driver of several ecosystem services of soils) composition of the soil at the start of the experiment, shown in mg C per liter soil solution for each DOC fraction. HA denotes humic acids, FA fulvic acids, Hy hydrophilic C compounds, HON hydrophobic neutrals, DOC the total DOC, and %OM the percentage of Organic Matter content, more information (Bin 2014).

<table>
<thead>
<tr>
<th>Agricultural Regime Treatment</th>
<th>HA</th>
<th>FA</th>
<th>Hy</th>
<th>HON</th>
<th>DOC</th>
<th>%OM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artificial Fertilizer</td>
<td>5.95</td>
<td>2</td>
<td>1.7</td>
<td>1</td>
<td>10.61</td>
<td>4.05</td>
</tr>
<tr>
<td>Biologic</td>
<td>7.05</td>
<td>3.35</td>
<td>1.85</td>
<td>1.35</td>
<td>13.6</td>
<td>4.56</td>
</tr>
<tr>
<td>Manure</td>
<td>8.5</td>
<td>3.55</td>
<td>2.75</td>
<td>1.05</td>
<td>15.88</td>
<td>3.98</td>
</tr>
</tbody>
</table>

**2.4 Experimental design**

**2.4.1 Treatments setup**

It has been shown in several studies that greenhouse bioassay results correlate well with field responses (Chaerle et al. 2004; Hillnhütter et al. 2010). Therefore, we conducted a bioassay to measure the effect of different kind of soil pathogens in sugar beet and corn and monitor the stage of the plant through time.

The pots were rectangular (80cm x 8cm, 3cm high) and they were separated with a metal bar to have two sides, they were filled with 2.75 kg of
soil each side with an initial moisture content of ± 20% (v/w). A row of 25 sterilized seeds were sown 1.5 cm apart and 0.5 cm deep (for sugar beet) or 1 cm deep (for corn). Over 9 weeks all plants were growing in a greenhouse under 24-22°C with a 16h photoperiod and 80% relative humidity (Mendes et al. 2011). The plants were watered weekly with demineralized water and 2 times during the experiment with standard Hoagland solution (macronutrients only) (Mendes et al., 2011). All pots were maintained at a soil water hold capacity (WHC) of 60%. To minimize spatial variance, the pots were ordered using a completely randomized design, which was re-randomized after four weeks. In order to mend observed nutrient deficiencies in the crop plants, 20 mmol NH₄NO₃ and 13 mmol KH₂PO₄ were added per pot in two application steps (on 28 October and 3 November), using 50 ml of a 200 mM NH₄NO₃ and 130 mM KH₂PO₄ solution per application step. On 25 November 2012, 54 days after the planting of the sugar beet seeds and corn seeds, the experiment was terminated by harvesting the plant, and the biomass was calculated after drying all the plants at 70°C for two days.


- X - Field soil with plants but no treatments;
- R - Field soil with plants and Rhizoctonia solani AG2.IIIB inoculated;
- P - Field soil with plants and Pratylenchus penetrans inoculated;
- N - Field soil with plants with Nutrients added (WHC realized with Hoagland solution during planting);
- G - Gamma radiated soil with plant + Rhizoctonia (60 kGray, Isotron, The Netherlands);
- E - Field soil without plants;
- NR - Field soil with plants, nutrient treatment + R. solani;
Of the treatments X, R, P, N and NR, there were 5 replicates and 3 replicates for E, G and L. In total resulted in 146 pots with 25 plants in each bioassay in a grand-total of 3650 plants.

2.5 Soil pathogen inoculation

*Rhizoctonia solani*

*Introduction of the fungi into the pots*

After 10 days of the seedling growth, the fungal pathogen *Rhizoctonia solani* (anastomosis group AG2-2IIIB) was placed just underneath the soil surface 1 cm from the first seedling, with the mycelial side towards to the plant. The culture was introduced in an agar plug of 5 mm of a one week-old potato dextrose agar (PDA). The spread of *R. solani* was evaluated at regular time intervals by scoring the distance between the inoculum and the most distal plant suffering from damping-off.

*Pratylenchus penetrans*

*Pratylenchus*

*Introduction of the nematodes into the pots*

The nematodes were introduced 7 days after sowing the seeds, after watering each pot. They were inoculated at the base of the first plant in a whole 0.5 cm deep, 0.5 cm wide. We introduced 18000 individuals of *P. penetrans* in a 500 ml solution and we introduced them using a 5 ml pipet into the whole (5 ml a 180 ind/ml). We made the same procedure for the control plants, but used demineralized water instead of nematode solution.
2.6 Quantitative Nematodes determination

2.6.1 Extraction of active nematodes from the soil, the Oostenbrink elutriator method


This equipment has been developed for an efficient and consistent recovery of active nematodes from soil. This method is used in many laboratories and is suitable for samples from 100 - 1000 ml. The separation of nematodes and soil particles is achieved in 3 steps and is based on the weight, the shape and the activity of the nematode.

We separate 4 samples from each type of soil with 100 ml each in total 12 glass jars. We start to fill the elutriator with clean water to the outlet of the funnel pipe. When is full we open the constant upward water stream which enters the base of the elutriator through a perforated pipe (flow set 1000 ml/min). We placed the moist soil sample in the top sieve and washed it into the elutriator via the funnel with the baffle plate until 2/3 of the column is filled up. We turned off the top nozzle and reduce the upward stream of water to 6000 ml/min, in order to fill at a constant water stream. As such the heavier particles in the suspension settle, the nematodes, the particles of the same weight and lighter float. After that the suspension was poured into a vertical rack of four sieves (φ 30 cm, 0.0045 mm pore size) and the sieved solution transferred to a plastic bowl. Afterwards that we decant the suspension over a sieve of φ16 cm, 0.385 mm pore size, in which a double cotton wool filters, was fixed. The sieve was placed in a shallow tray filled containing around 100 ml of tap water. One day later we removed the sieve with the filters from the extraction dish and the final suspension was store in the 4 °C fridge for analyzes.
2.6.2 Cold Staining of root material

Staining simplifies microscopic detection of nematode root infections and we will use this technique in order to count the root parasitic nematodes. This method is commonly used maintaining the condition of the nematodes extracted from the roots good to identify at high magnification (FAO 2009a).

After we carefully washed the root material with water we cut off a small part of the root of Sugar beet and Corn from the nematode infected and the control treatments. In a fume hood we inserted the small roots with forceps in a lacto phenol solution and stored in a cold chamber at 4 ºC for two months. The nematodes will stain blue while the plant tissue will not be colored. After two months we wash off the excess stain with water and the root material was transferred to pure lactophenol, afterwards in order to observe them under the microscope the rots were transferred to a petri dish filed with glycerin/water 1:1 for more information about the protocol see (Van Bezooijen 2006).

2.7 Spectral measurements

Spectral reflectance data were collected with an ASD Fieldspec 3 spectrometer with an ASD plant-probe and leaf-clip device attached (ASD Inc., Boulder CO, USA). The instrument has a spectral range between 350 and 2500 nm with 3 nm spectral resolution in the 350 nm – 1000 nm and 10 nm between 1000 nm and 2500 nm wavelengths. The plant-probe was designed for non-destructive data collection from live plants using a heat sensitive halogen light bulb (color and temperature 2901 ±10º% K). It has a spectral measurement spot size of 10 mm radius. The leaf clip has a gentle gripping system designed to hold the sample in place without inflicting damage or removing the sample. Since
we were interested in reflectance measurements the black panel background of the leaf-clip was used in each measurement (Carvalho 2013).

One of the objectives of the measurement was to monitor the differences between leaf reflectance over time, so we had chosen for corn 10 out 25 plants per pot to measure (plants 1, 2, 5, 7, 10, 12, 15, 17, 20, 21). To monitor the differences overtime in the Corn leaves through spectral reflectance we collect data 5 times during the experiment (10/10/2013 - 17/10/2013 – 25/10/2013 - 30/10/2013 - 08/11/2013). For each point of time we got 625 measurements.

For Sugar beet the measurements were different as the seedlings were too small to measure the leaves in the first weeks. As such we measure only 2 time points during the experiment (05/11/2013 – 14/11/2013) trying to follow the same sequence of plant has we did with Corn.

