Short-term changes in soil carbon dynamics, soil moisture, and soil biota in a newly established agroforestry system after incorporating organic mulch and cover crops

by

Rens van Dijke

Supervisor

Michiel in 't Zandt



A master's Thesis Submitted to the Department of Soil Biology Wageningen University 28 of February 2024

# Abstract

Finding relations between soil physical, chemical, and biological indicators is key for understanding soil functioning and evaluating soil quality. Short-term analysis of soil indicators can be of use to farmers who want to make management decisions based on specific plant needs within a growing season. This study investigated the short-term effects (62 days) of applying compost mulch and sowing clover cover crops on soil organic matter (SOM), soil moisture, and soil life. It was conducted in light clay soil at a newly established agroforestry system on 'De Biesterhof', a farm near Nijmegen, the Netherlands. SOM was divided into particulate organic matter (POM) and mineral-associated organic matter (MAOM) fractions using rapid particle size fractionation, nematodes were counted via the Oostenbrink method, and soil microbial community abundance and species composition were determined by PLFA extraction. Our results showed that applying compost mulch significantly increased the carbon content of the POM fraction of the soil. Highlighting possibilities for dividing SOM in POM and MAOM fractions for short-term evaluation, as well as the role mulching can have in increasing soil carbon stocks in the short term. Compost mulch addition also had significant positive effects on earthworm abundance and soil moisture content, showing its potential benefits for farmers. In contrast, the clover treatment had a positive effect on nematode abundance showing significantly higher numbers than the compost treatment. Additional research could provide information on short-term POM dynamics, and the role of macrofauna in increasing its fraction in the short term. Overall, this study shows that understanding short-term dynamics in soil properties can contribute to the ability of farmers to make informed management decisions.

# Contents

Abstract 2
Introduction
Soil organic carbon is crucial for assessing soil quality4
Assessing soil organic carbon by dividing it into particulate- and mineral-associated organic matter5
Soil moisture content
Biological indicators representing soil quality 6
Aim, research questions, and hypothesis7
Materials & Methods
Experimental setup
Soil collection9
Earthworms, field collection, and estimation of number and biomass
Separation of soil into POM and MAOM fractions10
Estimating soil moisture content
Oostenbrink method to determine nematode abundance11
Phospholipid fatty acid analysis to determine microbial biomass and community structure 11
Statistical methods
Results
Discussion
Most important findings16
Mulching provides multiple benefits for soil quality in the short term
The usefulness of knowing when to apply what management option
References
Appendix

# Introduction

Soils play an important role in sustaining life on earth and should be included in a holistic approach to address the ongoing challenges we encounter. They are essential for the provisioning of food, fiber, and fuel, nutrient cycling, cultural services, waste purification, and climate regulation (Millennium Ecosystem Assessment, 2005; Schulte et al., 2015). The capacity of soils to deliver such ecosystem services is under threat due to factors like loss of carbon stocks and nutrient depletion (FAO, 2015). Due to the multifunctional role of soils in biotic and abiotic ecosphere processes, the decline of (global) soil quality is closely related to climate change and the biodiversity of ecosystems (Schulte et al., 2015). Therefore, sustainable soil management can provide the basis for robust and future-proof ecosystems.

Agricultural intensification has increased global primary production by 25% from 2003 until 2019 (Potapov et al., 2022), but the linked practices put pressure on soil quality. Soil quality is commonly defined as "the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality and support human health and habitation" (Doran & Zeiss, 2000). Common intensive agricultural practices include tilling, the use of agrochemicals, and the use of artificial fertilizers (FAO, 2015). These practices appear particularly detrimental to the biodiversity of soil life, which is visible in decreased food web complexities and community-weighted mean body mass of soil fauna (Tsiafouli et al., 2015). Additionally, they reduce soil organic carbon (SOC) levels (Giuffré et al., 2021). Promising alternatives to unsustainable practices such as no-tillage, mulching, and using cover crops can positively affect soil quality and overcome the negative effects of intensive agriculture.

Evaluating soil quality can be challenging due to the complexity of soil processes and their interactions, making it difficult to identify suitable indicators. Soil functions such as water regulation and purification, habitat provisioning, nutrient cycling, carbon and climate regulation, and disease and pest regulation, can be used for this purpose (Creamer et al., 2022; Bünemann et al., 2018). Various soil functions (multifunctionality) can be achieved, but often, when emphasizing one specific function, trade-offs arise among the different soil functions (Vazquez et al., 2021). Soil processes, formed by interactions of the soil's physical, chemical, and biological properties determine the functioning of the soil (Bünemann et al., 2018; Vogel et al., 2018). Making connections between the soil processes is essential for identifying indicators to assess and manage soil quality effectively.

Conservation agriculture, with a focus on minimal soil disturbance and permanent ground cover, can strongly influence soil processes. This study will focus on how the practices of mulching and cover cropping could influence multiple soil quality indicators. Mulching is linked to reduced evaporation, soil moisture conservation, weed growth suppression, soil structure, and temperature control, the addition of organic material, and providing habitat for soil organisms (Kader et al., 2017).Cover cropping is said to influence soil quality by adding organic material to the soil via root exudates and the addition of organic material via above and belowground litter material (Teravest (2007). It also improves soil structure via root systems and provides a habitat for soil organisms. Both have been shown to have significant effects on soil chemical, physical, and biological indicators.

# Soil organic carbon is crucial for assessing soil quality

Soil organic carbon (SOC) is a measurable component of soil organic matter (SOM) and forms a key ingredient of soils. Its appearance can have positive effects on soil quality by influencing biological, chemical, and physical processes. For example, it enhances microbial activity, supports nutrient

availability, contributes to structural stability, and improves the water-holding capacity (Wiesmeier et al., 2019). SOC levels in the soil are determined by the balance between organic matter inputs by soil amendments, plant residues, roots, and root exudates, and losses due to decomposition, erosion, and leaching (Six et al., 2006). Additionally, it is influenced by nitrogen availability in the soil due to SOC being stored in chemical compounds containing nitrogen(Cotrufo et al., 2019).

Land use management is strongly related to SOC changes (Guo & Gifford, 2002; Poeplau et al., 2011). For example, vegetation type strongly influences carbon stocks in the soil by both influencing carbon input and decomposition (Wiesmeier et al., 2019). When forests or grasslands are converted to cropland, a decline of 30 to 80% of SOC stocks is seen (Guo & Gifford, 2002; Poeplau et al., 2011). In general, the use of cover crops and perennial crops in agricultural systems leads to higher SOC levels (Poeplau & Don, 2015). Changing management practices can increase SOC contents in the soil and improve soil quality.

# Assessing soil organic carbon by dividing it into particulate- and mineral-associated organic matter

Particulate organic matter (POM) and mineral-associated organic matter (MAOM) have very different ways in which they function in the soil and with which they stimulate soil faunal and plant growth. MAOM is in general more nutrient-dense, but it is more difficult to access due to its association with soil minerals (Lavallee et al., 2020). It can serve as a direct source of energy and nutrients for plants and microorganisms when released from its bond with minerals. POM is more abundant in the soil and has on average larger particles that need to be depolymerized before decomposition making its consumption more energy-intensive (Cotrufo et al., 2022; Lavallee et al., 2020). Once it is depolymerized it is directly available to plants and microorganisms as a source of carbon and nutrients (Lavallee et al., 2020). POM is more abundant, but it has a low nutrient content, particularly nitrogen, and a higher energy cost of consuming it (von Lützow et al., 2007). It is a source of food for macro and microorganisms that have decomposing roles in the soil food web (Kauer et al., 2021).

Separating SOM into POM and MAOM allows for a more detailed understanding of carbon dynamics in the soil. MAOM is a small fraction consisting of single molecules or tiny fragments bonded to soil minerals. POM consists of undecomposed fragments that are less protected within the soil environment. This distinction leads to a difference in turnover rate with MAOM persisting for decades to centuries and POM for less than a decade to decades(Lavallee et al., 2020; von Lützow et al., 2007). The nature of plant input (structural or soluble) is related to the formation of POM and MAOM. Structural plant inputs are believed to increase the relative amount of POM in soils. While soluble plant inputs seem to increase the formation of MAOM in the soil (Cotrufo et al., 2022).

### Soil moisture content

Soil moisture dynamics are important because they directly link to soil quality and soil functions (Creamer et al., 2018). Moisture excess and droughts can cause a series of unwanted processes such as plant stomatal closure, anaerobicity, and reduced land accessibility (Creamer et al., 2018). Additionally, moisture contents are highly important for the soil biological community because they are dependent on the availability of water. Soil moisture can be evaluated through soil moisture deficit, or the water holding capacity. Soil moisture deficit is linked to water inputs from precipitation and/or irrigation compared to water outputs through evapotranspiration and/or drainage. Water holding capacity is influenced by soil texture, the presence of organic matter, and the depth of plant root systems (Wall et al., 2020).

Soil moisture content is highly related to agricultural practices. Tillage, lower C levels, and leaving the soil uncovered promote evaporation and reduce soil water holding capacity and moisture contents (Kader et al., 2017). Applying mulches can improve the soil moisture content by reducing evaporation and regulating soil temperature(Greenly & Rakow, 1995; Kader et al., 2017). The use of cover cropping has fewer clear effects on soil moisture levels. In the long term, cover crops have a positive effect on soil moisture by improving the soil structure and raising SOC levels. However, in the short term, it can reduce soil moisture levels due to increased evaporation. The effect depends on factors like the type of cover crop, sowing time, infiltration levels, evaporation levels, and the amount of organic matter added by the cover crops(Mendis et al., 2022).

