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ORIGINAL RESEARCH

Evaluation of the microBIOMETER® field test kit and other soil health indicators in three different soils of the Western Cape Province

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There is growing concern about degradation of the health and quality of soil, due to, amongst other things, climate change and increasing pressure on farmers to produce more food. In order to monitor something, it must be measured. Several soil health tests have seen the light of day in recent decades. The majority of these tests currently available in South Africa are laboratory-bound and necessitates transportation and storage of soil samples that can change soil biology. The microBIOMETER® is a field test kit that can measure microbial biomass, percentage of fungi and bacteria, as well as the fungi:bacteria ratio in minutes, using a mobile phone application. This kit was tested in three different soil types with different management practices in the Western Cape Province, South Africa. At the same time, five currently available laboratory-based tests were also carried out on the same soil, namely: active carbon, microbial activity, protozoa, percentage organic carbon, ammonium nitrogen and microbial respiration. The different soil types responded differently to the tests. All the tests, including the microBIOMETER®, were able to distinguish some of the soil types and management practices. It is clear that all the tests in this study measured different aspects of the soil. It is recommended that more than one test be used to measure soil health and quality and that comparisons be made within soil types and land use and over the course of seasons. Further research may be able to identify a set of tests for a specific soil type and/or use.

Keywords: active carbon, aggregate stability, microbial activity, microBiometer®, protozoa, soil health, Solvita® CO_2-Burst

Evaluering van die microBIOMETER®-veldtoetsstel en ander grondgesondheidsaanwysers in drie verskillende gronde van die Wes-Kaap Provinsie: Daar is toenemende kommer oor agteruitgang van grondgesondheid en -kwaliteit, onder andere as gevolg van klimaatsverandering en toenemende druk op landbouers om meer voedsel te produseer. Ten einde iets te monitor, moet dit gemeet word. Verskeie aanwysers vir grondgesondheid het die afgelope dekades die lig gesien. Die meerderheid van hierdie toetse is laboratoriumgebonde en genoodsaak vervoer en berging van grondmonsters wat die grondlewe beïnvloed. Die microBIOMETER® is 'n veldtoetsstel wat die mikrobiese biomassa, persentasie swamme en bakterieë, asook die swam:bakterie-verhouding binne minute met behulp van 'n selfoontoepassing kan meet. Hierdie toetsstel is in drie verskillende grondtipes met verskillende bestuurspraktyke in die Wes-Kaap Provinsie, Suid-Afrika, getoets. Terselftertyd is vyf laboratoriumgebaseerde toetse wat tans beskikbaar is, ook op dieselfde grond uitgevoer, naamlik: aktiewe koolstof, mikrobiese aktiwiteit, protosoë, persentasie organiese koolstof, ammoniumstikstof en mikrobiese respirasie. Die verskillende grondtipes het verskillend op die toetse gereageer. Al die toetse, insluitend die microBIOMETER®, kon sommige van die grondtipes en bestuurspraktyke uitwys. Dit is duidelik dat al die toetse in hierdie studie verskillende aspekte van die grond getoets het. Dit word aanbeveel dat meer as een toets gebruik word ten einde grondgesondheid en -kwaliteit te meet en dat vergelykings binne grondtipes en grondgebruik en oor die verloop van die seisoene gedoen word. Verdere navorsing mag moontlik 'n stel toetse vir 'n spesifieke grondtipe en/of gebruik kan identifiseer.

Trefwoorde: aggregaatstabiliteit, aktiewe koolstof, grondgesondheid, microBIOMETER®, mikrobiese aktiwiteit, protosoë, Solvita® CO_2-Burst

Introduction

In light of growing concerns about potential effects of climate change (Erasmus et al., 2000), as well as the anthropogenic effects of, amongst other things, industrial agriculture (Emmert et al., 2021; Reinecke and Reinecke, 2018), the search for sustainable farming practices has been elucidated in many ways in recent years. Although soil health and quality are not synonyms, the definition of soil quality by Schloter et al., (2006) seems to be a simple description that pins down the important aspects of healthy soil: "The capacity of a soil to function presently and in the future, for an indefinite period". Sustainable farming needs healthy, high-quality soils (Guo, 2021).

In order to monitor something, it needs to be measured. A quick internet search with keywords "ways to measure soil health" offered the following possibilities, among others: Cornell University (Moebius-Clune et al., 2017) suggested active carbon, soil respiration (measured by Solvita® CO_2-Burst), soil organic matter, pH, total carbon and total nitrogen as well as aggregate stability, as important tests. The United States Department of Agriculture (USDA, 2015) listed earthworms, organic matter of biological origin between 0.053 – 2 mm in size, potentially mineralisable nitrogen, soil enzymes, microbial respiration and aggregate stability as possible indicators. A soil testing laboratory in the United States, called AgSource® (Anon, 2023a), saw soil health as a combination of biological, chemical, and physical aspects and offered a basic test for a soil health index, carbon dioxide respiration, and C:N ratio. An Australian soil testing facility, Nutrient Advantage® (Anon, 2021), relied on the results of measuring total carbon and nitrogen, C:N ratio, aggregate stability and slaking, active carbon and microbial respiration (Solvita® CO_2-Burst). The Woods End laboratory (Anon, 2022), manufacturers and distributors of the Solvita® test kits, presented their basic soil health test which included Solvita® CO_2-Burst, Solvita® labile-amino-N, water-stable aggregates, soil C:N ratio and a respiratory quotient. A local Western Cape company, Soil Health Support Centre (Anon, 2024), offered tests in soil respiration, volumetric aggregate stability, community structure (PFLA - phospholipid fatty acids) as well as the Haney analyses (standard macro and micronutrients for plants as well as estimates of nutrients for microbial consumption).