2.8 Spectral data processing

All the spectral measurements were offset corrected with software ViewSpec Pro 5.6.10 (ASD Inc. Boulder, USA). The 350-359 nm spectral range was removed after visual inspection because they were highly noisy (MacCann 2001). The spectral range used for all the analysis was equal for all leaf samples in order to ensure that the models and band selection could be compared within reflectance measurements.

2.9 Spectral data analysis

The spectral data was analyzed using vegetation indices. The indices were chosen according to the literature (Tab. S6). The indices for further discussion and analyzes were chosen with a PEARSON correlation test to avoid autocorrelation statistical issues, often found between indices (Tab. S7). Were selected the Normalized Vegetation index (NDVId) (Tucker 1979; Gamon et al.
Plant stress index (PSa) (Carter 1994) and Modified Red-edge Position (mREP) (Sims and Gamon 2002; Hamzeh et al. 2012). For more information consult Chapter 6 - supplementary information. To analyze the temporal scale in the disease spread the difference between the initial time of the experiment and the harvest time was calculated.

2.10 Data analysis

The effects of soil biota were analyzed in a multivariate ANOVA analysis with plant species (Zea mays, Beta vulgaris), soil regime (AF, B, M) and soil treatment (gamma radiation, nutrients, nutrients + R. solani, R. solani, Penetrans, control) as fixed factors. The normality test was analyzed. Shoot dry weight and vegetation indices were used as dependent variable in the ANOVA for all the analysis. Post-hoc Tuckey tests were performed to analyze which groups were significantly different from each other. Pearson Correlation test between spectral indices was done to avoid autocorrelation statistical issues. The ANOVA analysis and Pearson correlation was performed in IBM SPSS Statistics 22 for windows 8.
CHAPTER 3

Results
This chapter describes the results of the research and these are:

3.1 Soil treatment effects on plant biomass
3.2 Spectral Reflectance in Harvest
3.3 Temporal Difference in Spectral Indices
3.1 Soil treatment effects on plant biomass

The studied parameters (soil, treatment and species) had a significant influence on the total plant biomass. The soil type (Tab. 2, P= 0.003), species (Tab. 2, P< 0.001), and treatment (Tab. 2, P< 0.001), significantly influenced plant biomass, but, regarding interactions of the factors, the three-way interaction (soil*species*treatment), the soil*treatment did not result in a significant effect (Tab. 2, P> 0.05). On other hand, the treatment*species (Tab. 2, P< 0.001) interaction resulted in significant effects on the total average plant biomass (Tab. 2). The total biomass in the Corn was always higher than the sugar beet (Fig. 5).

Figure 5 Average total biomass per pot in dry biomass (left - corn -Zea mays; right - Sugar beet - beta vulgaris) in six different treatments (G - gamma radiation; N - nutrients; NR – nutrients + R. solani; P - P. penetrans; R - R. solani; X - control). Error bars are standard errors. Statistically different values between applied treatments are denoted by different letters.
Plants grown in Artificial fertilized (AF) soil were significantly smaller than plants from Manure (M) and the Biologic (B) type of soil, however there were no significant differences in the average total biomass between Manure and Biologic soils independently of the species studied (Fig. 5). In both plant species the fertilization treatment significantly increased shoot biomass when compared to the other treatments (Fig. 5, P< 0.001), however, in both species, Nutrients (N) and Nutrients + R. solani (NR) treatment did not have a significant difference between each other (Fig. 5, P> 0.05). In the Manure soil type, the total biomass of the Corn species was higher in the treatment P. penetrans (P), R. solani (R) and Control (X). In the same species in the treatment Gamma (G), Nutrients (N)

![Figure 6](image_url)  
**Figure 6** Average shoot biomass per pot in dry leaves (left - corn - Zea mays; right - Sugar beet - Beta vulgaris) in six different treatments (G - gamma radiation; N - nutrients; NR – nutrients + R.Solani; P - P. penetrans; R - R.Solani; X - control). Error bars are standard errors. Statistically different values between applied treatments are denoted by different letters.
and Nutrients + *R. solani* (NR) in the Biologic soil type had a higher biomass than the other types of soil. The Artificial fertilizer type of soil had always a lower biomass when compared to others types of soils, with the exception in the *R. solani* treatment, where the lowest biomass was in the Biologic soil. In the Sugar beet the Gamma treatment the Manure type of soil had higher total biomass than the Biologic type of soil (Fig. 5). In the same species there was increment on total biomass in the Biologic type of soil when was added nutrients in the *R. solani* treatment.

In both plant species shoot biomass was significantly affected by the different types of soil (Tab. 3, *P* = 0.015) and different treatments (Tab. 3, *P* < 0.001). No interaction was found between the plant species, type of soil and treatment was not significant (Tab. 3, *P* > 0.05). Manure type of soil had significant differences from the Artificial fertilizer (Fig. 6, *P* < 0.001) but not from the Biologic (Fig. 6, *P* > 0.05) in both plant species. There were no significant changes in shoot biomass between the treatments with *P. penetrans*, Gamma radiation, *R. solani* and Control (Fig. 6, *P* < 0.001). Significant differences were found between the *P.penetrans* treatment and Gamma radiation, Nutrients, and Nutrients + *R. solani* (Fig. 6, *P* < 0.05). In Corn, this difference resulted in less biomass in the Gamma treatment when compared to the Nutrients, Nutrients + *R. solani* and *P. penetrans* treatment. Furthermore, in the same species, the *P.penetrans* treatment had less biomass than Nutrients and Nutrients + *R. solani* in the Biologic soil type. In Corn species, the Control, *R. solani*, *P. penetrans* and Gamma treatment in Manure type of soil higher than in the other treatments was found, with the exception of Nutrients and Nutrients + *R. solani* treatment, where the Biologic soil had higher shoot biomass (Fig. 6). The Artificial Fertilizer soil type had always lower shoot biomass than the other types of soil. The Control treatment had lower shoot biomass values when compared to disease treatments (*R. solani* and *P. penetrans* treatment) in the same species in the Manure type of soil. The Gamma treatment had the lowest shoot biomass in this
species (Fig. 6). In Sugar beet the significant differences are in the higher shoot biomass of the Nutrients + *R. solani* and Nutrients treatment both in the Biologic soil type (Fig. 6). As in Corn species, the Artificial Fertilizer type of soil had always the lowest shoot biomass (Fig. 6). The treatment Gamma, *R. solani*, and Control had no significant differences in this species. The shoot biomass was always higher than the roots biomass, independent of treatment or species (data not shown). In general the total biomass was higher in the first segment of the pot (Fig. 5). The highest shoot biomass was found in the Corn Biologic type of soil, which was 10 times larger if compared to Corn grown in the Manure soil type (Fig. 6). The highest root biomass was found in Corn species, *R. solani* treatment of the Manure soil type which was 30 times bigger than in the Artificial Fertilizer soil type in the Gamma treatment. The lowest root biomass was in *R. solani* treatment in Sugar beet species, in the Manure type of soil (data not shown).

Table 2 ANOVA analysis of the effects of species, treatments and soils on the average total biomass. The significant P-values are highlighted in bold

<table>
<thead>
<tr>
<th>df</th>
<th>F</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>27</td>
<td>34.08</td>
</tr>
<tr>
<td>Intercept</td>
<td>1</td>
<td>1268.53</td>
</tr>
<tr>
<td>Soil</td>
<td>2</td>
<td>6.08</td>
</tr>
<tr>
<td>Species</td>
<td>1</td>
<td>722.35</td>
</tr>
<tr>
<td>Treatment</td>
<td>5</td>
<td>9.07</td>
</tr>
<tr>
<td>Soil * Treatment*Species</td>
<td>6</td>
<td>0.80</td>
</tr>
<tr>
<td>Treatment*Species</td>
<td>5</td>
<td>6.04</td>
</tr>
<tr>
<td>Soil*Species</td>
<td>2</td>
<td>5.48</td>
</tr>
<tr>
<td>soil*treatment</td>
<td>6</td>
<td>0.52</td>
</tr>
<tr>
<td>Error</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>127</td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>126</td>
<td></td>
</tr>
<tr>
<td>Mean Square</td>
<td></td>
<td>0.59</td>
</tr>
</tbody>
</table>
Table 3 ANOVA analysis of the effects of species, treatments and soil on the shoot biomass. The significant P-values are highlighted in bold.