## Biological indicators representing soil quality

Quantitative indicators on soil biota, due to their involvement in many processes, are crucial for soil quality evaluation (Bünemann et al., 2018; Creamer et al., 2022). They meet the conditions set by Doran & Zeiss (2000), for good indicators because they are sensitive to variations in management, well correlated with beneficial soil functions, useful for elucidating ecosystem services, comprehensible and useful to land managers, and easy and inexpensive to measure. Currently, soil biological indicators are not as well understood because related indicators are less developed or available compared to physical and chemical ones (Bünemann et al., 2018; Martin et al., 2022; O'Neill et al., 2021). Increasing data on how soil biota respond to changes in biotic and abiotic factors could improve our understanding of their role in soil processes and as indicators of soil quality.

Earthworms play important roles in the soil ecosystem as decomposers of organic material and are considered 'ecosystem engineers'. They help incorporate surface litter into deeper layers of the soil, improving soil structure and water regulation (Blouin et al., 2013). This leads to the modification of microbial communities and nutrient dynamics such as mineralization of C and N (Aira et al., 2008). Earthworms can be linked to soil processes of bioaccumulation, macropore formation, fragmentation, aggregation, food web assimilation, and bioturbation (Creamer et al., 2022). Bioturbation is known to affect soil functioning by changing the soil's pore and particle size structure (Wilkinson et al., 2009). When there is abundant POM available, earthworms ingest POM-rich soil and spread it toward other areas of the soil with their castings (Ruiz et al., 2021).

Nematodes are the most abundant of all animals on earth  $(4.4 \pm 0.64 \times 10^{20} \text{ nematodes})$  (with a total biomass of approximately 0.3 gigatons), with roles in all trophic levels in the soil food web (van den Hoogen et al., 2019). They are proposed as biological indicators for assessing soil quality due to their role in functions of carbon sequestration, nutrient cycling, and the provision of habitat for biodiversity (Creamer et al., 2022; Stone et al., 2016). They are involved in processes of microbial grazing, food web assimilation, predation, resistance and defense, and parasitism (Creamer et al., 2022). Additionally, they are useful indicators because they are sensitive to perturbations and disturbances (Chen et al., 2010).

Relations between SOC dynamics and nematode communities remain an underdeveloped field in soil (Ferris, 2010; Gan & Wickings, 2020). Root herbivory by nematodes is thought to have an impact on soil SOC dynamics by inducing C inputs by transforming plant materials to waste products and cadavers through the root herbivory pathway, increased root C input due to sheared root litter and leaking exudates, and by trophic interactions with other organisms (Gan & Wickings, 2020). Song et al., (2020), found a negative correlation between the use of organic mulches and nematode abundance. Others

found a small increase in nematode abundance after the addition of organic mulches (Blanco-Pérez et al., 2022).

Of all the soil organisms, fungi and bacteria are involved in most soil processes making them a suitable indicator for soil quality (Creamer et al., 2022). They can regulate nutrient availability, C sequestration, aggregate stability, plant disease prevalence, and plant growth promotion (Fierer et al., 2021). Soil microbial biomass is often linked to SOM contents in the soil, with higher inputs of SOM leading to higher soil microbial biomass. In general, a stronger correlation with inputs is found in soils with lower SOM contents (Wiesmeier et al., 2019). A decrease of easily available C leads to favor microbial communities with low biomass, enzymatic activities, and respiration rates (Fanin & Bertrand, 2016). The Gram-positive to Gram-negative bacteria ratio in the soil seems to be a good indicator of these two microbial groups. Easily available C in the soil correlates with Gram-negative bacteria (Fanin et al., 2019). While Gram-positive bacteria are more associated with recalcitrant C sources in the soil (Fanin et al., 2019).

# Aim, research questions, and hypothesis

In this study, we investigated the short-term effects of compost mulch application and cover cropping with clover on SOM dynamics, soil moisture levels, and soil life. It was an in-field study, in a newly established agroforestry system in a light clay soil in the Netherlands. The analysis will focus on multiple biological, chemical, and physical indicators, providing possibilities to find relations between outcomes of the different indicator values.

The following research questions were the focus of our study:

• What are the short-term effects (62 days) of applying compost mulch, and cover cropping with clover on soil quality?

This research question will be divided into the following sub-questions:

• What is the effect of the treatments on the C/N contents of the POM/MAOM fractions?

If there is an effect on the POM/MAOM fractions, it could be that the POM carbon (POM-C), and or POM nitrogen (POM-N) levels will increase for the mulched plots compared to the clover and control plots. This is expected because of bioturbation which can move compost down to the upper layers of the soil which could be added to the POM-C fraction. For the MAOM fraction, we expect no significant changes due to the short term of the experiment. Changes in MAOM occur in general over longer periods in the soil and are often followed by changes in POM over time(Angst et al., 2023).

• What is the effect of the different treatments on the soil moisture deficit?

The soil moisture content is expected to be highest in the mulched plot due to reduced evaporation levels and increased water retention by the mulch.

• What are the effects of the treatments on earthworm abundance, nematode abundance, and microbial community composition and abundance?

Earthworm abundance is expected to be highest in the mulched plots, lower in the clover plots, and lowest in the control plots. This is expected mainly due to a higher soil moisture content in the mulched plots and higher availability of organic material in the mulched and clover plots.

Nematode abundance is expected to be higher in the mulched plots and the clover plots than in the control treatment. This is expected due to the higher availability of organic material in the mulch and clover treatments and higher soil moisture levels in the mulched plots.

An increase of gram-negative bacteria for the mulch and clover treatments could be found since they use more labile, plant-derived SOM sources. An increase of gram-positive bacteria for the control plots could be found since they use more recalcitrant SOM sources. Furthermore, for the clover and control plots, an increase in the fungal-to-bacterial ratios could be found due to dryer conditions compared to the mulched plots (Osburn et al., 2022). The overall microbial biomass is expected to be highest in the mulch or clover treatment depending on the weather conditions. If changes could be found, they could be related to weather patterns and the influence of the mulch on the soil moisture deficit. Dryer conditions could lead to an increase in total PLFAs for the mulch treatment.

# Materials & Methods

# **Experimental setup**

This study aimed to find short-term differences (62 days) in soil biological, physical, and chemical indicators for sites that used clover cover cropping or compost mulch and a control plot with bare soil. It is the first measurement of a longer-term experiment that takes place on de Biesterhof, a farm in Millingen aan de Rijn in the Netherlands (51.84821562618432, 6.023288168604211). One year before sampling the farm transitioned to organic farming practices.

The sampling field consists of an agroforestry system in which rows of trees and shrubs (apple, plum, hazel, gooseberry, autumn olive, red currant, and black currant) are alternated with arable crops (Figure 1). On the side of the tree rows (2.5 meters on one side and 1.5 meters on the other, herbs or green manure crops were sown (Figure 1). Four randomly chosen plots within the tree rows were set out in the field. They all contained a combination of the mulch and clover treatments and a control plot. Two blocks within the plum row and two blocks within the apple row were chosen with a random number generator by picking a random tree number in the rows. Per treatment, there were 12 different measurement locations, two times 4 locations in a plot two times 2 locations in a plot (figure 2). This resulted in a total of 36 soil sampling locations.



figure 1: Top view of the agroforestry system in which the experiment took place. On the top left corner, a satellite image shows the field on 16-05-2023 in which the treatments are visible. The treatments and their order are visible in the center of the figure. On the right side, the setup of the tree systems is explained with alternating trees and bushes, cover crops and herbs sown next to the rows, and the two different distances between the trees.

On 15-04-2023 white clover (Trifolium repense) was sown in the tree rows under the trees and 1.5 and 2.5 meters next to the trees (Figure 1). On 16-06-2023, a drip irrigation system was placed to water the trees and shrubs. The system was placed so water was added directly around the trees and shrubs, up to 30 cm apart from the stems. A 5.3 cm layer of compost mulch was added 16 days after the first sampling date on 05-05-2023 to all of the tree rows except for the clover cover crop and control plots (Figure 1). The compost was Substrado Houtmulch, consisting of composted pruning waste with a fraction of 0-30 mm, organic matter content of >30%, and 50-65% dry matter content (See appendix). For the control plots, the clover was shoveled away by hand on 08-05-2023.

The field was a permanent grassland (*Lolium perenne*), for the past 5 years. On 09/03/2023 the grass was rototilled, slurry was injected, and plowed under until 23cm. On 05/04/2023 the top 5 cm of the tree rows was rototilled. On 13-10-2022 resulting from a mixed sampling of the whole field, it had a pH of 6.7, and an SOC content of 1.4% (Eurofins, 2022) (See appendix). The soil is a light clay soil consisting of 20% clay, 40% silt, and 36% sand.

### Soil collection

For the first measurement, per sample 15 soil cores were taken with a 10 cm grass plot sampler. For the second measurement the grass plot sampler could not enter the soil due to dry conditions and a gouge auger for hard soils was used. The plots were 1m2 and were located on the northeast side of selected trees and the samples were randomly taken in this area. Per tree that was indicated, one sampling plot started 50 cm next to the tree on the northeast side in the middle of the row, and another, in between the tree and the shrub that was placed in the middle of the trees (Figure 1). For the plum row 2.5 meters from the tree and for the apple row 1.5 meters from the tree (Figure 1). For the mulch treatment, the mulch was shoved aside thoroughly with a broom to avoid mixing the mulch layer on top of the soil, with the soil samples. The samples were mixed in a plastic bag and stored in a cool box

in the field, after which they were stored at 4 °C in a fridge on the sampling day. All the samples were sieved with a 10-millimeter sieve and mixed for homogenization. The samples in the plum row were collected on 19-04-2023 (1-18). On 20-04-2023 the samples in the apple row were collected (19-36). The second collection of soil samples was done on 03-07-2023 (plum row (37-54)) and 04-07-2023 (apple row (55-72)).

Figure 2 shows the rainfall over time from one month before the first sampling moment (19-03-2023), until the last sampling moment (07-07-2023) in Nijmegen, 10 km from the sampling location (KNMI, 2023) (See appendix).