Obviously, many different tests exist. As a soil's health is specific to a specific ecosystem (Guo, 2021; Schloter et al., 2006), different soils will respond differently to the same test and thus it seems prudent to use more than one test (Kibblewhite et al., 2008).

The United States Department of Agriculture (USDA, 1991) had developed a field soil health test kit that appears to be able to test the following aspects: respiration, infiltration, bulk density, electrical conductivity, pH, nitrates, aggregate stability, slaking, earthworm counts, and resistance to penetration. However, most of the tests currently available in South Africa are performed in laboratories. It usually involves sampling, transport and storage of the soil – all actions that can alter the biological aspects of soils (Cui et al., 2014; Lane et al., 2022).

Microbial biomass (live fraction) is one of many soil health indicators. This is one of the fractions of soil organic carbon (SOC) that changes easily in the short term (Kibblewhite et al., 2008). The microBIOMETER® (Anon 2023c) was developed by an American company, Prolific Earth Sciences, as a cost-effective test for microbial biomass, % fungi and % bacteria. The fact that it is a field test makes its use very attractive, as it will eliminate the necessity of transporting and storing soil samples. Two studies that evaluated the microBIOMETER®'s accuracy of microbial biomass measurement, achieved mixed results. Gordon (2021) came to the conclusion that the microBIOMETER® measured aspects of soil health. Even though this method did not correlate significantly with other tests for microbial biomass, this author found positive correlations between measurements of the microBIOMETER® with active carbon as well as soil protein content. Sain (2022) reported that the microbial biomass, as measured with the microBIOMETER®, varied more between treatments and was less able to distinguish between treatments than other methods of determining microbial biomass were. This author advised against using the microBIOMETER® as the sole test for soil health.

Kibblewhite et al. (2008), identified the following critical processes in the soil system: transformation of carbon, cycling of nutrients, maintenance of the structure and material of the soil, as well as biological regulation of soil communities. Figure 1 depicts the interrelationships between different aspects of soil that are relevant to soil quality and health, as explained by Kibblewhite et al., (2008). These processes should be kept in mind if any type of soil health test is considered, as one should endeavour to include at least one test for each of these ecosystem functions.

Microbial biomass (as measured by the microBIOMETER® in this study), as well as permanganate oxidisable carbon (called active carbon in this study), are both seen as sensitive indicators of land use change and management practices (Hurisso, et al., 2016), as these fractions of soil organic carbon have a 1-5 year turnover time (Ramesh et al., 2019). These two measurements should therefore be indicative of C transformations and nutrient cycling (Figure 1:1 C-transformations, 1:2 – nutrient cycling). Ning et al., (2021) also reported positive correlations between these two carbon fractions.

Soil structure is an indicator of the stability of soil aggregates (Ramesh et al., 2019), which in turn appears to have a direct influence on soil organic carbon accumulation and total nitrogen (Mustafa et al., 2020). Aggregate stability is linked to the maintenance of the soil structure (Figure 1:3 – soil structure maintenance).

Protozoa are involved in habitat and ecological changes and should therefore be a good indicator of soil health (Chitra, 2017; Luu, 2019). They are often correlated with microbial activity (Luu, 2019). They hunt and consume other microbes selectively and actively, thus playing an integral role in maintaining the equilibrium of soil life (Figure 1:4 – biological population regulation; Johns, 2017). Protozoa increase nutrient flow in the