<table>
<thead>
<tr>
<th>df</th>
<th>F</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>27</td>
<td>43.44</td>
</tr>
<tr>
<td>Intercept</td>
<td>1</td>
<td>1934.64</td>
</tr>
<tr>
<td>Soil</td>
<td>2</td>
<td>5.67</td>
</tr>
<tr>
<td>Species</td>
<td>1</td>
<td>923.97</td>
</tr>
<tr>
<td>Treatment</td>
<td>5</td>
<td>12.10</td>
</tr>
<tr>
<td>Soil*Species</td>
<td>6</td>
<td>0.35</td>
</tr>
<tr>
<td>Treatment*Species</td>
<td>5</td>
<td>8.94</td>
</tr>
<tr>
<td>Soil*Species</td>
<td>2</td>
<td>4.35</td>
</tr>
<tr>
<td>Soil*treatment</td>
<td>6</td>
<td>0.32</td>
</tr>
<tr>
<td>Error</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>127</td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>126</td>
<td></td>
</tr>
</tbody>
</table>

Mean Square = 0.02

### 3.2 Spectral Reflectance

In this analysis we focused in three Spectral Vegetation Indices (SVI’s) the Normalized Difference Vegetation Index (NDVI), the Plant Stress index (PSa) and the Modified Red Edge Position (mREP). All other indices were correlated with the three selected (Tab. S7).

The soil factor was a significant factor influencing mREP (Tab. 4, P= 0.01), while treatment factor influenced NDVIc (Tab. 4, P= 0.02), mREP (Tab. 4, P= 0.001) and Ps (Tab. 4, P< 0.001). Species were significantly different (P< 0.001). Indeed, the tested factors influenced all of the analyzed indices, with the exception of DSWI (Tab. 4). The interaction between factors also resulted in significant effects in the chosen indices. The three-way interaction (soil*species*treatment) did not influenced the SVI’s chosen (Tab. 4, P> 0.05), while two-way interactions had effect in the SVI’s chosen. The interaction between treatment and species resulted in significant effects in NDVIa (Tab. 4, P< 0.001), MCARI (Tab. 4, P< 0.001), mREP (Tab. 4, P< 0.001) and Ps (Tab. 4, P<
0. 001). The interaction of the soil factor with treatment and species influenced three of the analyzed indices. In the first case (soil*treatment), a significant higher effect was detected in NDVId (Tab. 4, P= 0. 003), while in the second case (soil*species) the affected indices were NDVId (Tab. 4, P= 0. 03), Psa (Tab. 4, P= 0. 05) and mREP (Tab. 4, P= 0. 04), with less effect in the Psa index.

The Manure type of soil was significantly different from the Biologic and Artificial fertilizer (Fig. 7- a). In the Corn species the NDVI decreased in the Biologic comparing to the others soil types (Fig. 7- a), with less effect in the P. penetrans treatment. In the same species Gamma-radiation treatment there was a significant increase in the NDVI in the Artificial fertilizer soil (Fig. 7- a). The NDVI increased in the Manure type of soil in the treatment P. penetrans, R. solani , and Control and decreased in the Biologic type of soil (Fig. 7- a). In the Sugar beet species, the soil type with higher positive NDVI effect was the Manure in all the treatments and the Biologic soil was always lowest positive effect. Treatment wise Gamma had a significantly more NDVI effect while the less effect outcome was in the Biologic type of soil in the R. solani treatment.

In the Plant Stress index (PSa) significant effect of species and treatment (Tab. 4, P< 0. 001) were found. The Manure and the Biologic types of soil were significantly different from each other in both plant species with a significant increase of Psa in the Biologic type of soil (fig. 7- c, P< 0. 001). In the Corn species the PSa was always higher in Biologic soil than in the other types of soil especially in the R. solani treatment (Fig. 7- c). Treatment wise PSa was always higher in P. penetrans, R. solani and Control treatments than in the Gamma radiation treatment (Fig. 7- c). In both species Nutrients + R. solani treatment had a significant increase in Psa index in the Biologic type of soil. In the Control treatment and in the Pratylenchus treatment the positive trend in effect was from Biologic to Artificial fertilizer and to Manure types of soil. The lowest Psa effect in Corn species was in the Artificial fertilizer type of soil, especially in treatment Gamma (Fig. 7- c). In the Sugar beet species the lowest Psa effect
was found in the *R. solani* treatment in the Manure type of soil and the highest Psa effect in Biologic soil in the same treatment (Fig. 7- c). Also in this species the Psa increased in the Nutrients + *R. solani* comparing to the *R. solani* treatment.

The analyses of the Modified Red Edge Position (mREP) index showed significant differences between species, soil types and treatments (Tab. 4, P< 0.05). There was a highly significant interaction between treatment and species (Tab. 4, P< 0.001). In corn species Gamma treatment there was a significant increase in mREP from Biologic to Manure to Artificial fertilizer (Fig. 7- b). There was a significant decrease in mREP in the *P. penetrans* treatment in Biologic soil type comparing to the other types of soil. The Control treatment and the diseases treatments (*R. solani* and *P. penetrans*) increase the mREP trend from Manure, to Artificial Fertilizer to Biologic type of soil. In the Sugar beet there was an increase the mREP effect in the Control treatment of the Manure soil type comparing to all the others treatments. And there was a decrease in mREP effect in the *R. solani* treatment of the Biologic soil comparing to the other treatments (Fig. 7- b). In the Gamma treatment mREP had a significant increase from Artificial Fertilizer to Biologic to Manure (Fig. 7- b). The treatment Control, *R. solani* and *P. penetrans* increase the mREP trend from Manure, to Artificial Fertilizer to Biologic type of soil. In the sugar beet species the mREP increased in the Nutrients treatment comparing with Nutrients + *R. solani* treatment (Fig. 7- b).
Monitoring the impact of soil management on plant spectral reflectance and soil-borne disease resistance - ML
**Figure 7** Mean per pot of leaves reflectance indices (a– NDVI, b– mREP, c– PSa) per soil treatment (g). Soil origins are Biologic (B) in dark grey with stripes, Artificial Fertilizer (AF) in light grey and Manure (M) in dark grey. On average, there were 5 samples per species per treatment. Error bars are standard errors. Statistically different values between applied treatments are denoted by different letters.
3.3 Temporal Difference in Spectral Indices (initial time-harvesting time)

Species were significantly different in the NDVIs temporal analyzes (Tab. 4, P < 0.001). There was a two-way interaction between species*treatment, soil*treatment and species*soil (Tab. 4, P ≤ 0.003). The effect of treatment or type of soil alone was not significant (Tab. 4, P > 0.05). In Corn species the Gamma radiation treatment in the Artificial fertilizer soil the mean NDVI temporal difference was approximately null, while there was a positive NDVI temporal difference in the other two soil regimes. In the same species P. penetrans treatment showed an increase in NDVI temporal difference, especially in the Biologic soil, when compared to other treatments and soils (Fig. 8- a). In the Corn species Biologic soil had a significant increase in NDVI temporal difference when compared to the other soils type (Fig. 8- a). The only exception was the R. solani treatment in the Artificial fertilizer soil which had a superior positive NDVI temporal difference (Fig. 8- a). While in Corn species the Nutrients + R. solani treatment, had always a positive NDVI temporal difference, in Sugar beet the NDVI temporal difference was always negative (Fig. 8- a). The Biologic type of soil showed the highest negative NDVI temporal difference in the R. solani treatment, the lowest negative NDVI temporal difference was in Gamma radiation treatment in Sugar beet species. In sugar beet, the lowest negative NDVI temporal difference was found in Manure type of soil (Fig. 8 – a). This temporal difference is especially notorious in Control and pathogen treatments. The NDVI temporal difference in the Artificial Fertilizer soil was lower in the Gamma treatment.