*Figure 2: Precipation data in mm from one month before the sampling (19-03-2023), until the last sampling date (07-07-2023). A drought period is visible from te middle of May until the middle of June.* 

### Earthworms, field collection, and estimation of number and biomass

To evaluate the number and biomass of the earthworms the standard WUR protocol was used (SOPSBL-012, 2017). A soil sample of 20\*20\*20 cm was dug out and the soil was taken apart by hand to form small pieces (about 1 cm<sup>3</sup>) in the field. The earthworms were collected and kept in a plastic jar with water-moist tissue at 4 °C. They were stored at 18 °C for 48 hours to empty their guts. The outside of the earthworms was dried with a dry tissue after which they were counted, and the total fresh weight biomass was measured. The samples were collected on 01-05-2023 (1-10), 02-05-2023 (samples 11-26), and 03-05-2023 (samples 27-36). The second sampling round was on 06-07-2023 (samples 37-54) and 07-07-2023 (samples 55-72).

# Separation of soil into POM and MAOM fractions

Rapid particle size fractionation method following the standard WUR operating protocol (SOPSBL-024, 2021) was used to separate the soil samples into particle sizes associated with POM and MAOM. It is one of the most time-efficient methods with a good differentiation that allows for estimation of Carbon (C), and Nitrogen (N) in the separated fractions(Baldock et al., 2013; Lavallee et al., 2020; Sanderman et al., 2013).

First, the samples were dried overnight at 40 °C after which they were sieved over a 2 mm sieve. 10 grams of soil per sample were taken and mixed with 40 ml, 5.00 g/L NaHMP demi-water solution and shaken at 180 rpm for 17 hours. Next, the samples were sieved at 50  $\mu$ m using an automated wet sieving machine (1mm amplitude, 20 s interval, 3-minute sieving). The fractions were collected in glass beakers and dried for 48 hours at 105 °C. The fractions were weighed and stored in plastic airtight containers. To correct for soil moisture contents, representative sub-samples of 20g soil were dried between 40°C and 105°C and the moisture loss was calculated. The soil recovery rate was calculated with the following formula:

## Soil recovery (%)

 $= \frac{(Fine \ fraction \ (g) - NaHMP \ (g)) + Coarse \ fraction \ (g)}{Total \ soil \ weight \ at \ 40^{\circ}C \ (g) - (Total \ soil \ weight \ at \ 40^{\circ}C \ * \ Moist \ loss \ correction \ (\%))}$ 

### \* 100%

A soil recovery rate of 95% and above was accepted, in other cases the procedure was repeated.

For estimation of the C and N contents of the POM/MAOM fractions, they were ground for 1 minute (VWR star beater) at 20 Hz. 4-7  $\mu$ g of the samples was weighed for the analysis and placed in tin capsules. The total carbon and total nitrogen contents in the samples were examined with the micro-Flash 2000 Organic Elemental C/N analyzer machine.

# Estimating soil moisture content

Soil moisture content was evaluated by sieving 50 grams of fresh soil over a 5mm sieve and drying the samples overnight at 105°C in aluminum cups. The moisture content was determined by weighing the soil before and after drying.

# Oostenbrink method to determine nematode abundance

To estimate the number of nematodes per kg of soil the standard WUR Standard Operating Protocol for Nematodes sampling and extraction was used (WURSOP-SBL-010, 2017). 45-55 grams of soil was weighed 19 days after the first sampling date and the extraction was finished 7 days later. The nematodes were extracted following the Oostenbrink method (Oostenbrink, 1960). The Oostenbrink elutriator was used to extract nematodes and they were captured by four stacked 45  $\mu$ m mesh size sieves. Demi-water in a laboratory wash bottle was used to carefully wash the nematodes out of all the sieves. The extraction was placed in a milk filter (Hygia 220mm rapid filters) in an iron tray to filter the nematodes overnight. This extraction was placed in airtight plastic containers, and the amount of liquid ranged between 95-130 grams. For counting the extraction liquid was stirred and 9,00 milliliters was transferred to a petri dish. The nematodes were identified two times, when the difference between the first two was higher than 10%, they were counted three times.

# Phospholipid fatty acid analysis to determine microbial biomass and community structure

Phospholipid fatty acid (PLFA) analysis is a method to study the community structure and biomass of soil microbial taxonomical groups (arbuscular mycorrhizal fungi, fungi, gram-negative bacteria, gram-positive bacteria, eukaryotes, and actinomycetes) (Willers et al., 2015). Concentration levels of total PLFA provide insights into the microbial biomass, while the composition of specific fatty acid peaks provides insights into the microbial community structure (Joergensen & Wichern, 2008).

Extraction of PLFAs was done following the WUR standard operating protocol for PLFA (WURSOP-SBL-023, 2023). The samples were freeze-dried and stored at -20°C within one week after collection to overcome losses of PLFAs. In brief, lipids were first extracted with a Bligh and Dyer solution (chloroform (HPLC): methanol (HPLC): citrate buffer 1:2:0.8 v/v/v) following the Bligh and Dyer method (Bligh & Dyer, 1959). The fatty acids were liberated from the polar lipids and derivatized to form fatty acid methyl esters (FAME) with 19:0 FAME/ml (internal standard) to allow for examination by GC methods. PLFA concentrations were determined by gas chromatography with flame-ionization detection (Agilent Technologies, 6890N Network CG systems). Concentrations of PLFA were determined by Sherlock PLFA Analysis Software (MIDI, Inc and USDA-ARS) following the method developed by Buyer & Sasser, (2012). The software automatically provides total PLFAs for each of the microbial taxonomical groups and ratios by linking the fatty acid biomarkers to the fatty acids found in the soil samples (Buyer & Sasser, 2012).

# Statistical methods

The data was analyzed to find statistically significant results in the program R version 4.3.2. For the estimation of changes in mean values for all the indicators, linear mixed-effects modeling was used, with the two different sampling distances from the trees as a random factor to control for possible effects of tree planting and drip irrigation. For the C/N contents of the POM/MAOM fractions, another random factor of the batch in which they were prepared was added. Two-way Anova's were run on the model to compare the means for the different treatments control, clover, and mulch, for the two different sampling moments. They were analyzed with Tukey's Honestly Significant Difference tests. The residuals of the models were checked for normality and homogeneity of variances. When these assumptions were not met, the data was transformed by taking the log, square root, or exponent. For correlations between the soil moisture content, C/N content of the POM/MAOM fractions, and the biological indicators linear regression analysis was used with Pearson's correlation coefficient. P-values of 0.05 were used as limits.

# Results

The estimated means for carbon contents of the POM fraction increased for all the treatments (Table 1), but only significantly for the mulch treatment (p < 0.05) with an increase of 70%. MAOM-C also increased for all the treatments (Table 1) but showed no significant differences for any of the treatments.

Tabel 1: Means and Standard Deviations for the C- and N-contents of the POM- and MAOM fractions and the Total PLFA microbial biomass measured for the three different treatments and two moments in time. For the POM- and MAOM fractions, the C- and N-contents show the estimated means, controlled for the batch number in which they were analyzed in the C/N analyzer.

Variable:	Treatment:	Clover1	Clover2	Control1	Control2	Mulch1	Mulch2
	Mean	0.35	0.50	0.25	0.39	0.30	0.51
POM-C (%)	(SD)	(0.10)	(0.10)	(0.10)	(0.10)	(0.10)	(0.10)
	Mean	0.05	0.05	0.02	0.04	0.03	0.06
POM-N (%)	(SD)	(0.03)	(0.03)	(0.03)	(0.03)	(0.03)	(0.03)
	Mean	1.39	1.49	1.46	1.48	1.48	1.53
MAOM-C (%)	(SD)	(0.09)	(0.09)	(0.09)	(0.09)	(0.09)	(0.09)

	Mean	0.21	0.21	0.20	0.21	0.21	0.21
MAOM-N (%)	(SD)	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)
Total PLFA	Mean	35105	36332	34867	35690	36033	33592
(Picomolar per g)	(SD)	(5032)	(4733)	(5545)	(6736)	(5501)	(4666)

Soil moisture content at the second measurement was highest in the mulched treatment (15.72%), significantly higher than the clover (12.3%) (p < 0.01), and the control (13.5%) (p < 0.01) treatments. The mulch treatment did not significantly decrease the soil moisture content (p < 0.025), but the clover and control treatments did (p > 0.05).



Soil Moisture Content

*Figure 3: Boxplot of the soil moisture variable with on the y-axis the mean percentage of soil moisture, on the x-axis the different treatments and moments in time represented by the blue and pink colors.* 

Worm numbers were higher in the mulch treatment than in the clover (p < 0.05) and control (p < 0.025) treatments at the second measurement (figure 4). Indicating a positive effect on the number of worms in the mulched treatment compared to the clover treatment. Worm numbers for the clover (p < 0.05) and control (p < 0.01) treatments significantly decreased, for the mulch (p > 0.05) treatment it did not.

Worm biomass was also highest in the mulch treatment (Figure 5). Worm biomass was higher after the 62 days in the mulch treatment than the control (p < 0.025) treatment, also higher than the clover treatment, but not significant (p = 0.08). In the mulch (p > 0.05) and clover (p > 0.05) treatments worm biomass did not decrease after 62 days, for the control (p < 0.025) treatment it did.

Soil moisture content and worm number showed a positive correlation (Pearson's correlation coefficient: 0.52, p < 0.01) (figure 6). Worm biomass also showed a positive correlation (Pearson's correlation coefficient: 0.56, p < 0.01) (Figure 7). Indicating that worm abundance is related to the soil moisture content in the 62 days.



figure 4: Boxplot of the number of worms with on the y-axis the mean of the absolute number of worms found per taken worm sample, on the x-axis the different treatments and moments in time represented by the blue and pink colors.



figure 5: Boxplot of the worm biomass with on the y-axis the mean of the worm biomass in grams found per taken worm sample, on the x-axis the different treatments and moments in time represented by the blue and pink colors.