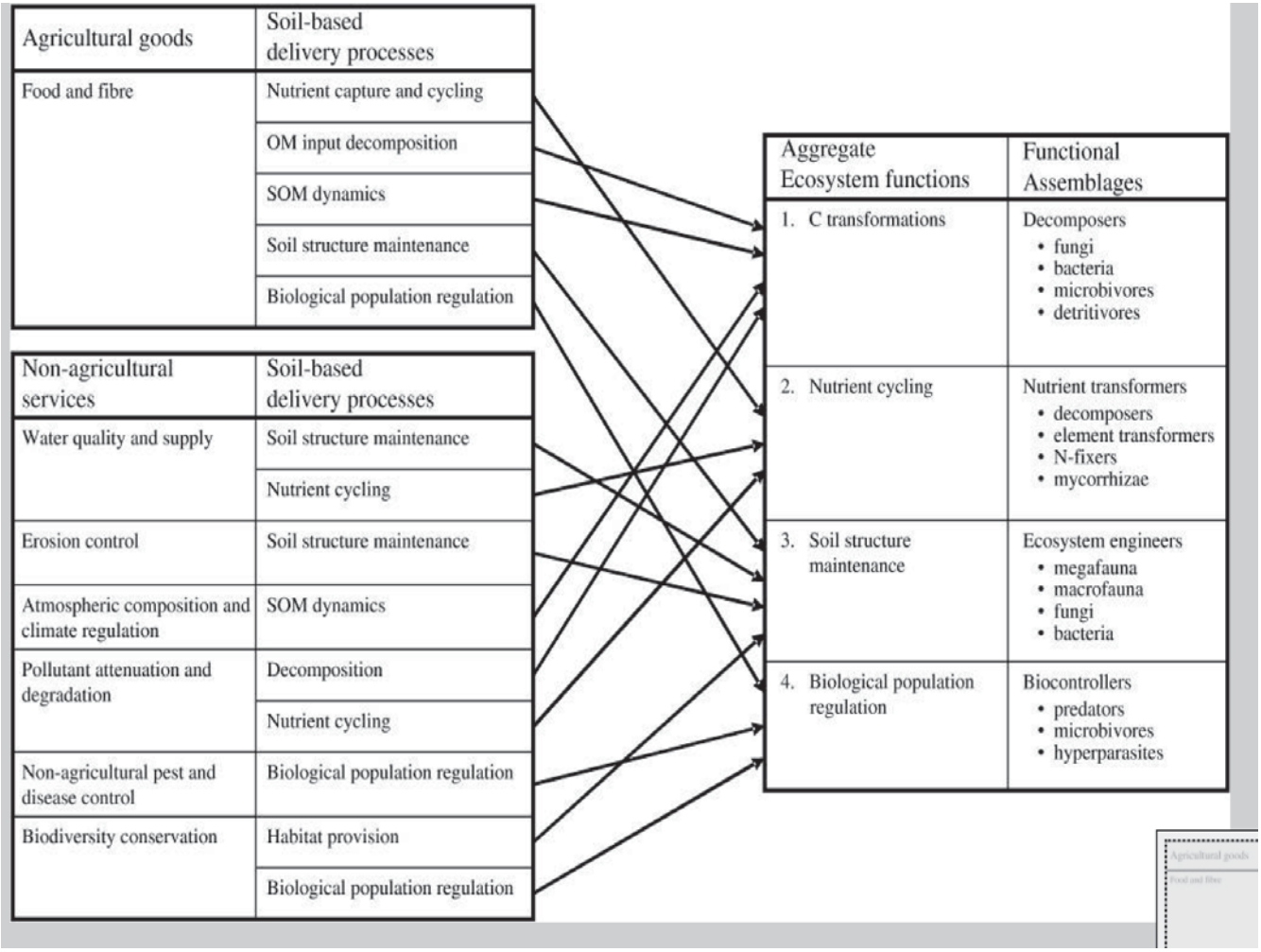


Figure 1: Relationships between the activities of the soil biological community and a range of ecosystem goods and services that society can expect from agricultural land. OM, organic matter; SOM, soil organic matter (Kibblewhite et al., 2008).

soil ecosystem (Foissner, 1999), as they release nutrients (mineralisation) that were in the bodies of their prey (Figure 1:2 – nutrient cycling).

Adam and Duncan (2001) proposed that the spectrophotometric determination of the hydrolysis of fluorescein diacetate (FDA) as a simple, sensitive and quick method for determining total microbial activity in soil, because it includes several enzyme classes such as lipases, esterases, and proteases. These enzymes are abundant in soil (Patle et al., 2018) and play roles in conversion of many substrates (Figure 1:1 – C transformation, 1:2 – nutrient cycling, 1:3 – soil structure maintenance).

The Solvita® CO_2-Burst test kit was developed by the Woods End laboratories (Anon 2022). The test kit measures the sudden burst of carbon dioxide that microbes produce after a disturbance event (drying and then rewetting). The CO_2 produced in this way is related to soil health in that it is an indicator of biological activity (Figure 1:1 – C transformation, 1:2 – nutrient cycling, 1:3 – soil structure maintenance). This also mimics the natural cycle of drying and rewetting in nature when it rains.

The composition of soil microbial communities and characteristics and soil organic matter are interrelated (Figure 1; Domeignoz-Horta et al., 2021; Tecon and Or, 2017).

The aim of this investigation was to evaluate the microBIOMETER® against more traditional (laboratory) soil health indicators currently used in South Africa and specifically in agricultural soils of the Western Cape. In order to test the different soil health indicators, the locations selected, were in different regions of the Western Cape Province with different climates, soil types and management practices (Table 1). The following tests were performed (specific methods in parentheses): microbial biomass, (microBIOMETER®), active carbon ($KMnO_4$ oxidation), wet soil aggregate stability (Royal Eijkelkamp, n.d.), protozoa (most-probable-number, or MPN), total microbial activity (fluorescein diacetate, or FDA) as well as CO_2 respiration (Solvita® CO_2-Burst). The soil samples' total organic carbon content (% C, Walkley-Black's method) and ammonium nitrogen (% N, Kjeldal method) were also determined (Anon, 1990).

Materials and methods

The Western Cape Province of South Africa mainly experiences a typical Mediterranean climate with hot, dry summers and cool, wet winters. Three research farms of the Western Cape Department of Agriculture (Anon, 2023b) were selected as locations for the aforementioned tests.

Nortier (32.035147 S, 18.331839 E), near Lamberts Bay, is located on the West Coast. This farm had the least intensive agricultural practices of the three chosen farms and is mainly used for grazing on natural vegetation. The soils are from the Namib form with Aeolian non-red regic sand. The soils have limited pedological development and are greyish, sandy and very well drained. Clay content is between 1 - 4% with a depth of more than 750 mm. The average precipitation is 200 mm, most of which occurs in the winter months (Soil classification working group, 1991; Booysen et al., 2009, unpublished).

Outeniqua (33.987630 S, 22.420645 E) is located south west of George in the Eden District and is known for research into dairy production on planted pastures. The soils have a well-developed texture and is described as Cape Granite (Witfontein form) with a depth of 450-750 mm. The clay content is less than 15%. Annual precipitation is 700 mm per year, occurring throughout the year, with peaks in March, April and August.

Langgewens (33.276981 S, 18.703856 E), near Moorreesburg, is located in the Swartland, in one of the main wheat-producing areas under dryland conditions. The soils are shallow, weathered rock of Malmesbury shale and have limited pedological development. Glenrosa and/or Mispah forms are dominant, with lime usually found throughout the landscape. Clay content varies between 15 and 35%, with a depth of less than 750 mm (Soil classification working group, 1991). The soils have good lateral drainage, but weak vertical drainage, often leading to waterlogged low-lying areas (Wiese et al., 2016). Average precipitation is just under 400 mm per year, of which about 80% occurs during the period April to September

Four plots under different management practices were identified on each farm. The site called 'veld' is as natural and undisturbed by agriculture as could be found on the particular farm (Table I).