The treatment (P < 0.001) and the species (P < 0.001) significantly influenced the temporal differences in the Psa index. Regarding the interaction of the factors, the tree-way interaction (soil*treatment*species) did not result in a significant effect (P > 0.05), neither the interaction between treatment*soil
Thus, the two-way interaction species*soil (Tab. 4, P > 0.05) and treatment*species (Tab. 4, P < 0.001) did result in an effect in the temporal differences. There was no significant effect of soil type into PSa temporal differences (Tab. 4, P > 0.05). In the Corn species the temporal differences were always in the negative range with the higher Psa negative temporal difference in the Gamma radiation treatment and lower Psa negative temporal difference in the R. solani treatment both in the Biologic soil (Fig. 8-c). The Biologic soil had always the higher Psa negative temporal differences. On the other hand, Sugar beet plants had the Psa temporal differences in the positive range; the Biologic type of soil had the higher Psa positive temporal difference in the R. solani, P. penetrans and Control treatment, the smaller Psa temporal difference in the R. solani treatment, in the Manure soil (Fig. 8-c). In sugar beet species Gamma radiation treatment had an increase in Psa positive temporal difference from Manure, to Artificial fertilizer, to Biologic (Fig. 8-c).

In the mean temporal difference in the mREP index treatments (Tab. 4, P = 0.001), soil type (Tab. 4, P = 0.007) and species (Tab. 4, P < 0.001) affected the index. Concerning the interaction of the factors, the three-way interaction (soil*treatment*species) did not result in a significant effect (P > 0.05), neither the interaction between treatment*soil (Tab. 4, P > 0.05). The soil*species interaction was found (Tab. 4, P < 0.004) between initial and harvesting time. There was also a significant effect in two-way interaction between treatment and species. In mREP there was an increase in mREP temporal differences in the corn grown in biologic soil when compared to the others agricultural regimes where the differences were lower (Fig. 8). Treatment wise, Nutrients and R. solani treatment had an increase in the mREP temporal difference when compared to the other treatments in Corn species. The comparison between the treatment Nutrients and Nutrients + R. solani showed a significant positive mREP temporal difference between them, from Nutrients to Nutrient + R. solani (Fig. 8-b, P < 0.001). In Corn species Gamma treatment had a decrease of mREP temporal difference from
Biologic to Manure, to Artificial fertilizer. In Sugar beet species all the mREP temporal differences were in the negative domain (Fig. 8- b). In the same species *R. solani* treatment had a highly decrease of mREP temporal differences from Artificial fertilizer, to Biologic, to Manure. In the other hand Gamma treatment had the lowest decrease of mREP temporal difference in the Biologic type of soil (Fig. 8- b). There was no effect of nutrient fertilization in the *R. solani* infected treatment (i.e. Nutrients + *R. solani* vs *R. solani*).
Figure 8 Leaves temporal differences (T_initial – T_harvesting time) in: panel a– NDVI, panel b– mREP and panel c– PSa) per soil treatment. Soil origins are Biologic (B) in dark grey with stripes, Artificial Fertilizer in light grey and Manure in dark grey. Error bars are standard errors. Statistically different values between applied treatments are denoted by different letters.
Table 4 ANOVA analysis of the effect of species (Zea mays, Beta vulgaris), Soil origin (M, B, AF), And Soil Treatment (N, NR, X, G, P, R) on the different vegetation indices. The significant P-values are highlighted in bold.

<table>
<thead>
<tr>
<th>Fixed actors</th>
<th>df</th>
<th>NDVId</th>
<th>NDVId</th>
<th>NDVIc</th>
<th>NDVIc</th>
<th>MCARI</th>
<th>MCARI</th>
<th>NRI</th>
<th>NRI</th>
<th>EVI</th>
<th>EVI</th>
<th>mREP</th>
<th>mREP</th>
<th>PRIa</th>
<th>PRIa</th>
<th>DSWI</th>
<th>DSWI</th>
<th>PsA</th>
<th>PsA</th>
<th>Nitrogen Index b</th>
<th>Nitrogen Index b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>27</td>
<td>34.20</td>
<td>&lt;0.001</td>
<td>7.79</td>
<td>&lt;0.001</td>
<td>128.00</td>
<td>&lt;0.001</td>
<td>2.50</td>
<td>0.001</td>
<td>2.10</td>
<td>0.01</td>
<td>121.52</td>
<td>&lt;0.001</td>
<td>139.63</td>
<td>1.54</td>
<td>0.07</td>
<td>64.28</td>
<td>&lt;0.001</td>
<td>1.68</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>1</td>
<td>26.50</td>
<td>&lt;0.001</td>
<td>13.41</td>
<td>&lt;0.001</td>
<td>2720.60</td>
<td>&lt;0.001</td>
<td>0.00</td>
<td>0.00</td>
<td>35.56</td>
<td>0.01</td>
<td>1062.52</td>
<td>&lt;0.001</td>
<td>4174.93</td>
<td>&lt;0.001</td>
<td>2.34</td>
<td>0.07</td>
<td>666.13</td>
<td>&lt;0.001</td>
<td>2.41</td>
<td>0.04</td>
</tr>
<tr>
<td>Soil</td>
<td>2</td>
<td>0.65</td>
<td>0.52</td>
<td>1.69</td>
<td>0.19</td>
<td>11.75</td>
<td>&lt;0.001</td>
<td>2.28</td>
<td>0.11</td>
<td>0.56</td>
<td>0.58</td>
<td>5.29</td>
<td>0.01</td>
<td>2.22</td>
<td>0.12</td>
<td>2.00</td>
<td>0.14</td>
<td>1.25</td>
<td>0.29</td>
<td>1.78</td>
<td>0.18</td>
</tr>
<tr>
<td>Species</td>
<td>1</td>
<td>622.72</td>
<td>&lt;0.001</td>
<td>121.17</td>
<td>&lt;0.001</td>
<td>2613.25</td>
<td>&lt;0.001</td>
<td>2.43</td>
<td>0.00</td>
<td>14.45</td>
<td>&lt;0.001</td>
<td>2621.55</td>
<td>&lt;0.001</td>
<td>3136.86</td>
<td>&lt;0.001</td>
<td>2.40</td>
<td>0.13</td>
<td>1283.33</td>
<td>&lt;0.001</td>
<td>7.47</td>
<td>0.01</td>
</tr>
<tr>
<td>Treatment</td>
<td>5</td>
<td>2.10</td>
<td>0.07</td>
<td>2.85</td>
<td>&lt;0.001</td>
<td>14.82</td>
<td>&lt;0.001</td>
<td>3.82</td>
<td>0.00</td>
<td>0.52</td>
<td>0.76</td>
<td>4.53</td>
<td>0.00</td>
<td>2.94</td>
<td>0.02</td>
<td>1.33</td>
<td>0.26</td>
<td>5.87</td>
<td>&lt;0.001</td>
<td>1.03</td>
<td>0.41</td>
</tr>
<tr>
<td>Soil * Treatment</td>
<td>6</td>
<td>1.75</td>
<td>0.12</td>
<td>0.86</td>
<td>0.53</td>
<td>0.38</td>
<td>0.89</td>
<td>1.59</td>
<td>0.16</td>
<td>1.14</td>
<td>0.35</td>
<td>1.67</td>
<td>0.14</td>
<td>1.36</td>
<td>0.24</td>
<td>2.34</td>
<td>0.04</td>
<td>1.17</td>
<td>0.33</td>
<td>2.14</td>
<td>0.06</td>
</tr>
<tr>
<td>Soil * Treatment * Species</td>
<td>5</td>
<td>7.82</td>
<td>&lt;0.001</td>
<td>1.00</td>
<td>0.43</td>
<td>13.35</td>
<td>&lt;0.001</td>
<td>0.24</td>
<td>0.95</td>
<td>1.99</td>
<td>0.09</td>
<td>7.10</td>
<td>&lt;0.001</td>
<td>0.85</td>
<td>0.52</td>
<td>1.33</td>
<td>0.26</td>
<td>7.97</td>
<td>&lt;0.001</td>
<td>1.38</td>
<td>0.24</td>
</tr>
<tr>
<td>Soil * Treatment * Species</td>
<td>2</td>
<td>6.39</td>
<td>0.00</td>
<td>2.15</td>
<td>0.12</td>
<td>9.54</td>
<td>&lt;0.001</td>
<td>0.02</td>
<td>0.98</td>
<td>1.63</td>
<td>0.20</td>
<td>5.96</td>
<td>0.00</td>
<td>1.28</td>
<td>0.28</td>
<td>1.99</td>
<td>0.14</td>
<td>5.62</td>
<td>0.01</td>
<td>1.73</td>
<td>0.18</td>
</tr>
<tr>
<td>soil * treatment</td>
<td>6</td>
<td>3.58</td>
<td>0.00</td>
<td>2.16</td>
<td>0.06</td>
<td>0.35</td>
<td>0.91</td>
<td>0.99</td>
<td>0.44</td>
<td>1.54</td>
<td>0.18</td>
<td>2.14</td>
<td>0.06</td>
<td>1.11</td>
<td>0.37</td>
<td>2.34</td>
<td>0.04</td>
<td>0.64</td>
<td>0.70</td>
<td>2.43</td>
<td>0.03</td>
</tr>
<tr>
<td>Error</td>
<td>87</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>115</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>114</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Square</td>
<td>0.00</td>
<td></td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>684.87</td>
<td>0.02</td>
<td>5.45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Mean Square             | 0.00|       | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 684.87  | 0.02    | 5.45    |         |         |         |         |         |         |         |         |         |         |         |
CHAPTER 4
Discussion
Soil supports a diverse community of organisms, both functionally and taxonomically that interact in a complex belowground foodweb. In agroecosystem, biodiversity performs a variety of ecological services beyond the production of food, including recycling of nutrients, regulation of microclimate and local hydrological processes, suppression of undesirable organisms and detoxification of noxious chemicals (Altieri 1999; Collins and Qualset 2010). These above-belowground interactions are the main focus in this study, and how the plant spectral properties can change in relation with the different agricultural regimes.