20 10 0

12

Correlation Worm Number and Soil Moisture

Figure 6: Graph representing the correlation between on the y-axis the worm biomass in grams and on the x-axis the soil moisture content in percentage

Figure 6: Graph representing the correlation between on the y-axis the worm biomass in grams and on the x-axis the soil moisture content in percentage

15

Soil Moisture Content

21

18

Nematode abundance (nematodes per kg) was highest in the clover treatment after the 62 day period (14122 kg-1), followed by the control (10570 kg-1) and the mulch (8246 kg-1) treatments (Figure 8). Nematode numbers in the clover treatment significantly increased (p < 0.01), as did the control (p < 0.01). Nematode numbers in the mulch (p > 0.05) treatment did not significantly increase.



figure 8: Boxplot of the nematode abundance with on the y-axis the mean of the number of nematodes found per kg of soil, on the x-axis the different treatments and moments in time represented by the blue and pink colors.

Microbial community composition and total PLFA showed no significant changes after 62 days. There were some trends detected with an increase in the gram-positive to gram-negative bacteria ratio for

the mulch and clover treatments. However, this was also the case for the control treatment. Also, for the absolute numbers of PLFA, there were no significant effects found in any of the treatments.

# Discussion

# Most important findings

Our observations reveal significant changes in soil physical, chemical, and biological indicators within 62 days after applying compost mulch or the use of clover as a cover crop in light clay soil, shortly after plowing.

A significant increase of POM-C for the mulch treatment shows the possibilities for adding compost on top of the soil to quickly improve this carbon fraction in light clay soil. This adds to the work of Haddix et al., (2020), who found an increase in POM-C levels, six-months after the addition of plant-derived litter (fresh organic material < 1.5 cm). Building on their findings that fast POM-C formation due to the addition of plant material is unrelated to soil type, it could be that the findings in this study apply to multiple soil types. Compost is the plant-derived litter in the current study with particle sizes down to 500  $\mu$ m (López & Cabrera, 2002), falling within the range of POM-C (< 2,000  $\mu$ m) could potentially influence rates of POM-C formation. Especially in the uppermost layers (0-2cm) of the soil.

Worm abundance, staying higher in the mulched plots indicates the ability of compost mulch to sustain worm numbers compared to a cover crop system and the control plots. Vršič et al., (2021) looked at short-term changes (79 days) in earthworm abundance in heavy loam soil and found similar results for mulch (straw) and cover crops (grass) in a vineyard system, both being higher than the control treatments. However, they found only a slight difference in soil moisture content between the cover crop and mulch systems, also after drought periods, revealing differences in the sensitivity of different cover crop systems to precipitation patterns. Our findings are in line with Teravest et al., (2011), finding significantly higher earthworm abundance in mulched plots (woodchips) than a legume cover crop. The correlation of worm numbers with soil moisture, asks for further research to improve understanding of the impact of different types of cover crops and different mulch material on the short-term soil dynamics under different precipitation patterns in different soil types.

Our findings indicate a positive effect of cover cropping with white clover (Trifolium repense) on nematode abundance. Nematode numbers showed a larger increase for the clover cover crop plots compared to the mulched plots (Figure 8). The control plot also shows increased nematode numbers, which could be due to the establishment of some of the clover plants in that treatment after manual removal (See appendix). Increased nematode numbers in the clover treatment could be attributed to an increase in plant parasitic nematodes. A known herbivory species (M. brevidens) has been shown to increase by 215% within one growing season in a legume cover crop (vetch, Vicia sativa) system (Garba et al., 2024). In general, grass and legumes have been shown to sustain high levels of plant-parasitic nematodes (Garba et al., 2024). This increase in nematode numbers due to the use of legumes could be an explanation of why (Grabau et al., 2017) found no changes in nematode abundance for multiple sampling moments in a growing season, in two different sites for oats (Avena sativa), radish (Raphanus sativus), and rape (Brassica napus) cover crops. This shows the possibility of an increase in nematodes due to the legume cover crop in the current study, instead of cover cropping in general.

Although no significant correlation between earthworm abundance and POM-C levels was established, it would be in line with a previous study finding earthworms can improve short-term POM-C contents (Vidal et al., 2019). Furthermore, visual evaluation of earthworm castings clearly showed incorporation

of the mulch material into deeper layers of the soil (See appendix). Although not significant, the clover treatment also showed an increase in POM-C levels and the final estimated means were almost similar between the mulch and cover crop treatments (Table 1). Therefore, an explanation for the absence of the correlation between worm abundance and POM-C increase could be due to alternative pathways of POM-C formation due to grazing of herbivorous nematodes. Although such pathways have been proposed (Ferris, 2010), evidence of SOC formation due to herbivorous nematodes in general seems lacking (Gan & Wickings, 2020). Future research could better establish the effects of mulch, and cover crop applications on soil macrofauna and short-term POM-C dynamics.

We did not find changes in soil microbial communities or biomass following PLFA extraction. This could be attributed to the absence of changes in the soil microbial communities in the short term. It can also show the limits of PLFA extraction for short-term changes in microbial communities in field trials. Significant changes found in this study in POM-C fractions, soil moisture, and megafauna, would make it reasonable to the treatments also affected soil microbes. Especially because soil microbial communities are known to be good indicators of short-term changes in different management techniques (Bünemann et al., 2018; Creamer et al., 2022; Eze et al., 2023). An increase in biomass does not mean there was no increase in microbial activity (Fierer et al., 2021). Therefore, substrate incubation assays could provide more detailed insights concerning microbial activity. Further research on short-term dynamics with PLFA or other DNA sequencing methods could provide information on links to mulching, cover cropping, soil moisture, and soil mesofauna.

### Mulching provides multiple benefits for soil quality in the short term

This study provides evidence of mulch and cover cropping practices influencing short-term changes in soil biological, chemical, and physical properties. Highlighting that short-term, intraseasonal measurements on soil indicators can be viable for assessing changes in soil quality.

A significant increase in the POM-C fraction for the mulch treatment shows the relevance of dividing carbon fractions into POM/MAOM as an indicator for changes in SOC dynamics in the short term. POM, generally consisting of smaller concentrations of SOC in the soil than the MAOM fraction, seems suitable to detect changes resulting from short-term measurements. As an increase in POM-C is a precursor for increases in MAOM-C (Angst et al., 2023), being able to detect short-term changes in SOC dynamics could improve knowledge on how to best increase SOC contents of both fractions in the longer term.

Effects of the mulch treatment on soil moisture content, earthworm abundance, and the POM-C fraction, show the ability of mulching as a management practice to stimulate soil biology and soil structure. Increases in POM-C can improve soil structure because POM-C can act as nuclei of aggregate formation, through the promotion of microbial activity, leading to the deposition of microbial byproducts and further proliferation of saprotrophic hyphae, which further promotes aggregation (Cotrufo & Lavallee, 2022). The findings of the current study seem useful with increasing attention to soil multifunctionality (Creamer et al., 2022). This study shows promising results for compost mulch to improve soil functions such as water regulation, habitat provisioning, nutrient cycling, and carbon regulation in the short term.

### The usefulness of knowing when to apply what management option

The impact of management practices on short-term changes in soil quality can be an important body of knowledge for farmers. Such short-term effects can be very useful for farmers to evaluate the cost-

effectiveness of management implications. Farmers need data that supports their management decisions in the short term, preferably within a growing season to coerce with specific plant needs at certain moments in time. For example, in establishing orchard systems sensitivity to drought can vary over time. In the first year, tree failure due to drought conditions can be a major problem (Brèteau-Amores et al., 2023; Hirons & Percival, 2011). While in later stages, with more developed root systems, drought stress is less experienced. However, mulching can have negative effects on crop performance due to its insulating capacities and reducing warming of the soil in spring. Understanding short-term dynamics can provide tools for farmers to make estimations on such trade-offs between different management practices. Mulching with organic amendments could be attractive when providing multiple benefits and reducing costs due to minimized tree failure while improving SOC contents. However, it is a more expensive option than cover cropping and might be useful at different moments in time. Therefore, knowing when to implement what option can be of great use to farmers.

# References

- Aira, M., Sampedro, L., Monroy, F., & Domínguez, J. (2008). Detritivorous earthworms directly modify the structure, thus altering the functioning of a microdecomposer food web. *Soil Biology and Biochemistry*, 40(10), 2511–2516. https://doi.org/10.1016/j.soilbio.2008.06.010
- Angst, G., Mueller, K. E., Castellano, M. J., Vogel, C., Wiesmeier, M., & Mueller, C. W. (2023). Unlocking complex soil systems as carbon sinks: multi-pool management as the key. *Nature Communications*, *14*(1). https://doi.org/10.1038/s41467-023-38700-5
- Baldock, J. A., Sanderman, J., MacDonald, L. M., Puccini, A., Hawke, B., Szarvas, S., & McGowan, J. (2013). Quantifying the allocation of soil organic carbon to biologically significant fractions. *Soil Research*, *51*(7–8), 561–576. https://doi.org/10.1071/SR12374
- Blanco-Pérez, R., Vicente-Díez, I., Pou, A., Pérez-Moreno, I., Marco-Mancebón, V. S., & Campos-Herrera, R. (2022). Organic mulching modulated native populations of entomopathogenic nematode in vineyard soils differently depending on its potential to control outgrowth of their natural enemies. *Journal of Invertebrate Pathology*, 192. <u>https://doi.org/10.1016/j.jip.2022.107781</u>
- Bligh, E. G., & Dyer W. J. (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol. 37(8):911-7. doi: 10.1139/o59-099. PMID: 13671378.
- Blouin, M., Hodson, M. E., Delgado, E. A., Baker, G., Brussaard, L., Butt, K. R., Dai, J., Dendooven, L., Peres, G., Tondoh, J. E., Cluzeau, D., & Brun, J. J. (2013). A review of earthworm impact on soil function and ecosystem services. In *European Journal of Soil Science* (Vol. 64, Issue 2, pp. 161– 182). https://doi.org/10.1111/ejss.12025
- Brèteau-Amores, S., Brunette, M., & Andrés-Domenech, P. (2023). A Cost Assessment of Tree Plantation Failure under Extreme Drought Events in France: What Role for Insurance? *Forests*, 14(2). https://doi.org/10.3390/f14020308
- Bünemann, E. K., Bongiorno, G., Bai, Z., Creamer, R. E., De Deyn, G., de Goede, R., Fleskens, L.,
  Geissen, V., Kuyper, T. W., Mäder, P., Pulleman, M., Sukkel, W., van Groenigen, J. W., & Brussaard,
  L. (2018). Soil quality A critical review. In *Soil Biology and Biochemistry* (Vol. 120, pp. 105–125).
  Elsevier Ltd. https://doi.org/10.1016/j.soilbio.2018.01.030
- Buyer, J. S., & Sasser, M. (2012). High throughput phospholipid fatty acid analysis of soils. *Applied Soil Ecology*, *61*, 127–130. https://doi.org/10.1016/j.apsoil.2012.06.005
- Chen, X. Y., Daniell, T. J., Neilson, R., O'Flaherty, V., & Griffiths, B. S. (2010). A comparison of molecular methods for monitoring soil nematodes and their use as biological indicators. *European Journal* of Soil Biology, 46(5), 319–324. https://doi.org/10.1016/j.ejsobi.2010.05.002
- Cotrufo, M. F., Haddix, M. L., Kroeger, M. E., & Stewart, C. E. (2022). The role of plant input physicalchemical properties, and microbial and soil chemical diversity on the formation of particulate and mineral-associated organic matter. *Soil Biology and Biochemistry*, *168*. https://doi.org/10.1016/j.soilbio.2022.108648
- Cotrufo, M. F., & Lavallee, J. M. (2022). Soil organic matter formation, persistence, and functioning: A synthesis of current understanding to inform its conservation and regeneration. In *Advances in*