Five soil samples (each 40 mm diameter and 150 mm deep) were randomly collected in each of the three subplots (repetitions) in the four trial plots (management practices) per farm, using a metal pipe. The five soil samples were bulked and mixed to provide soil for each of the three subplots for all analyses of all trial plots. These soil samples were used for the microBIOMETER® measurements in the field, after which the remaining soil was stored in new plastic bags, transported to the laboratory in a cooler bag and then stored at 4 °C until analyses.

MicroBIOMETER®

The microBIOMETER® test kit (Anon, 2023c) comes equipped with everything needed to do the test, except for the water needed to mix with the soil and the extraction solution. Before the measurement, the microBIOMETER® application had to be installed on a smartphone. The simple instructions were accessible on the website, but a printed version was also

Table I: Description of the different management practices as well as the percentage organic carbon (C) and ammonium nitrogen (N) of the four plots per farm as used in this study.

NORTIER **32.035147 S, 18.331839 E**	**OUTENIQUA** **33.987630 S, 22.420645 E**	**LANGGEWENS** **33.276981 S, 18.703856 E**
Veld = undisturbed, natural area near the sea. Utilised by naturally occurring wildlife. Sometimes grazed by sheep. % C = 0,703 % N = 0,094	**Veld** = undisturbed, natural area, some alien plants. Sometimes grazed by cattle and naturally occurring wildlife. % C = 2,59 % N = 0,216	**Veld** = undisturbed, natural area, some alien plants. Utilised by naturally occurring wildlife. % C = 1,53 % N = 0,127
Hill = undisturbed, natural area on a hill. Utilised by naturally occurring wildlife. Sometimes grazed by sheep. % C = 0,647 % N = 0,088	**Kikuyu** = irrigated planted kikuyu pasture grazed by dairy cows. Yearly NPK fertiliser and lime as recommended. % C = 2,747 % N = 0,367	**WWWW** = wheat monoculture; reduced tillage last 16 years. NPK fertiliser and lime as recommended; herbicides and fungicides when necessary. % C = 1,35 % N = 0,135
SBG = old salt bush and grass pasture. Utilised by sheep at time of sampling. % C = 0,623 % N = 0,081	**Vlei** = undisturbed marshy area. Utilised by naturally occurring wildlife. % C =5,097 % N = 0,488	**McWMcW** = medic/wheat/medic/wheat rotation; sampling in wheat phase. Reduced tillage last 16 years. NPK fertiliser and lime as recommended; herbicides and fungicides when necessary. % C = 1,587 % N = 0,153
Pasture = irrigated planted pasture (mixture of rye, triticale, fodder barley, vetch, lupines and peas). No agrochemicals. % C = 0,553 % N = 0,081	**Mulch** = Cover crop trial, with a dead *Eragrostis teff* mulch at the time of sampling. NPK fertiliser and lime as recommended during the growing season of the cover crop. % C = 1,563 % N = 0,146	**LWCW** = lupine/wheat/canola/wheat-rotation; sampling done in wheat phase. Reduced tillage the last 16 years. NPK fertiliser and lime as recommended; herbicides and fungicides when necessary. % C = 1,44 % N = 0,151

included in the test kit. The whole procedure took about 25 minutes per sample to complete. The soil samples had to be sieved, measured and added to the extraction solution (supplied salt compound) and water, mixed and allowed to settle. A drop of the supernatant of the soil solution was then placed on the enclosed special filter paper. This was then analysed using the mobile phone application. The resulting % fungi, % bacteria, fungi:bacteria ratio and soil microbial biomass were available within seconds. For this evaluation, only the microbial biomass was used.

Active carbon (ActC)

The $KMnO_4$ oxidation method is easy to perform and does not need hazardous chemicals in large quantities. Following a modified procedure (Marais et al. 2020), soil was mixed with a $KMnO_4$ solution and allowed to react with the carbon in the sample while being shaken horizontally. After the reaction was completed, the colour intensity of the solution was measured on a spectrophotometer at 550 nm. By applying the specified formula, the amount of active carbon was determined (parts per million, ppm). This was done in duplicate for each soil sample, after which the average was used in the statistical analyses.

Wet soil aggregate stability

The procedure for the wet aggregate stability determination was obtained from the website (Royal Eijkelkamp, n.d.) of the machine's manufacturers. Four grams of previously air-dried and sieved (between 1-2 mm diameter) soil samples were placed in an Eijkelkamp® wet soil sieving apparatus and moistened, before being moved up and down by ways of the machine, first in distilled water, which washed out the unstable aggregates, then in a NaOH solution, which washed out the stable aggregates. These soil solutions were then dried in an oven at 100 ˚C for 24 hours (until all the water had evaporated) and then weighed. The application of the specified formula led to the determination of the percentage of stable aggregates for the samples. This was done twice for each soil sample, after which the average was used in the statistical analyses. This procedure could not be done on the sandy soils of Nortier, as the sand fell through the 1 mm sieve. This sandy soil therefore had no aggregates of between 1 - 2 mm.

Protozoa (most probable number, MPN)

In this method (Briones and Reichardt, 1999), a soil dilution series was allowed to incubate for five days (22 ˚C) with a sterile soil extract, prepared from the experimental soil samples, which served as food for the microbes. The soil solution of the different dilutions was then observed under a 200 x magnification lens of an inverted microscope. The final protozoan population was estimated using a formula of Rønn et al. (1995).