Land use and management can change the soil as habitat, influencing the soil food web and consequently soil biodiversity and productivity in different ways. In general, intensification leads to the decrease of soil organisms abundance and biomass, specially the soil organisms that drive processes of the C and N cycling (Vitousek and Walker 1989; Bardgett and Wardle 2003). Nevertheless, groups as pathogenic fungi and nematodes can benefit with the intensification of the land use, as R. solani and P. penetrans. The total biomass of these pathogens increase with a more intensive agriculture, in accordance with Van de Putten and coworkers (2001). The right choices of the agricultural practices and management, that promote net carbon accumulation, have been considered as an important mitigation option to the global warming (Freibauer et al. 2004; Gelfand and Robertson 2015; Kontopoulou et al. 2015). The impact of soil biota feedback on plant biomass interacts with soil nutrient availability, generally in a positive way (Van der Putten and Peters 1997; Wardle 2002; Reynolds et al. 2003; De Deyn et al. 2004). Therefore, the shoot biomass in both plant species studied increased with disposal of the nutrient supply (Fertilization treatment) in the biologic soil. In the fertilized and infected treatment the biomass was also high with no soil pathogen effect in the plant biomass independent of the species. This lack of pathogen effect is known to
occur, as high nutrient concentration reduces soil biota effects in plant biomass (Van der Putten and Peters 1997; Carvalho et al. 2012). Troelstra et al. (2001) concluded in several greenhouse experiments, with sterilized dune soils, that ectoparasitic nematode species affect negatively plant growth. When comparing the infected and control treatments, no losses in biomass due to infection were found in the Manure regime. The application of manure compost that is rich in nitrogen may have reduced soil-borne diseases effects by releasing allelochemicals generated during soil storage and subsequent microbial decomposition (Lazarovits et al. 1999; Bailey and Lazarovits 2003). This accumulation of nitrogen and carbon also stimulate the increment of soil microbial diversity, also conferring protection against soil-borne diseases (DOC 15.88, table 1) (Conn and Lazarovits 1999; Kumar et al. 2012). It was also recognized that manure composts have an acid pH (HA = 8.5/FA = 3.55, table 1), helping biotic control activity, such as antifungal activity due to the formation of nitrous acid (Tenuta and Lazarovits 2002; Timmusk 2003) but crop, soil type and management also play a role (Hoitink and Boehm 1999; Brussaard et al. 2007). The soil sterilization, using Gamma radiation amendment was used to show soil’s pathogenic potential in several studies (Oremus and Otten 1981; Maas et al. 1983; Van der Putten et al. 1988; Putten and Troelstra 1990; De Rooij-Van der Goes et al. 1995; Zoon 1995; Troelstra et al. 2001). In this study the lowest biomass was found in the sterilized treatments suggesting that the soil biota has influenced the plant performance (Wardle et al. 2004) in this experiment.

The Biologic type of soil had the highest shoot biomass which was, in some cases, 10 times larger than in the Manure type of soil. The fact that biological type of soil had the highest organic matter content (Bin 2014) suggests once again that the organic materials also has impact on plant health and crop productivity (Magdoff et al. 1997). In contrast with several studies where biological type of soil can reduce disease incidence, (Abawi and Widmer 2000; Gamlil et al. 2000; Bailey and Lazarovits 2003; Lazarovits et al. 2014) in this
experiment we did not see this reduction. Birkhofer et al. (2008) and van Diepeningen et al. (2006) compared two different types of soil management: the Organic and the Artificial fertilizer. Concluding that Organic systems are more efficient in crop growth, soil quality and pest resilience. In contrast, the artificial fertilizers and herbicide/pesticide application affects the potential for top-down control of aboveground pests negatively reducing the organic carbon levels as well of both nitrate and soluble nitrogen in the soil. The worst plant performance was found in the Artificial fertilizer type of soil which supports earlier results. This demonstrates that this type of management is not the most suitable to grow Corn or Sugar beet, because of the high level of pathogen attacks of these two species. One of the tested hypotheses was that the best practice in soil management would have a positive effect in the growth of the plants, in our case, the Biologic type of soil management. The biological management had a significant interaction on species biomass even within the fungus attack treatment.

In our hypothesis the best soil management would lead to lower peak in the visible region from 400 to 700nm in the spectral signature, because of the lower plant stress. Plant stress results in lower chlorophyll concentration with an increment of the reflectance in the visible region of the spectra (Adams et al. 1999; Pinter et al. 2003). Hillnhütter et al. (2011) showed that NDVI is a suitable index for the assessment of the symptoms caused by R. solani in Sugar beet plants under controlled conditions. In this experiment, the infection due R. solani was detected with the decrease of NDVI in biologic type of soil. This did not hold for the other types of soil management at the initial time. However, all the Sugar beet plants in the other types of soil management were already in severe conditions and not measured due to lack of living tissue. In the Biologic soil with the R. solani treatment the plants were less stressed than in the other types of soils as lower mortality rate occurred (personal observation). Accordingly with Garbeva et al. (2004) the microbiota in a “rich” soil tend to reduce the severity
of the attack of soilborne pathogens (general disease suppression). So disease suppression can be influenced by cropping and management practices (Huber and Watson 1970).

The pathogen treatment *P. penetrans* increase the NDVI difference between initial and final time, especially in Biologic type of soil in Corn species. It suggests that at the initial point the plant was in less stress than in the harvest time, supporting that the in plants biological managed resist longer to the infection. In Sugar beet species the NDVI negative temporal difference indicates that the Artificial Fertilizer had more stress in the plant due the pathogen. However, the lack of stress in the Sugar beet might be an artifact of the Sugar beet mortality rate. Plants that died were not possible to measure in the subsequent spectral measurements. As a result, plants that survive infection may indicate a lack of stress while what we find is a higher mortality. As such further tests need to be done in order to overcome this side effect. This condition has the effect of broadening the green reflectance peak, increasing visible reflectance (Adams et al. 1999).

Ollinger (2011) showed that plants grown without nutrient addiction revealed a different reflectance pattern than plants with nutrients addiction, as our experiment. During the experiment was seen physical modification in this treatment in plant vigor. The Fertilization treatment when applied with the pathogen *R. solani* in Biologic type of soil resulted in lower temporal differences suggesting the system responded positively to the addiction of nutrients.