*Agronomy* (Vol. 172, pp. 1–66). Academic Press Inc. https://doi.org/10.1016/bs.agron.2021.11.002

- Cotrufo, M. F., Ranalli, M. G., Haddix, M. L., Six, J., & Lugato, E. (2019). Soil carbon storage informed by particulate and mineral-associated organic matter. *Nature Geoscience*, *12*(12), 989–994. https://doi.org/10.1038/s41561-019-0484-6
- Creamer, R. E., Barel, J. M., Bongiorno, G., & Zwetsloot, M. J. (2022). The life of soils: Integrating the who and how of multifunctionality. *Soil Biology and Biochemistry*, *166*. https://doi.org/10.1016/j.soilbio.2022.108561
- Creamer, R., O', L., & Editors, S. (n.d.). *World Soils Book Series The Soils of Ireland*. http://www.springer.com/series/8915
- Doran, J. W., & Zeiss, M. R. (2000). Soil health and sustainability: managing the biotic component of soil quality. In *Applied Soil Ecology* (Vol. 15).
- Eze, S., Magilton, M., Magnone, D., Varga, S., Gould, I., Mercer, T. G., & Goddard, M. R. (2023). Metaanalysis of global soil data identifies robust indicators for short-term changes in soil organic carbon stock following land use change. In *Science of the Total Environment* (Vol. 860). Elsevier B.V. https://doi.org/10.1016/j.scitotenv.2022.160484
- Fanin, N., & Bertrand, I. (2016). Aboveground litter quality is a better predictor than belowground microbial communities when estimating carbon mineralization along a land-use gradient. *Soil Biology and Biochemistry*, 94, 48–60. https://doi.org/10.1016/j.soilbio.2015.11.007
- Fanin, N., Kardol, P., Farrell, M., Nilsson, M. C., Gundale, M. J., & Wardle, D. A. (2019). The ratio of Gram-positive to Gram-negative bacterial PLFA markers as an indicator of carbon availability in organic soils. *Soil Biology and Biochemistry*, *128*, 111–114. https://doi.org/10.1016/j.soilbio.2018.10.010
- Ferris, H. (2010). Ó The Society of Nematologists. In Journal of Nematology (Vol. 42, Issue 1).
- Fierer, N., Wood, S. A., & Bueno de Mesquita, C. P. (2021). How microbes can, and cannot, be used to assess soil health. In *Soil Biology and Biochemistry* (Vol. 153). Elsevier Ltd. https://doi.org/10.1016/j.soilbio.2020.108111
- Gan, H., & Wickings, K. (2020). Root herbivory and soil carbon cycling: Shedding "green" light onto a "brown" world. In *Soil Biology and Biochemistry* (Vol. 150). Elsevier Ltd. https://doi.org/10.1016/j.soilbio.2020.107972
- Garba, I. I., Stirling, G. R., Stirling, A. M., & Williams, A. (2024). Cover crop functional types alter soil nematode community composition and structure in dryland crop-fallow rotations. *Applied Soil Ecology*, *194*, 105196. https://doi.org/10.1016/j.apsoil.2023.105196
- Giuffré, Giovanna., Ricci, Andrea., Bisoffi, Stefano., Dönitz, Ewa., Voglhuber-Slavinsky, Ariane.,
   Helming, Katharina., Evgrafova, Alevtina., Ratinger, Tomas., Robinson, D. A., & European
   Commission. Directorate-General for Research and Innovation. (n.d.). *Mission area : soil health and food : foresight on demand brief in support of the Horizon Europe mission board.*
- Grabau, Z. J., Thu, Z., Maung, Z., Noyes, D. C., Baas, D. G., Werling, B. P., Brainard, D. C., &Melakeberhan, H. (2017). Effects of Cover Crops on Pratylenchus penetrans and the Nematode Community in Carrot Production. In *Journal of Nematology* (Vol. 49, Issue 1).

- Greenly, K. M., & Rakow, D. A. (1995). THE EFFECT OF WOOD MULCH TYPE AND DEPTH ON WEED AND TREE GROWTH AND CERTAIN SOIL PARAMETERS. In *Journal of Arboriculture* (Vol. 21, Issue 5).
- Guo, L. B., & Gifford, R. M. (2002). Soil carbon stocks and land use change: A meta analysis. *Global Change Biology*, *8*(4), 345–360. https://doi.org/10.1046/j.1354-1013.2002.00486.x
- Haddix, M. L., Gregorich, E. G., Helgason, B. L., Janzen, H., Ellert, B. H., & Francesca Cotrufo, M. (2020). Climate, carbon content, and soil texture control the independent formation and persistence of particulate and mineral-associated organic matter in soil. *Geoderma*, 363. https://doi.org/10.1016/j.geoderma.2019.114160
- Hirons, A., & Percival, G. (2011). *Fundamentals of tree establishment: a review*. https://www.researchgate.net/publication/274953179
- Joergensen, R. G., & Wichern, F. (2008). Quantitative assessment of the fungal contribution to microbial tissue in soil. *Soil Biology and Biochemistry*, *40*(12), 2977–2991. https://doi.org/10.1016/j.soilbio.2008.08.017
- Kader, M. A., Senge, M., Mojid, M. A., & Ito, K. (2017). Recent advances in mulching materials and methods for modifying soil environment. In *Soil and Tillage Research* (Vol. 168, pp. 155–166).
   Elsevier B.V. https://doi.org/10.1016/j.still.2017.01.001
- Kauer, K., Pärnpuu, S., Talgre, L., Eremeev, V., & Luik, A. (2021). Soil particulate and mineral-associated organic matter increases in organic farming under cover cropping and manure addition. *Agriculture (Switzerland)*, 11(9). https://doi.org/10.3390/agriculture11090903
- Lavallee, J. M., Soong, J. L., & Cotrufo, M. F. (2020). Conceptualizing soil organic matter into particulate and mineral-associated forms to address global change in the 21st century. *Global Change Biology*, 26(1), 261–273. https://doi.org/10.1111/gcb.14859
- López, R., & Cabrera, F. (n.d.). Compost properties related to particle size.
- Martin, T., Wade, J., Singh, P., & Sprunger, C. D. (2022). The integration of nematode communities into the soil biological health framework by factor analysis. *Ecological Indicators*, *136*. https://doi.org/10.1016/j.ecolind.2022.108676
- Mendis, S. S., Udawatta, R. P., Anderson, S. H., Nelson, K. A., & Cordsiemon, R. L. (2022). Effects of cover crops on soil moisture dynamics of a corn cropping system. *Soil Security*, 8. https://doi.org/10.1016/j.soisec.2022.100072
- Millennium Ecosystem Assessment (Program). (2005). *Ecosystems and human well-being : synthesis*. Island Press.
- O'Neill, B., Sprunger, C. D., & Robertson, G. P. (2021). Do soil health tests match farmer experience? Assessing biological, physical, and chemical indicators in the Upper Midwest United States. *Soil Science Society of America Journal*, *85*(3), 903–918. <u>https://doi.org/10.1002/saj2.20233</u>
- Oostenbrink M (1960). Estimating nematode populations by some selected methods. In: Sasser J N, Jenkins W R, eds. Nematology. Chapel Hill: University of North Carolina Press, 85–102
- Osburn, E. D., McBride, S. G., Kupper, J. V., Nelson, J. A., McNear, D. H., McCulley, R. L., & Barrett, J. E. (2022). Accurate detection of soil microbial community responses to environmental change

requires the use of multiple methods. *Soil Biology and Biochemistry*, *169*. https://doi.org/10.1016/j.soilbio.2022.108685