Microbial activity (fluorescein diacetate, FDA)

The method of Adam and Duncan (2001) involves incubating the soil sample with buffer and FDA for a specific time at a set temperature (28 ˚C). During the incubation, the amount of fluorescent colour formation is an indication of the enzymatic activity of the microbial community in the sample. The intensity of the colour was measured on a spectrophotometer at 450 nm and compared to a standard graph to determine the relative microbial activity in a sample, using the specified formula. Each sample was run in triplicate, while the mean was used in the final statistical analyses.

Microbial respiration (Solvita® CO_2-Burst)

Dried and sieved soil was weighed into a plastic cup according to the protocol on the website (Anon, 2022). A specified volume of distilled water was added and the plastic beaker was placed in a glass jar with an airtight screw cap. A paddle coated with carbon chromatographic gel was placed in the wet soil and the glass jar was sealed. After incubation for 24 hours at a constant temperature of 22 ˚C, the paddle was removed and read on a *Solvita® Digital Color Reader*. The microbes responded to the rewetting with a sudden burst of CO_2, due to microbial activation after a disturbance event of desiccation followed by rapid rewetting. The parts per million (ppm) of CO_2 thus formed by microbial respiration, served as an overall indicator of aerobic soil's microbial potential. This test was conducted on two of the three subplots per experimental site.

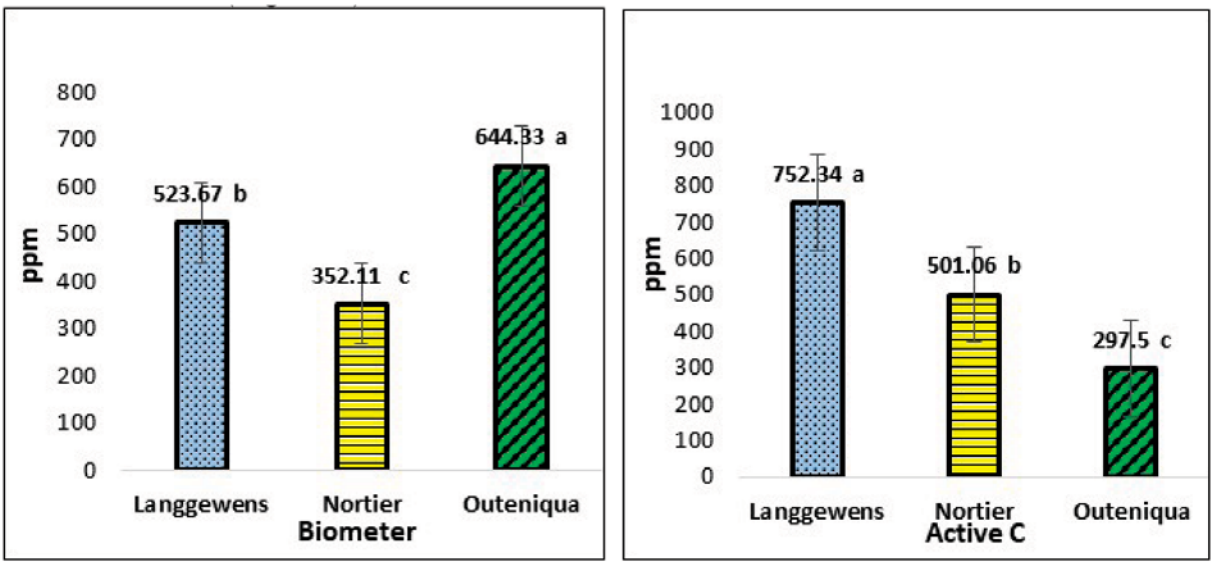
Statistical analyses

The data from the three farms were analysed separately, because the treatments between farms differed. The farms were however jointly analysed for the treatment "veld". An analysis of variance (ANOVA) was applied to test the hypothesis that there were no within-farm and between-farm soil differences for "veld" treatment. Fisher's t-test with LSD (least significant difference) at 5% significance level (p=0.05) was further applied to determine differences between treatment means. The Shapiro-Wilk test was used to determine whether the standardised residuals of the model were acceptable. The Proc GLM procedure of the statistical package SAS (version 9.4; SAS Institute Inc, Cary, USA) was used. Furthermore, Pearson correlation coefficients were calculated to determine the linear relationship between the variables. This test was performed using the statistical package XLSTAT (Addinsoft, 2022).

Results and discussion

Based on the means of the ANOVA, only the microBIOMETER® and the active carbon tests showed significant differences between all three farms when only the undisturbed plot (veld) was considered (Figure 2).

The different farms were then analysed individually. At Nortier, the only test that could distinguish the veld from the others was the estimated population of protozoa, while the microBIOMETER® could distinguish the two most undisturbed plots (veld and hill) from the salt bush pasture (Table II). This farm has fairly homogeneous sandy soil with not many different management practices while no chemical inputs were applied.



a: **b:**

Figure 2: Microbial biomass as measured by the (**a**) microBIOMETER® (Biometer) and (**b**) active carbon (Active C) in parts per million (ppm) in the soils of the undisturbed plots (veld) on the different farms. Means from ANOVA. Bars depict standard error. Different letters indicate significant differences at $p = 0.05$.