The temporal differences of the Corn species in NDVI and mREP were positive, and in the Sugar beet were negative differences. In the other hand the Psa temporal differences in Corn were negative and in Sugar beet positive. This result shows that the temporal differences with SVI’s can have disadvantages only living plants can be analyzed.
CHAPTER 6

Conclusion
Conclusion and Recommendations

It was possible to detect the symptoms of pathogenic soil borne diseases on plant spectral analysis through Spectral Vegetation Indices. The temporal data was as informative as the harvest time, and could lead to better understanding on plant disease spread and dynamics. It allowed to see the difference between the stress in the beginning of the experiment and in the end, especially in the Biologic type of soil where less stressed occurred at the beginning than in the harvest time. The biological soil higher temporal variance suggested a better resilience to pathogen attack and was considered the best type of soil for plant quality. It was also found that changes in nutrient supply can decrease disease symptoms or damage by pathogens in greenhouse experiment.

I propose that further studies are needed to develop this method for use at the field scale as part of a strategy to detect early development of soil-borne diseases. It also can improve this study, the understanding of the interactions and synergisms between plant and soil with hyperspectral sensing method, using time series experiments on host-pathogen interactions and synergism as well as in screening systems for crop resistance.

The root nematode collecting methods could also be revised, since we failed to detect live nematodes after the cold staining root method. This method might be little effective.
CHAPTER 6

Supplementary information
This chapter contains the supplementary information:

6.1 Major pathogen groups

   6.1.1 Plant parasitic nematodes and fungal pathogens

6.2 Plant spectral properties

   6.2.1 The visible region
   6.2.3 The red-edge
   6.2.4 Near-infrared region
   6.2.4 Mid-infrared region
   6.2.5 Vegetation indices
   6.2.6 Ratio indices (RI)

6.3 Extra Information
6.1 Major pathogen groups

6.1.1 Plant parasitic nematodes and fungal pathogens

The soil borne pathogens are a diverse group. Fungi are considered the most important pathogen group with a great diversity. A fungus is a Eukaryote (cells having true nuclei), spore-forming (reproduction), heterotrophs organisms (obtain their carbon and energy from other organisms) and most of the true fungi are filamentous and branched (Hogg 2013). They digest food externally and they absorb nutrients directly through its cells walls. Some fungi obtain their nutrients from a living host – biotrophs, others obtain from dead host – saprotrophs, and some infect a living host, but they kill host cells in order to obtain their nutrients – necrotrophs (Deacon 2005).

Over 20,000 species of fungi are parasites and cause disease in crops and plants. All plants are attacked by one species or another of phytopathogenic fungi. Individual species of fungi can parasitize one or many different kinds of plants. Plant fungal pathogens can be divided into five main taxonomic classes based on morphological and biological characteristics: 1- Plasmodiophoromycetes, endoparistic slim mold – e.g.: Plasmodiophora brassicae, causing club root of crucifers - Spongospora subterranean, causing powdery scab of potato tubers; 2- Oomycetes, water molds. They cause several kinds of diseases including seedling blights, damping-off, root rots, foliar blights and downy mildews. Some notable diseases are the late blight of potato, downy mildew of grape vine, sudden oak death and root and stem rot of soybean, with some important pathogens including Aphanomyces, Bremia, Phytophthora and Pythium; 3- Ascomycetes, phytopathogenic fungal diseases such as peach leaf curl (Taphrina sp.), powdery mildew (Podosphaera, (apple), microsphaera (azalea) and vascular wilt diseases (Verticillium, Fusarium). Normally these diseases are recognize by signs and symptoms in leaf illnesses, fruit and vegetable rots, root, stem and soft rots, brown rot (Monilinia, cancers,
anthracnose); 4- Basidiomycetes, which are club and mushroom fungi, that cause smut fungi, corn smut, loose smut of barley, bunt of wheat, hollyhock, cedar-apple, white pine blister, needle rusts, cereal rusts. Some of the species of Ascomycetes and Basidiomycetes grow another type of spore that is asexually generated, which put them in a different class called 5- Fungi Imperfecti. The most known pathogens in this group are Fusarium, Rhizoctonia, and Verticillium. Most of the soil borne fungi persists in the soil for a long term period; usually they produce resilient survival structures like a melanised mycelium, clamydospores, oospores and sclerotia. The ones that just produce the thin mycelium usually just survive for short periods.

Nematodes are small, non-segmented roundworms. Soil borne plant-parasitic nematodes spend the greatest part of their lives in the soil. Usually in soil, plant parasitic nematodes have three ways of living, either freely, as an egg or as a durable cyst. They can be external feeders on plant roots, or residents inside roots. This group of plant parasitic pathogens disturb crops by decreasing plant vigour and growth. In the field we can see some differences in the infection, some plants are severely infested and others don’t. Therefore, in the global yield, crops will mature irregularly and the quality of the production will decrease. Root knot nematodes in general cause the drop in vigour, consequently severe distortions and swellings of roots can affect the marketability of the root crops. Cyst nematodes can survive for long periods of time, the female body dries in the form of cyst, protecting the eggs within. Needle nematodes can feed at the tips of the roots, affecting root tips to swell and causing roots to fork or branch out. On the other hand, stubby root nematodes reduce the length of the roots. In this experiment we used two different plant pathogen, as a nematode the Pratylenchus penetrans and as Fungi the Rhizoctonia solani.
6.2 Plant spectral properties

The primary source of energy for numerous biological processes of plants is the solar radiation. This radiant energy is essential in order to photosynthesize, grow and develop during its ontogeny and keep the thermodynamic balance in relation to the environment. So the interaction between solar radiation and plant is divided into three categories: 1) thermal effects, where more than 70% of the solar radiation is affected by the plants’ temperature and transpiration processes (Gates 1965; Boyer 1967; Gates 1968); 2) photosynthetic effects, as photosynthetically active radiation is used to transform simple inorganic compounds such as water, carbon dioxide, minerals, salts in complex organic compounds like sugars, fats, proteins and 3) photomorphogenic effects, where the solar radiation plays an important role regulating and controlling the growth and development. Influencing many changes in plant morphology, physiology and biochemistry (Schmidt and Skidmore 2003). The optical properties of the leaves depend, therefore, on a number of characteristics such as chlorophyll, carotenoid content and water,
cellulose and lignin and leaf internal structure, among others (Ross 1981; Schmidt and Skidmore 2003). The radiation incident upon the leaf surface may be reflected, absorbed, transmitted and scattered by leaf tissues, cells and their organelles. As such, the quantity of reflection, absorption and transmission depends on the wavelength of radiation, angle of incidence, surface, optical properties and biochemical content of the leaf (Schmidt and Skidmore 2003).

In this study the focus will be in the features of leaf reflectance patterns which can be subdivided in four main spectral regions (Ollinger 2011): Visible, red-edge, near infra-red and mid-infrared.

6.2.1 The visible region

Most of the radiation emitted by the sun appear in the range of 200 to 2500 nm (Belward 1991; Carvalho 2013). Roughly half of the energy in incident radiation that reaches the Earth's surface is in the visible wavelengths (Fig. 2). As such, plants adapted their foliar pigments to use the energy in the visible region for photosynthesis (Kumar et al. 2002). This pigment influence in reflectance patterns is mostly due to chlorophyll as it is around 5 to 10 times greater than others pigment, such as carotenoids or xanthophyll. Green vegetation pigments use electron transitions (Kumar et al. 2002; Ollinger 2011), as mechanism to absorb the radiation. The energy resulted from this electron transition will be used for photochemical reactions.

\[ \text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{CH}_2\text{O} + \text{O}_2 \]

Thus, some pigments adapted in this direction to be more effective in the use of radiation and the wavelength of absorbed light depends upon the specific pigment. We know that the lowest reflectance in the visible portion of the spectrum is between 400-700nm due to the normally called spectral absorption maxima of Chlorophyll around 420, 490 and 660nm (Knipling 1970; Belward 1991; Verdebout et al. 1994; Kumar et al. 2002; Belward and Valenzuela...
The table 4 gives the main pigments found in higher plants and their absorption maxima.