- Poeplau, C., & Don, A. (2015). Carbon sequestration in agricultural soils via cultivation of cover crops -A meta-analysis. In *Agriculture, Ecosystems and Environment* (Vol. 200, pp. 33–41). Elsevier. https://doi.org/10.1016/j.agee.2014.10.024
- Poeplau, C., Don, A., Vesterdal, L., Leifeld, J., Van Wesemael, B., Schumacher, J., & Gensior, A. (2011). Temporal dynamics of soil organic carbon after land-use change in the temperate zone - carbon response functions as a model approach. In *Global Change Biology* (Vol. 17, Issue 7, pp. 2415– 2427). Blackwell Publishing Ltd. https://doi.org/10.1111/j.1365-2486.2011.02408.x
- Potapov, P., Turubanova, S., Hansen, M. C., Tyukavina, A., Zalles, V., Khan, A., Song, X. P., Pickens, A., Shen, Q., & Cortez, J. (2022). Global maps of cropland extent and change show accelerated cropland expansion in the twenty-first century. *Nature Food*, 3(1), 19–28. https://doi.org/10.1038/s43016-021-00429-z
- Report, M., Fao, ©, & Bizzarri, G. (n.d.). INTERGOVERNMENTAL TECHNICAL PANEL ON SOILS INTERGOVERNMENTAL TECHNICAL PANEL ON SOILS Status of the World's Soil Resources.
- Ruiz, S. A., Bickel, S., & Or, D. (2021). Global earthworm distribution and activity windows based on soil hydromechanical constraints. *Communications Biology*, 4(1). https://doi.org/10.1038/s42003-021-02139-5
- Sanderman, J., Fillery, I. R. P., Jongepier, R., Massalsky, A., Roper, M. M., MacDonald, L. M., Maddern, T., Murphy, D. V., & Baldock, J. A. (2013). Carbon sequestration under subtropical perennial pastures II: Carbon dynamics. *Soil Research*, *51*(7–8), 771–780. https://doi.org/10.1071/SR12351
- Schulte, R. P. O., Bampa, F., Bardy, M., Coyle, C., Creamer, R. E., Fealy, R., Gardi, C., Ghaley, B. B., Jordan, P., Laudon, H., O'Donoghue, C., Ó'hUallacháin, D., Lilian O'Sullivan, Rutgers, M., Six, J., Toth, G. L., & Vrebos, D. (2015). Making the most of our land: Managing soil functions from local to continental scale. *Frontiers in Environmental Science*, *3*(DEC). https://doi.org/10.3389/fenvs.2015.00081
- Six, J., Frey, S. D., Thiet, R. K., & Batten, K. M. (2006). Bacterial and Fungal Contributions to Carbon Sequestration in Agroecosystems. *Soil Science Society of America Journal*, 70(2), 555–569. https://doi.org/10.2136/sssaj2004.0347
- Song, D., Tariq, A., Pan, K., Chen, W., Zhang, A., Sun, X., Ran, Y., & Zeng, F. (2020). Effects of straw mulching practices on soil nematode communities under walnut plantation. *Scientific Reports*, 10(1). https://doi.org/10.1038/s41598-020-72530-5
- Stone, D., Costa, D., Daniell, T. J., Mitchell, S. M., Topp, C. F. E., & Griffiths, B. S. (2016). Using nematode communities to test a European scale soil biological monitoring programme for policy development. *Applied Soil Ecology*, 97, 78–85. https://doi.org/10.1016/j.apsoil.2015.08.017
- Teravest, D., Smith, J. L., Carpenter-Boggs, L., Granatstein, D., Hoagland, L., & Reganold, J. P. (2011).
   Soil Carbon Pools, Nitrogen Supply, and Tree Performance under Several Groundcovers and
   Compost Rates in a Newly Planted Apple Orchard. In *HORTSCIENCE* (Vol. 46, Issue 12).
- Tsiafouli, M. A., Thébault, E., Sgardelis, S. P., de Ruiter, P. C., van der Putten, W. H., Birkhofer, K., Hemerik, L., de Vries, F. T., Bardgett, R. D., Brady, M. V., Bjornlund, L., Jørgensen, H. B.,

Christensen, S., Hertefeldt, T. D., Hotes, S., Gera Hol, W. H., Frouz, J., Liiri, M., Mortimer, S. R., ... Hedlund, K. (2015). Intensive agriculture reduces soil biodiversity across Europe. *Global Change Biology*, *21*(2), 973–985. https://doi.org/10.1111/gcb.12752

- van den Hoogen, J., Geisen, S., Routh, D., Ferris, H., Traunspurger, W., Wardle, D. A., de Goede, R. G. M., Adams, B. J., Ahmad, W., Andriuzzi, W. S., Bardgett, R. D., Bonkowski, M., Campos-Herrera, R., Cares, J. E., Caruso, T., de Brito Caixeta, L., Chen, X., Costa, S. R., Creamer, R., ... Crowther, T. W. (2019). Soil nematode abundance and functional group composition at a global scale. *Nature*, *572*(7768), 194–198. https://doi.org/10.1038/s41586-019-1418-6
- Vazquez, C., de Goede, R. G. M., Rutgers, M., de Koeijer, T. J., & Creamer, R. E. (2021). Assessing multifunctionality of agricultural soils: Reducing the biodiversity trade-off. *European Journal of Soil Science*, 72(4), 1624–1639. https://doi.org/10.1111/ejss.13019
- Vidal, A., Watteau, F., Remusat, L., Mueller, C. W., Nguyen Tu, T. T., Buegger, F., Derenne, S., & Quenea, K. (2019). Earthworm cast formation and development: A shift from plant litter to mineral associated organic matter. *Frontiers in Environmental Science*, 7(APR). https://doi.org/10.3389/fenvs.2019.00055
- von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E., & Marschner, B. (2007). SOM fractionation methods: Relevance to functional pools and to stabilization mechanisms. *Soil Biology and Biochemistry*, *39*(9), 2183–2207. https://doi.org/10.1016/j.soilbio.2007.03.007
- Vršič, S., Breznik, M., Pulko, B., & Rodrigo-Comino, J. (2021). Earthworm abundance changes depending on soil management practices in slovenian vineyards. *Agronomy*, *11*(6). https://doi.org/10.3390/agronomy11061241
- Wall, D. P., Delgado, A., O'Sullivan, L., Creamer, R. E., Trajanov, A., Kuzmanovski, V., Bugge Henriksen, C., & Debeljak, M. (2020). A Decision Support Model for Assessing the Water Regulation and Purification Potential of Agricultural Soils Across Europe. *Frontiers in Sustainable Food Systems*, 4. https://doi.org/10.3389/fsufs.2020.00115
- Wiesmeier, M., Urbanski, L., Hobley, E., Lang, B., von Lützow, M., Marin-Spiotta, E., van Wesemael, B., Rabot, E., Ließ, M., Garcia-Franco, N., Wollschläger, U., Vogel, H. J., & Kögel-Knabner, I. (2019). Soil organic carbon storage as a key function of soils - A review of drivers and indicators at various scales. In *Geoderma* (Vol. 333, pp. 149–162). Elsevier B.V. https://doi.org/10.1016/j.geoderma.2018.07.026
- Wilkinson, M. T., Richards, P. J., & Humphreys, G. S. (2009). Breaking ground: Pedological, geological, and ecological implications of soil bioturbation. In *Earth-Science Reviews* (Vol. 97, Issues 1–4). https://doi.org/10.1016/j.earscirev.2009.09.005
- Willers, C., Jansen van Rensburg, P. J., & Claassens, S. (2015). Phospholipid fatty acid profiling of microbial communities-a review of interpretations and recent applications. In *Journal of Applied Microbiology* (Vol. 119, Issue 5, pp. 1207–1218). <u>https://doi.org/10.1111/jam.12902</u>
- WUR SOP 010. (2017). Nematodes sampling and Extraction
- WUR SOP 012. (2017). Hand sorting, counting, weighing and identification of earthworms. 0 Changes from previous version
- WUR SOP 023. (2023). Extraction PLFA and NLFA for GC analysis (based on Lund/NIOO protocol 2019

WUR SOP 024. (2021). Rapid particle size fractionation to determine fast (labile) vs. slow (stable cycling Soil Organic Carbon (SOC)

# Appendix

Variable:	Treatment:	Clover1	Clover2	Control1	Control2	Mulch1	Mulch2
POM-C (%)	Mean	0.35	0.50	0.25	0.39	0.30	0.51
	(SD)	(0.10)	(0.10)	(0.10)	(0.10)	(0.10)	(0.10)
POM-N (%)	Mean	0.05	0.05	0.02	0.04	0.03	0.06
	(SD)	(0.03)	(0.03)	(0.03)	(0.03)	(0.03)	(0.03)
MAOM-C (%)	Mean	1.39	1.49	1.46	1.48	1.48	1.53
	(SD)	(0.09)	(0.09)	(0.09)	(0.09)	(0.09)	(0.09)
MAOM-N (%)	Mean	0.21	0.21	0.20	0.21	0.21	0.21
	(SD)	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)
Soil Moisture (%)	Mean	18.20	12.3	17.11	13.5	17.8	15.7
	SD	(1.67)	(2.64)	(0.74)	(0.94)	(0.87)	(2.17)
Number of Worms	Mean	7.7	2.0	9.5	1.7	9.5	7.3
	(SD)	(6.9)	(2.5)	(8.2)	(1.4)	(5.1)	(4.2)
Biomass (g)	Mean	2.3	0.6	2.3	0.3	2.9	2.3
	(SD)	(1.9)	(1.1)	(2.4)	(0.4)	(2.3)	(2.1)
Nematodes (per kg)	Mean	5371	14122	5694	10569	5113	8246
	(SD)	(2886)	(8018)	(5253)	(5410)	(1567)	(2900)
Total PLFA	Mean	35105	36332	34867	35690	36033	33592
(Picomolar per g)	(SD)	(5032)	(4733)	(5545)	(6736)	(5501)	(4666)
Amffungi (%)	Mean	4.98	5.00	5.09	4.89	5.08	5.10
	(SD)	(0.44)	(0.23)	(0.47)	(0.21)	(0.19)	(0.31)
Fungi (%)	Mean	1.78	2.45	1.78	1.98	1.85	1.81
	(SD)	(0.31)	(1.33)	(0.59)	(0.21)	(0.19)	(0.31)
Gram-positive (%)	Mean	35.26	36.75	35.69	36.21	35.38	36.84
	(SD)	(1.89)	(0.82)	(2.10)	(1.05)	(0.97)	(0.76)
Gram-negative (%)	Mean	34.67	34.34	35.76	34.88	35.09	34.51
	(SD)	(1.33)	(0.91)	(0.78)	(0.76)	(0.61)	(0.70)
Actinomycetes (%)	Mean	19.44	20.15	18.69	19.61	19.40	20.48
	(SD)	(1.58)	(1.54)	(2.73)	(1.81)	(1.14)	(0.78)

Eukaryotes (%)	Mean	3.86	1.33	2.98	2.41	3.20	1.25
	(SD)	(2.90)	(1.28)	(3.14)	(2.33)	(2.69)	(0.98)

### **Productblad Substrado® Houtmulch**





#### Eigenschappen

Substrado® Houtmulch komt na het RHP composteringsproces vrij. Als de fijnere compost afgezeefd is blijven grovere stukken over die verder verkleint en geschoond worden. De fijne fractie 0-30 mm wordt na het verkleinen verder gecomposteerd en als houtmulch ingezet.