Table II: Means of ANOVA of variables determined for the treatments at the three different farms. Means within the same column with different letters indicate significant differences at $p = 0.05$.

	micro BIOMETER® Microbial biomass (ppm)	Active C (ppm)	FDA Microbial activity (ppm)	Log Population protozoa	%C	%N	SolvCO$_2$ Burst Microbial respiration (ppm)	% Aggregates
NORTIER								
Veld	347.67^{a}	489.39^{a}	28.30^{a}	3.82^{b}	0.703^{a}	0.094^{a}	13.5^{a}	n/a
Hill	244.33^{a}	481.23^{a}	34.616^{a}	6.281^{a}	0.647^{a}	0.088^{a}	19.05^{a}	n/a
Pasture	248.67^{ab}	473.57^{a}	31.192^{a}	6.062^{a}	0.553^{a}	0.081^{a}	18.8^{a}	n/a
SBG*	202^{b}	465.08^{a}	38.285^{a}	6.623^{a}	0.623^{a}	0.109^{a}	21.9^{a}	n/a
OUTENIQUA A								
Veld	644.3^{ab}	297.5^{b}	164.24^{a}	3.987^{ab}	2.59^{b}	0.216^{c}	51.74^{b}	50.145^{a}
Kikuya	848^{ab}	526.04^{a}	140.22^{ab}	5.042^{a}	2.747^{b}	0.367^{b}	110.55^{a}	50.197^{a}
Vlei	603.3^{b}	352.33^{ab}	178.5^{a}	3.719^{b}	5.097^{a}	0.488^{a}	78.4^{ab}	49.542^{a}
Mulch	916.7^{a}	-198.69^{c}	76.06^{b}	3.78^{b}	1.563^{b}	0.146^{d}	51.1^{b}	50.338^{a}
LANGGEWENS NS								
Veld	523.67^{ab}	752.3^{a}	46.366^{a}	3.46^{c}	1.53^{a}	0.127^{a}	27.75^{cb}	49.445^{b}
WWWW*	340.67^{b}	555.4^{a}	36.506^{a}	4.323^{bc}	1.35^{a}	0.135^{a}	88.04^{a}	50.361^{a}
McWMcW**	614^{a}	466.1^{a}	47.315^{a}	5.52^{ab}	1.587^{a}	0.153^{a}	47.8^{b}	48.592^{c}
LWCW*	345.33^{b}	643.1^{a}	45^{a}	6.263^{a}	1.44^{a}	0.151^{a}	15.4^{c}	48.708^{cb}

*Refer to Table I for clarification of treatments.

At Outeniqua, with its high carbon levels (Table I), all the tests, except aggregate stability, could distinguish some of the management practices. It is possible that this farm, with its well-developed soils and high carbon content, does not really show differences at the aggregate level any more, as was also found by Mbanjwa et al. (2022) in another South African study. At Outeniqua, the percentage of ammonium nitrogen showed significant differences between all the treatments.

None of the tests could distinguish all the different management practices at all the farms. If the undisturbed (veld) plot is ignored at Langgewens, the test for microbial respiration (Solvita® CO_2-Burst) was able to distinguish between the three different rotations. The microBIOMETER®, the estimated population of protozoa as well as the stable aggregates, were able to distinguish significant differences in some of the management practices at Langgewens.

microBIOMETER®

The procedure for the microBIOMETER® (Anon, 2023) was easy to understand, although in some instances problems were encountered where there was no cell phone signal. The supplier stated that a cell phone signal was not required, but the problem persisted at some of the locations.

The microbial biomass, as determined using the microBIOMETER®, was one of only two soil health indicators tested, that was able to distinguish between the three locations (Langgewens, Nortier, Outeniqua) when only the soil of the most undisturbed areas (veld) was taken into account (Figure 3), i.e. where no artificial inputs (cultivation, fertilisers, pesticides) could have made a difference to the soil biology.

From Figure 3 it is clear that the microBIOMETER® could not distinguish the different management practices (Table I) when all locations and all management practices were analysed together. This type of distinction between widely varying soil types and climate is almost impossible for any single test (Vos et al., 2013; Fierer, et al., 2021). This was also the case with the microBIOMETER®, as well as for the other tests used in this study. The trends of the microbial biomass at the different locations were expected, with Outeniqua's soils the highest and Nortier's sandy soils the lowest in organic carbon (Table I). The highest microbial biomass in the soils under the mulch at Outeniqua was not expected, as the mulch (dead material at the time of sampling) had no living roots that could feed the microbes with root exudates. Possibly the mulch had already started to decompose, which could have provided food for (other) microbes. Microbial biomass is the mass of all living microbes in the soil (Hoyle et al., 2023), including the decomposers.

Active carbon

As was in the case of the microBIOMETER®, active carbon was one of only two soil health indicators tested, which was able to distinguish between the three locations (Langgewens, Nortier, Outeniqua) when only the soil from the most undisturbed areas (veld), was taken into account (Figure 2).

At Nortier, the farm with the least intensive agriculture (no inputs of pesticides or fertilisers), active carbon correlated positively with microBIOMETER®, % organic carbon, as well as % ammonium nitrogen (Figure 4). Since active carbon is the readily available carbon for microbes and the microBIOMETER® measures microbial biomass, this positive correlation was expected, as was also found by Gordon, (2021) in a study to test the microBIOMETER®'s validity. The positive correlation with the organic carbon and ammonium nitrogen was also to be expected, as both are directly linked to microbial biomass and therefore microbes (Li et al., 2021).