**Table S5 Plant pigments and their absorption maxima (Schmidt 2003)**

<table>
<thead>
<tr>
<th>Type of pigment</th>
<th>Characteristic absorption maxima (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll a</td>
<td>420, 490, 660</td>
</tr>
<tr>
<td>Chlorophyll b</td>
<td>435, 643</td>
</tr>
<tr>
<td>B-Carotene</td>
<td>425, 450, 480</td>
</tr>
<tr>
<td>Δ-Carotene</td>
<td>420, 440, 470</td>
</tr>
<tr>
<td>Xanthophyll</td>
<td>425, 450, 475</td>
</tr>
</tbody>
</table>

**6.2.2 The red-edge**

Red edge refers to the region of rapid change in reflectance of vegetation in the near infrared range of the electromagnetic spectrum between the 690-720nm. The red edge is a unique characteristic feature of the living plant spectral patterns because it results from two special optical properties of plant tissue: high internal leaf scattering causing large near infrared reflectance and chlorophyll absorption giving low red reflectance. It is often related to the stress responses of vegetation and it is the most studied feature in the spectral curve (Collins 1978).

**6.2.3 Near-infrared region**

The near-infrared region (NIR) is in the electromagnetic region between the 700 -1300nm. In contrast with the visible region, plants have a high reflectance and transmittance in this region. The energy levels in near infrared region are not enough for photochemical reactions and some studies demonstrate that white and green leaves have the same reflectance in this region, confirming that the pigments do not affect infrared (Billings and Morris...
The main factor controlling the spectral responses in the near infrared region is the internal structure of the leaf (Gates et al. 1965; Sinclair et al. 1971) as each cell acts like an elementary corner reflector. The distribution of the air spaces, size, shape and arrangement of the cells affects the passage of the light in the leaf and the spectra in this region changes with the development, growth and senescence of the leaf (Jacquemoud and Baret 1990).

### 6.2.4 Mid-infrared region

In the Mid-infrared region (1300-2500nm) the reflectance is much lower than in Near Infra-red region (NIR). This area is characterized by strong water absorption features (Peñuelas et al. 1993; Schmidt 2003). The increase of water content in leaves will reduce the reflectance pattern not just in water absorptions bands but also in other regions. This phenomenon of water absorption is caused by transitions in the vibrational and rotational states of the water molecules within the leave (Belward 1991). This region is also known for absorption features of specific foliar biochemical lignin, cellulose, starch, proteins and nitrogen but usually they are camouflaged by water absorption in the fresh leaf spectra.

### 6.2.5 Vegetation indices

Vegetation indices are mathematical transformations of the original spectral reflectance signature. They are designed to diminish the additive and multiplicative errors linked with atmospheric effects, soil background effects, solar illumination, and sensor viewing geometry (Liang and Fang 2004). The different vegetation responses create different spectral signatures in diverse spectral regions, which have been used to develop several arithmetic formulae,
commonly known as spectral vegetation indices (SVI’s). With the disposal of hyperspectral data and reflectance for a great number of spectral bands, several new vegetation indices have been developed.

Over recent years, many indices vegetation have been developed for crop biophysical parameters estimation, to detect stress in plants, based on chlorophyll and water content (Peñuelas et al. 1993), pigment estimation (Penuelas et al. 1995; Blackburn 1998; Gitelson et al. 2003), and leaf area(Rouse et al. 1973). Zarco-Tejada et al. (2001) described chlorophyll content as a potential indicator of vegetation stress because of its direct role in the photosynthetic processes of light harvesting and initiation of electron transport and its responsiveness to a range of stresses. Knipling (1970) also specified that stress-induced alterations of spectral reflectance in the visible spectra result from the sensitivity of leaf chlorophyll concentrations to metabolic disturbance.

Spectral vegetation indices (SVIs) have been shown to be useful for an indirect detection of plant diseases (Thenkabail et al. 2000; Hatfield et al. 2008). Efficient use of spectral reflectance measurements for disease detection relies on the identification of most significant spectral wavelength, highly correlated to a specific disease. Mahlein et al. (2013) are trying to develop specific spectral disease indices (SDIs) for the detection of diseases in crops. This kind of indices can improve disease detection, identification and monitoring in precision agriculture applications (Mahlein et al. 2010).

Table 6 (extra information) gives a synoptic overview of spectral reflectance indices as developed and tested by different authors.

6.2.6 Ratio indices (RI)

Ratio indices are defined as quotients between measurements of reflectance in distinct parts of the spectra. These ratios are known to be effective in enhancing information, when there is an inverse relationship
between two spectral responses to the same biophysical occurrence. Though, an intrinsic weakness of these indices is the loss of uniqueness in information due to the fact that different leaves can have diverse spectral responses, however they have band ratio values that are similar (Delalieux et al. 2009).
### 6.3 Extra Information

The extra information contains the Table S6, Table S7, Table S8 and Table S9.

**Table S6** Examples of vegetation indices with the formula calculation and definition. In grey are the indices that we used in our study.