Het product is geschikt om in zware gronden op te mengen om meer structuur te krijgen. Tevens is Substrado® Houtmulch zeer geschikt om als mulchlaag te gebruiken om het bodemleven te activeren en onkruid te onderdrukken.

#### **Kwaliteit**

Door de temperatuur die tijdens het composteren bereikt wordt verliezen mogelijke ziektes en onkruidzaden hun kiemkracht. Doordat het hout gecomposteerd en gehygiëniseerd is, verdwijnt zacht materiaal als bast en twijgen en is de stikstof vastlegging laag. Substrado® Houtmulch bestaat voor 100% uit gecomposteerd hout en is dus uitermate geschikt voor langdurige structuurverbetering van de bodem.



Fruitteelt Kleigronden **Groenvoorziening** Tuinaanleg Tuinbouw **Productspecificaties** 50 - 65 Droge stofgehalte % Organische stofgehalte > 30 % Fractie 0 - 30mm Verwerkingsvoorschrift Op zware gronden met de grond mengen. Als mulchlaag 5-15 cm aanbrengen, mogelijk handmatig nalopen om vervuiling te verwijderen. Voor een goede onkruidwering dient de grond vlak te zijn en vrij van onkruiden, de ondergrond dient vrij te zijn van overblijvende onkruiden.

Hermalen 7 5481 XX Schijndel T+31(0)73 - 543 10 00 info@denoudengroep.com www.denoudengroep.com

f 🖸 🎔 🕨 in

#### Rapport

🔅 eurofins

Agro

**BemestingsWijzer** 

rijenteelt noord

Uw klantnummer: 9030093

De Biesterhof Zeelandsestraat 74 6566 JB MILLINGEN AD RYN Eurofins Agro Postbus 170 NL - 6700 AD Wageningen

- monstername: Patrick Bens: 0652002106 klantenservice: 088 876 1010 klantenservice.agro@eurofins.com www.eurofins-agro.com Т

Onderzoek Onderzoek-/ordernr: Datum monstername: Datum verslag: 804050/005876164 28-10-2022 13-10-2022 Resultaat Eenheid Resultaat Streeftraject vrij laag goed vrij hoog hoog laag 4790 3070 - 4600 N-totale bodemvoorraad kg N/ha Chemisch 13 - 17 95 - 145 C/N-ratio 9 N-leverend vermogen kg N/ha 95 o-piantbeschikbaar kg S/ha S-totale bodemvoorraad kg S/ha C/S-ratio 11 < 465 96 20 - 30 670 - 1245 50 - 75 20 - 30 S-leverend vermogen kg S/ha 5 kg P/ha kg P/ha P-plantbeschikbaar P-bodemvoorraad 5,6 - 9,3 470 - 605 4,6 310 K-plantbeschikbaar kg K/ha kg K/ha 170 470 215 - 340 345 - 580 K-bodemvoorraad kg Ca/ha kg Ca/ha 225 - 520 Ca-plantbeschikbaar 200 Ca-bodemvoorraad 8670 7685 - 9780 Mg-plantbeschikbaar Mg-bodemvoorraad 215 - 340 430 - 720 kg Mg/ha kg Mg/ha 475 670 71 36 Na-plantbeschikbaar Na-bodemvoorraad 46 - 93 kg Na/ha kg Na/ha 50 - 92 g Si/ha g Fe/ha g Zn/ha g Mn/ha g Cu/ha g Co/ha g B/ha g Mo/ha 18540 - 80340 7730 - 13910 1550 - 2320 3090 - 4020 Si-plantbeschikbaar 75430 Fe-plantbeschikbaar Zn-plantbeschikbaar < 6300 < 310 Mn-plantbeschikbaar Cu-plantbeschikbaar Co-plantbeschikbaar 1480 3090 - 4020 125 - 200 15 - 25 495 - 680 310 - 15450 11 - 14 110 < 10 B-plantbeschikbaar 940 Mo-plantbeschikbaar Se-plantbeschikbaar g Mo/ha g Se/ha < 10 12 Fysisch Zuurgraad (pH) 6,7 > 5,9 C-organisch Organische stof C/OS-ratio 1,4 3,1 0,45 % % 0,45 - 0,55 % 0,7 2,0 - 3,0 Koolzure kalk Klei (<2 μm) Silt (2-50 μm) Zand (>50 μm) Slib (<16 μm) 20 40 36 % % % 32 mmol+/kg % % % % % > 140 > 95 80 - 90 6,0 - 10 2,0 - 4,0 1,0 - 1,5 < 1,0 < 1,0 Klei-humus (CEC) 164 99 85 11 2,4 0,3 < 0,1 CEC-bezetting Ca-bezetting Mg-bezetting K-bezetting Na-bezetting H-bezetting Al-bezetting < 0,1

> Pagina: 1 Totaal aantal pagina's: 3 Rapportidentificatie: 804050/005876164, 28-10-2022



Eurofi

ning zijn van de i prakeliji /an EL ns Agro Wage uit het

Agro Testing Wageningen BV is ingeschreven in het RvA-register voor testlaboratoria zoals mschreven in de erkenning onder nr. L122 voor uitsluitend de monsternemings- en/of de methoden.

ŊQ.

#### rijenteelt noord

Resultaat		Eenheid	Resultaat	Streeftraject	laag	vrij laag	goed	zeer goed	ł
	Verkruimelbaarheid Verslemping Stuifgevoeligheid	rapportcijfer rapportcijfer rapportcijfer	7,0 4,9 8,8	6,0 - 8,0 6,0 - 8,0 6,0 - 8,0					
D. I I		Eenheid	Resultaat	Streeftraject	laag	vrij laag	goed	vrij hoog	hoog
Biologisch	Microbiële biomassa Microbiële activiteit Schimmel/bacterie-ratio	mg C/kg mg N/kg	290 55 1,0	155 - 465 31 - 52 0,6 - 0,9					

#### Bemestingsadviezen

Het resultaat wordt afgezet tegen het landbouwkundig streeftraject en krijgt een waardering; laag, vrij laag, goed, vrij hoog, hoog. Dit is geen beoordeling zoals bedoeld in ISO 17025 (par. 7.8.6).

Lever de resultaten van grondoniuerse u de volgende waarden doorgeven: P-bodemvoorraad (P-Al) = 23 mg P<sub>2</sub>O<sub>5</sub>/100 g Wetgeving Lever de resultaten van grondonderzoek ieder jaar opnieuw voor 15 mei van het betreffende jaar in bij RVO. Voor dit perceel kunt

Wilt u weten hoeveel fosfaat u mag toedienen op basis van deze analyseresultaten? Check dan de fosfaatgebruiksnormen voor dit jaar via https://www.eurofins-agro.com/nl-nl/fosfaatklasse-grasland-bouwland



De hier vermelde oppervlakte kan afwijken van de gegevens van RVO.nl; de oppervlakte gemeten door RVO.nl is leidend. Oppervlakte (ha): 8,3

Hoekpunten perceel: 198894 429136, 198910 429172, 198919 429190, 198925 429199, 198931 429202, 198940 429210,

Hoekpunten perceel: 198894 429136, 198910 429172, 198919 429190, 198825 429199, 198931 429202, 198940 429210, 198961 429205, 198975 429201, 199057 429246, 199091 429321, 198839 429528, 198826 429538, 198799 429487, 198783 429460, 198767 429436, 198712 429381, 198658 429329, 198894 429136 Monsternamepunten: 198670 429329, 198741 429382, 198772 429297, 198778 429333, 198782 429384, 198783 429259, 198787 429449, 198815 429349, 198822 429390, 198829 429255, 198835 429438, 198849 429258, 198849 429488, 198886 429170, 198889 429366, 198893 429350, 198896 429255, 198901 429406, 198803 429454, 198819 429310, 198937 429248, 198946 429269, 198950 429413, 198952 429317, 198960 429247, 199012 429260, 199013 429374, 199051 429304

Vanwege ruimtegebrek is het mogelijk dat niet alle coordinaten van de vastgelegde hoekpunten van het perceel op dit verslag zijn weergegeven. Deze zijn echter wel opgeslagen in onze database

Advies Gewas: Moestuin

Er is door u geen bemestingsadvies aangevraagd!

Toelichting Fosfaat:

Het berekende Pw-getal is voor dit perceel 22 mg P2O5/I.

Kali:

Het berekende K-getal is voor dit perceel 18 K-getal wordt niet meer gebruikt bij de adviesberekening.

#### Bodemleven:

De biologische bodemvruchtbaarheid wordt nu weergegeven via 3 kengetallen, te weten de microbiële biomassa, de microbiële activiteit en de schimmel/bacterie-ratio. Op basis van de huidige kennis wordt een waardering gegeven

die afhankelijk is van de hoeveelheid organische stof. Er wordt nu nog geen advies gegeven. Via diverse onderzoeksprojecten zal er meer informatie beschikbaar komen.