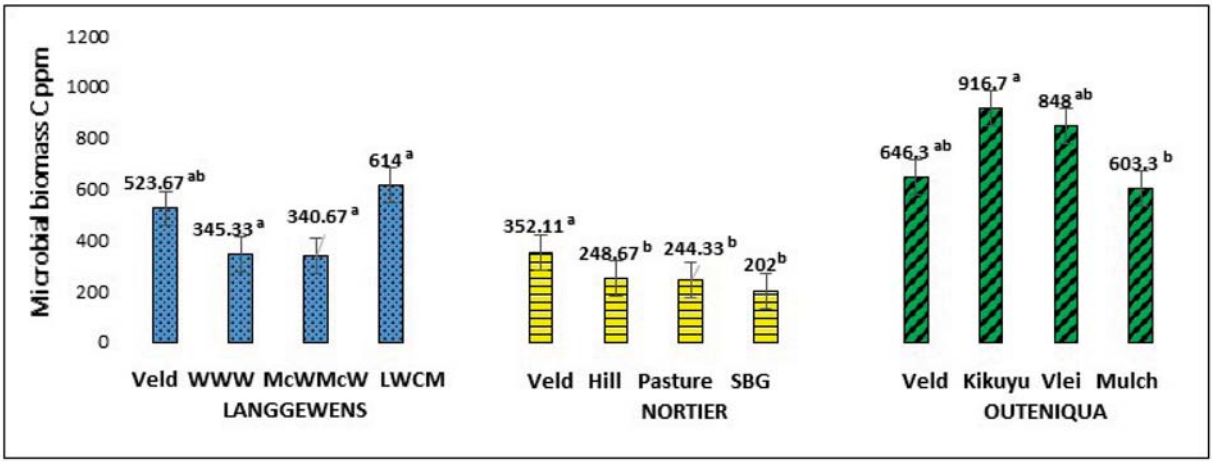
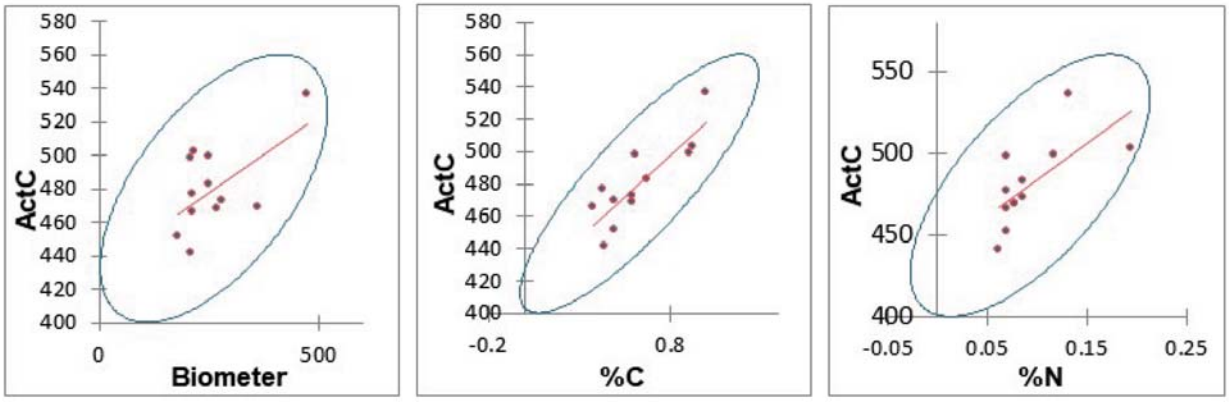
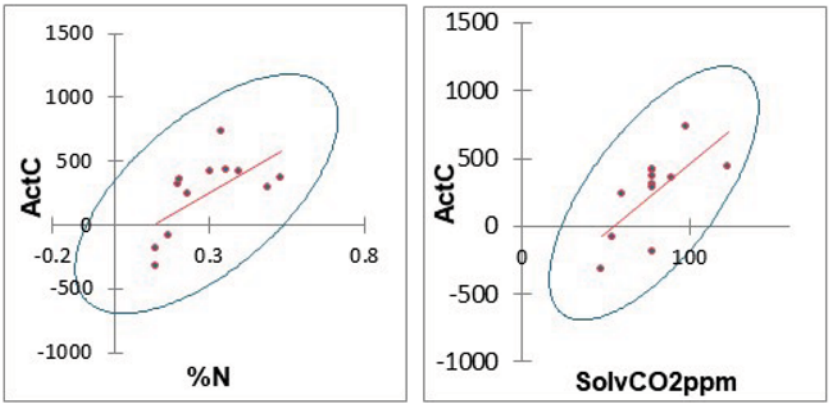


Figure 3: Management practice means of microbial biomass (parts per million) as measured by the microBIOMETER® in the soils of all management practices on all three different farms. Bars depict standard error. Different letters indicate significant differences in management practice means per farm at $p = 0.05$. Refer to Table I for clarification of treatments.



a: r = 0.587 p = 0.045 **b**: r = 0.846 p = 0.001 **c**: r = 0.667 p = 0.018

Figure 4: Nortier – positive correlation between active carbon (ActC) and (**a**) microbial biomass (Biometer), (**b**) organic carbon (%C) and (**c**) ammonium nitrogen (%N). Pearson's correlation coefficient (r) en *p* = specific significance of the coefficient.



a: r = 0.633 p = 0.027 **b**: r = 0.682 p = 0.014

Figure 5: Outeniqua – positive correlation between active carbon (ActC) and (**a**) ammonium nitrogen (%N) as well as (**b**) microbial respiration ($SolvCO_2$ppm). Pearson's correlation coefficient (r) en *p* = specific significance of the coefficient.

At Langgewens, the only farm in this study that was used for the dryland production of annual crops in rotation (wheat at the time of sampling), with the associated inputs of agricultural chemicals (Table I), active carbon did not correlate with any of the other measurements. This was unexpected, as the crops should have increased the microbial biomass (as was seen with the microBIOMETER®) and higher root exudates would have been expected, especially in the rotation plots. A possible explanation could be the high input of agrochemicals on these plots for the production of cash crops (Damoran et al., 2016). Chen et al., (2021) also reported a negative effect of inorganic nitrogen fertilisers, but also found that domestication of wheat plants in itself could have disturbed the microbial communities associated with these plants, which could also have added to the unexpected non-correlation. All the rotation systems were in the wheat phase at the time of this study.