<table>
<thead>
<tr>
<th>Code</th>
<th>Definition</th>
<th>Formula</th>
<th>Description (for leaf measurements)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRI</td>
<td>nitrogen reflection index</td>
<td>((\text{R570-R670})/(\text{R570+R670}))</td>
<td>relates to nitrogen content</td>
<td>(Filella et al. 1995; Abawi and Widmer 2000; Schleich er et al. 2001)</td>
</tr>
<tr>
<td>SR</td>
<td>simple ratio</td>
<td>((\text{R805/R710}))</td>
<td>Chlorophyll content</td>
<td>(Rouse et al. 1973; Gamon et al. 1995)</td>
</tr>
<tr>
<td>NDVId</td>
<td>normalized difference vegetation index</td>
<td>((\text{R805 - R555})/(\text{R805 + R555}))</td>
<td>Chlorophyll content</td>
<td>(Tucker 1979; Gamon et al. 1995; Peñuelas and Filella 1998)</td>
</tr>
<tr>
<td>NDVIa</td>
<td>normalized difference vegetation index</td>
<td>((\text{R805 - R710})/(\text{R805 + R710}))</td>
<td>Chlorophyll content</td>
<td></td>
</tr>
<tr>
<td>NDVIb</td>
<td>normalized difference vegetation index</td>
<td>((\text{R800 - R670})/(\text{R800 + R670}))</td>
<td>Chlorophyll content</td>
<td>(Peñuelas et al. 1993; Hamzeh et al. 2012) (Tucker 1979)</td>
</tr>
<tr>
<td>NDVIC</td>
<td>normalized difference vegetation index</td>
<td>((\text{R750 - R705})/(\text{R750 + R705}))</td>
<td>Chlorophyll content</td>
<td>(Gitelson and Merzlyak 1994; Hamzeh et al.)</td>
</tr>
<tr>
<td></td>
<td>Description</td>
<td>Formula</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>-------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>RDVI</td>
<td>re-normalized difference vegetation index</td>
<td>$(R805 - R670)/\sqrt{(R805 + R657)}$</td>
<td>(Haboudane et al., 2004; Main et al., 2011)</td>
<td></td>
</tr>
<tr>
<td>MCARI</td>
<td>modified chlorophyll absorption and reflectance index</td>
<td>$((R700 - R670) - 0.2*(R700 - R550)/(R700/R670))$</td>
<td>Daughtry et al., 2000</td>
<td></td>
</tr>
<tr>
<td>PPR</td>
<td>Plant pigment ratio</td>
<td>$(R550 - R450)/(R550 + R450)$</td>
<td>Metternicht, 2003</td>
<td></td>
</tr>
<tr>
<td>GI</td>
<td>greenness index</td>
<td>$(R554/R677)$</td>
<td>(Huete and Jackson, 1987)</td>
<td></td>
</tr>
<tr>
<td>EVI</td>
<td>enhanced vegetation index</td>
<td>$2.5*(R800 - R670)/(R800+(6<em>R670) - (7.5</em>R475)+1)$</td>
<td>Huete et al., 1994; Huete et al., 1997; Walker et al., 2014</td>
<td></td>
</tr>
<tr>
<td>TCARI</td>
<td>transformed chlorophyll absorption and reflectance index</td>
<td>$3*([R700 - R670] - 0.2*[R700 - R550]*(R700/R670))$</td>
<td>Daughtry et al., 2000; Hamzeh et al., 2012 (Haboudane et al., 2004)</td>
<td></td>
</tr>
<tr>
<td>REP</td>
<td>red-edge position</td>
<td>$R700 + 40*((R670 + R780)^2 - R700)/(R740 - R700)$</td>
<td>Clevers et al. (2002); Hamzeh et al., 2012 (Sims and Gamon, 2002)</td>
<td></td>
</tr>
<tr>
<td>mREP</td>
<td>modified red-edge position</td>
<td>$(R750 - R705)/(R750 + R705 - 2*R445)$</td>
<td>Sims and Gamon, 2002; Hamzeh et al., 2012 (Gamon et al., 1992; Penuelas et al., 1995)</td>
<td></td>
</tr>
<tr>
<td>PRIa</td>
<td>photochemical/physiological reflectance index</td>
<td>$(R531 - R570)/(R531 + R570)$</td>
<td>(Gamon et al., 1992; Penuelas et al., 1995; Clevers et al., 2002)</td>
<td></td>
</tr>
<tr>
<td>Metric</td>
<td>Description</td>
<td>Formula</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>-----------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------</td>
<td>---------------------------------</td>
<td></td>
</tr>
<tr>
<td>PR Ib</td>
<td>Photochemical/physiological reflectance index</td>
<td>((R_{570} - R_{539})/(R_{570} + R_{539}))</td>
<td>(Gamon et al., 1997)</td>
<td></td>
</tr>
<tr>
<td>MSI</td>
<td>Moisture stress index</td>
<td>((R_{1599}/R_{819}))</td>
<td>(Hamzeh et al., 2012)</td>
<td></td>
</tr>
<tr>
<td>WI</td>
<td>Water index</td>
<td>((R_{900}/R_{970}))</td>
<td>(Hamzeh et al., 2012)</td>
<td></td>
</tr>
<tr>
<td>DSWI</td>
<td>Disease-water stress index</td>
<td>((R_{800}/R_{1660}))</td>
<td>(Apan et al., 2004; Hamzeh et al., 2012)</td>
<td></td>
</tr>
<tr>
<td>PS a</td>
<td>Plant stress index</td>
<td>((R_{695}/R_{420}))</td>
<td>(Carter, 1994)</td>
<td></td>
</tr>
<tr>
<td>PS b</td>
<td>Plant stress index</td>
<td>((R_{605}/R_{760}))</td>
<td>(Carter, 1994)</td>
<td></td>
</tr>
<tr>
<td>PS c</td>
<td>Plant stress index</td>
<td>((R_{695}/R_{760}))</td>
<td>(Carter, 1994)</td>
<td></td>
</tr>
<tr>
<td>PS d</td>
<td>Plant stress index</td>
<td>((R_{710}/R_{760}))</td>
<td>(Carter, 1994)</td>
<td></td>
</tr>
<tr>
<td>Nitrogen 1</td>
<td>Nitrogen stress index</td>
<td>((R_{415}/R_{710}))</td>
<td>(Prabhakar et al., 2012)</td>
<td></td>
</tr>
<tr>
<td>Nitrogen 2</td>
<td>Nitrogen stress index</td>
<td>((R_{517}/R_{413}))</td>
<td>(Zhao et al., 2005; Prabhakar et al., 2012)</td>
<td></td>
</tr>
<tr>
<td>ARI</td>
<td>Anthocyanin reflectance index</td>
<td>((1/R_{550}) - (1/R_{700}))</td>
<td>(Hamzeh et al., 2012)</td>
<td></td>
</tr>
<tr>
<td>DBlue</td>
<td>Max value 1st Derivative in the blue edge</td>
<td>Blue edge is between 490-530nm</td>
<td>(Gong et al., 2002; Zhang et al., 2012)</td>
<td></td>
</tr>
<tr>
<td>Dyellow</td>
<td>Max value 1st Derivative in the yellow edge</td>
<td>Yellow edge is between 550-582nm</td>
<td>(Gong et al., 2002)</td>
<td></td>
</tr>
<tr>
<td>Dred</td>
<td>yellow edge</td>
<td>max value 1st Derivative in the red edge</td>
<td>red edge is between 670-737nm</td>
<td></td>
</tr>
<tr>
<td>------</td>
<td>-------------</td>
<td>-----------------------------------------</td>
<td>-------------------------------</td>
<td></td>
</tr>
</tbody>
</table>

Table S7 Pearson Correlation matrix between several plant vegetation indices. **. Correlation is significant at the 0.01 level; *. Correlation is significant at the 0.05 level.

<table>
<thead>
<tr>
<th></th>
<th>NDVId</th>
<th>NDVId</th>
<th>MCARI</th>
<th>NRI</th>
<th>EVI</th>
<th>mREP</th>
<th>PRIa</th>
<th>DSWI</th>
<th>PSa</th>
<th>Nitrogen Index b</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDVId</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NDVIdc</td>
<td>.671**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCARI</td>
<td>-.692**</td>
<td>.013</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NRI</td>
<td>-.139**</td>
<td>.536**</td>
<td>.674**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EVI</td>
<td>.514**</td>
<td>.769**</td>
<td>.183**</td>
<td>.440**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mREP</td>
<td>.978**</td>
<td>.511**</td>
<td>-.805*</td>
<td>-.295**</td>
<td>.389**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRIa</td>
<td>.588**</td>
<td>.274**</td>
<td>-.520*</td>
<td>-.172**</td>
<td>.127**</td>
<td>.604**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DSWI</td>
<td>.257**</td>
<td>.526**</td>
<td>.323**</td>
<td>.391**</td>
<td>.761**</td>
<td>.135**</td>
<td>.002</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PSa</td>
<td>-.812**</td>
<td>-.212*</td>
<td>.915**</td>
<td>.438**</td>
<td>-.100*</td>
<td>-.900**</td>
<td>-.584**</td>
<td>.185*</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Nitrogen Index b</td>
<td>-.638**</td>
<td>.033</td>
<td>.931**</td>
<td>.719**</td>
<td>.122**</td>
<td>-.759**</td>
<td>-.442**</td>
<td>.342**</td>
<td>.905**</td>
<td>1</td>
</tr>
</tbody>
</table>
Table S8  Rhizoctonia Solani spread during the experiment in 3 different soil managements
Table S9 Overall of the experiment in pictures
Monitoring the impact of soil management on plant spectral reflectance and soil-borne disease resistance - ML
CHAPTER 7

References


Altieri, M.A. (1999). The ecological role of biodiversity in agroecosystems. Agriculture, Ecosystems & Environment, 74, 19-31


Bonanomi, G., Antignani, V., Capodilupo, M., & Scala, F. (2010). Identifying the characteristics of organic soil amendments that suppress soilborne plant diseases. Soil Biology and Biochemistry, 42, 136-144


Carvalho, S. (2013). Jacobaea through the eyes of spectroscopy: Identifying plant interactions with the (a)biotic environment by chemical variation effects on spectral reflectance patterns. In: Wageningen University


distribution of the barnacle Chthamalus stellatus. Ecology, 42, 710-723


FAO (2009b). Maize, rice and wheat: area harvested, production quantity, yield

FAO (2011). Statistics


Hedlund, K. (2012). *SOILSERVICE*. In


Mendes, R., Kruijt M, de Bruijn I, Dekkers E, van der Voort M, Schneider JH, Piceno YM, DeSantis TZ, Andersen GL, Bakker PA, & JM., R. (2011). Deciphering the Rhizosphere Microbiome for Disease-Suppressive Bacteria. In Science (Ed.) (pp. 1097-1100): Laboratory of Phytopathology, Wageningen University


OECD Section 8 - Sugar Beet (BETA VULGARIS L.). OECD Publishing


protein and polyphenols for trees and grass using hyperspectral imagery. Remote Sensing of Environment, 114, 64-72

Statistiek, C.B. (2014). Agriculture; crops, livestock and land use by general farm type, region. In


Zhang, W., Ricketts, T.H., Kremen, C., Carney, K., & Swinton, S.M. (2007). Ecosystem services and dis-services to agriculture. Ecological economics, 64, 253-260