Pagina: 2 Totaal aantal pagina's: 3 Rapportidentificatie: 804050/005876164, 28-10-2022

#### Rapport

#### rijenteelt noord

Contact & info	Romonetordo	10
COMPACE ALLING	Demonsterue	ം

Bemonsterde laag:	0 - 25 cm
Grondsoort:	Zavel
Monster genomen door:	Eurofins Agro, Sander Schuurman
Contactpersoon monstername:	Patrick Bens: 0652002106
Bemonsteringsmethode:	volgens Eurofins Agro standaard MIN 1030
Specificatie monstername:	Gestratificeerd

Indien de volgende informatie wordt getoond op de rapporten kan deze informatie verstrekt zijn door de opdrachtgever en van invloed zijn op de waardering, advisering en/of het analyseresultaat: bemonsteringsdiepte, gewas, teelttype/ras.

Q

Methode		Resultaat	Eenheid	Methode	RvA
Analyse	N-totale bodemvoorraad	1550	mg N/kg	Em: NIRS	Q
esultaten	S-plantbeschikbaar	3,4	mg S/kg	Em: CCL3 (Gw NEN 17294-2)	
	S-totale bodemvoorraad	< 150	mg S/kg	Em: NIRS	Q
	P-plantbeschikbaar	1,5	mg P/kg	Em: CCL3 (Gw NEN 15923-1)	Q
	P-bodemvoorraad	23	mg P <sub>2</sub> O <sub>2</sub> /100 g	PAL1: Gw NEN 5793	Q
	K-plantbeschikbaar	55	mg K/kg	Em: CCL3 (Gw NEN 17294-2)	
	K-bodemvoorraad	3,9	mmol+/kg	Em: NIRS	
	Ca-plantbeschikbaar	0,8	mmol Ca/l	Em: NIRS	
	Ca-bodemvoorraad	140	mmol+/kg	Em: NIRS	
	Mg-plantbeschikbaar	154	mg Mg/kg	Em: CCL3 (Gw NEN 17294-2)	
	Mg-bodemvoorraad	17,9	mmol+/kg	Em: NIRS	
	Na-plantbeschikbaar	23	mg Na/kg	Em: CCL3 (Gw NEN 17294-2)	
	Na-bodemvoorraad	0,5	mmol+/kg	Em: NIRS	
	Si-plantbeschikbaar	24410	µg Si/kg	Em: CCL3 (Gw NEN 17294-2)	
	Fe-plantbeschikbaar	< 2040	µg Fe/kg	Em: CCL3 (Gw NEN 17294-2)	
	Zn-plantbeschikbaar	< 100	ug Zn/kg	Em: CCL3 (Gw NEN 17294-2)	
	Mn-plantbeschikbaar	480	µg Mn/kg	Em: CCL3 (Gw NEN 17294-2)	
	Cu-plantbeschikbaar	35	µg Cu/kg	Em: CCL3 (Gw NEN 17294-2)	Q
	Co-plantbeschikbaar	< 2.6	µg Co/kg	Em: CCL3 (Gw NEN 17294-2)	Q
	B-plantbeschikbaar	304	µg B/kg	Em: CCL3 (Gw NEN 17294-2)	
	Mo-plantbeschikbaar	< 4	µg Mo/kg	Em: CCL3 (Gw NEN 17294-2)	
	Se-plantbeschikbaar	3,9	µg Se/kg	Em: CCL3 (Gw NEN 17294-2)	
	Zuurgraad (pH)	6,7		Em:PHC3(Cf NEN ISO 10390)	Q
	C-organisch	1,4	%	Em: NIRS	Q
	Organische stof	3,1	%	Em: NIRS	Q
	C-anorganisch	0,08	%	Em: NIRS	
	Koolzure kalk	0,7	%	Em: NIRS	
	Klei (<2 µm)	20	%	Em: NIRS	
	Silt (2-50 µm)	40	%	Em: NIRS	
	Zand (>50 µm)	36	%	Em: NIRS	
	Klei-humus (CEC)	164	mmol+/kg	Em: NIRS	
	Microbiële biomassa	290	mg C/kg	Em: NIRS	
	Microbiële activiteit	55	mg N/kg	Em: NIRS	
	Schimmel biomassa	119	mg C/kg	Em: NIRS	
	Bacteriële biomassa	114	mg C/kg	Em: NIRS	
	Bulkdichtheid	1236	kg/m <sup>3</sup>	Em: NIRS	
	De op pagina 1 en 2 bij Resu	Itaat vermelde waard	en zijn berekend uit bove	nstaande analyseresultaten.	

Q Methode geaccrediteerd door RvA Em: Eigen methode, Gw: Gelijkwaardig aan, Cf: Conform

De resultaten zijn weergegeven in droge grond. Alle verrichtingen zijn binnen de gestelde houdbaarheidstermijn tussen monstername en analyse uitgevoerd. Het monster is geanalyseerd in het Eurofins Agro laboratorium in Wageningen, tenzij anders is vermeld. De resultaten hebben uitsluitend betrekking on het monster dat Eurofins Agro heeft genomen, ontvangen en op het materiaal dat in behandeling is genomen op 21-10-2022 en daarmee op het geanalyseerde monster. Nadere omschrijving van de toegepaste monstername en analyse methoden is te vinden op www.eurofins-agro.com

Pagina: 3 Totaal aantal pagina's: 3 Rapportidentificatie: 804050/005876164, 28-10-2022



Ing. Alge n van toepassing. Op v n. Eurofins Agro Testir evolgen voortvloeiend worden deze en/of de specific Wageningen BV stelt zich niel uit het gebruik van door of nam oden toegezonden. uele schadelijke gevolgen

ifa.

Eurofin nader o fins Agro Testing Wageningen BV is ingeschreven in het RvA-register voor testlaboratoria zoals r omschreven in de erkenning onder nr. L122 voor uitsluitend de monsternemings- en/of de zemethorder







KNMI precipation data of Nijmegen:
539,20230310, 355, 0,
539,20230311, 156, 0,

539,20230312,	2,	0,
539,20230313,	16,	0,
539,20230314,	25,	0,
539,20230315,	58,	0,
539,20230316,	1,	0,
539,20230317,	1,	0,
539,20230318,	10,	0,
539,20230319,	10,	0,
539,20230320,	1,	0,
539,20230321,	15,	0,
539,20230322,	5,	0,
539,20230323,	15,	0,
539,20230324,	35,	0,
539,20230325,	52,	0,
539,20230326,	41,	0,
539,20230327,	40,	0,
539,20230328,	5,	0,
539,20230329,	5,	0,
539,20230330,	3,	0,
539,20230331,	55,	0,
539,20230401,	100,	0,
539,20230402,	144,	0,
539,20230403,	0,	0,
539,20230404,	0,	0,
539,20230405,	0,	0,
539,20230406,	0,	0,
539,20230407,	51,	0,
539,20230408,	11,	0,
539,20230409,	0,	0,
539,20230410,	0,	0,

539,20230411,	68,	0,
539,20230412,	75,	0,
539,20230413,	68,	0,
539,20230414,	5,	0,
539,20230415,	0,	0,
539,20230416,	1,	0,
539,20230417,	0,	0,
539,20230418,	0,	0,
539,20230419,	0,	0,
539,20230420,	0,	0,
539,20230421,	102,	0,
539,20230422,	66,	0,
539,20230423,	50,	0,
539,20230424,	101,	0,
539,20230425,	43,	0,
539,20230426,	0,	0,
539,20230427,	0,	0,
539,20230428,	0,	0,
539,20230429,	19,	0,
539,20230430,	0,	0,
539,20230501,	0,	0,
539,20230502,	0,	0,
539,20230503,	0,	0,
539,20230504,	0,	0,
539,20230505,	0,	0,
539,20230506,	41,	0,
539,20230507,	7,	0,
539,20230508,	16,	0,
539,20230509,	139,	0,
539,20230510,	111,	0,

539,20230511,	9,	0,
539,20230512,	49,	0,
539,20230513,	12,	0,
539,20230514,	1,	0,
539,20230515,	0,	0,
539,20230516,	6,	0,
539,20230517,	2,	0,
539,20230518,	0,	0,
539,20230519,	0,	0,
539,20230520,	0,	0,
539,20230521,	0,	0,
539,20230522,	18,	0,
539,20230523,	4,	0,
539,20230524,	0,	0,
539,20230525,	0,	0,
539,20230526,	0,	0,
539,20230527,	0,	0,
539,20230528,	0,	0,
539,20230529,	0,	0,
539,20230530,	0,	0,
539,20230531,	0,	0,
539,20230601,	0,	0,
539,20230602,	0,	0,
539,20230603,	0,	0,
539,20230604,	0,	0,
539,20230605,	0,	0,
539,20230606,	0,	0,
539,20230607,	0,	0,
539,20230608,	0,	0,
539,20230609,	0,	0,

539,20230610,	0,	0,
539,20230611,	0,	0,
539,20230612,	0,	0,
539,20230613,	0,	0,
539,20230614,	0,	0,
539,20230615,	0,	0,
539,20230616,	0,	0,
539,20230617,	0,	0,
539,20230618,	0,	0,
539,20230619,	0,	0,
539,20230620,	0,	0,
539,20230621,	99,	0,
539,20230622,	0,	0,
539,20230623,	213,	0,
539,20230624,	0,	0,
539,20230625,	0,	0,
539,20230626,	0,	0,
539,20230627,	0,	0,
539,20230628,	19,	0,
539,20230629,	2,	0,
539,20230630,	9,	0,
539,20230701,	4,	0,
539,20230702,	24,	0,
539,20230703,	3,	0,
539,20230704,	72,	0,
539,20230705,	86,	0,
539,20230706,	63,	0,
539,20230707,	0,	0,
539,20230708,	0,	0,
539,20230709,	0,	0,

539,20230710, 40, 0,