At Outeniqua, active carbon correlated positively with ammonium nitrogen, (as was also observed at Nortier), as well as with the microbial respiration ($SolvCO_2$ppm) (Figure 5). The active carbon is the form of carbon that is easily used by and secreted by microbes, while ammonium is formed by microbial degradation. Ohio State University states in their fact sheet that soil organic matter is the products of, among other things, the carbon and nitrogen cycles maintained by soil organisms (Hoorman and Islam, 2010). These living organisms respire, so these correlations are understandable.

Wet soil aggregate stability

Aggregate stability, which was not determined at Nortier, due to the sandy soils, only showed significant correlations at Langgewens and indeed negatively with microbial activity (FDA) and positively with soil respiration ($SolvCO_2$ppm), as shown in Figure 6. In this study, only the stability of 1-2 mm-sized aggregates was determined. Although it is assumed that more stable soil aggregates are an indication of higher microbial activity, like all other processes in the soil, they are very dynamic and are influenced by a host of factors (season, temperature, moisture, parent material, plant types, external inputs in the form of agrochemicals) (Wilpiszeski et al., 2019). These authors found that there were even significant differences between microbial activities of micro- and macro-aggregates from the same soil sample. The unexpected negative correlation between aggregate stability and microbial activity can possibly be attributed to a variety of undetermined factors, of which the high input of agricultural chemicals (the highest of the three farms in this study) could be one. Basak et al. (2022) reported

that input of fertiliser negatively affected the macro-aggregates in their experiment. These authors also found that the microbial activity (as measured by FDA) was much higher in their plots where organic nutrition was applied together with compost, than those plots that only received fertiliser (as was in this case), while Wang et al. (2022) found negative correlations with fertiliser and the speed of potential nitrogen cycling. As for the positive correlation with microbial respiration, this may also be due to only the 1-2 mm size aggregates being determined, as Yang et al., (2019) found that these sized aggregates showed the highest respiration of all the aggregate size classes they tested.

Protozoa (MPN)

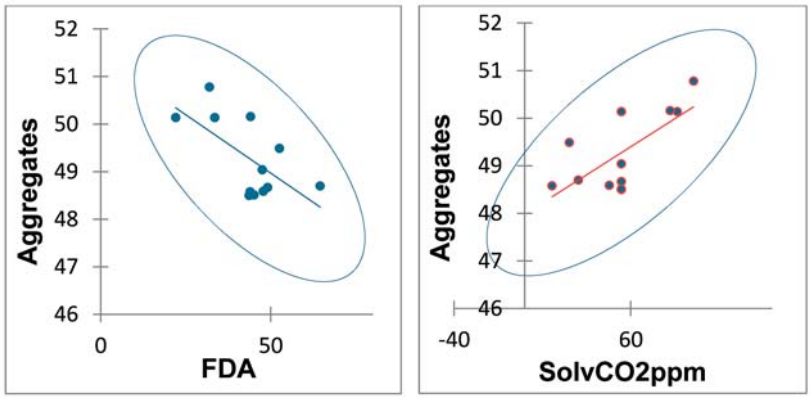
The log transformation of the estimated population of the protozoa had positive correlations on only two farms and indeed with ammonium nitrogen at Langgewens and microbial respiration at Outeniqua (Figure 7).

Protozoa numbers are a good indicator of the speed of the nutrient cycling, as they directly release minerals to the soil (mineralisatIon), plants and other microbes that were trapped in the bodies of their prey (Foissner, 1999). This is most likely why it showed a positive correlation with ammonium nitrogen at Langgewens.

The positive correlation with microbial respiration ($SolvCO_2ppm$) at Outeniqua, but not at Nortier and Langgewens, can possibly be explained on the basis of the carbon content, which is much higher at Outeniqua (Table 1). The microbes that protozoa prey on, feed on various carbon fractions (Hoyle et al. 2011). Soil with more organic carbon will therefore be able to support more microbes.

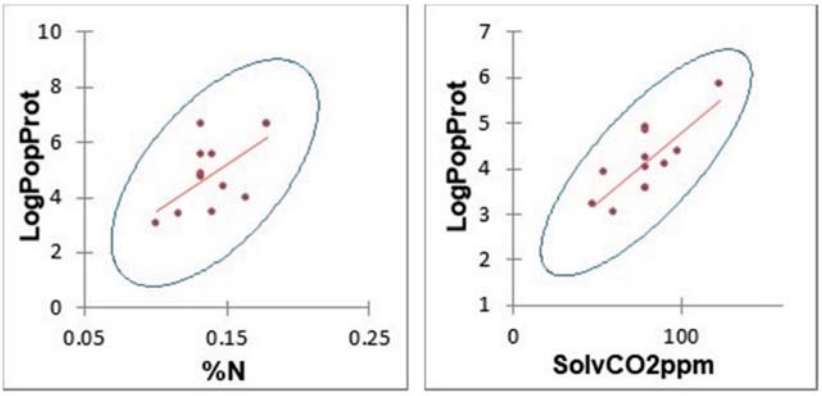
Microbial activity (FDA)

Microbial activity, as measured with the fluorescein diacetate method (FDA), correlated positively with % organic carbon at Langgewens and Nortier, while the only significant correlation at Outeniqua was a negative correlation with microbial biomass, as measured with the microBIOMETER® (Figure 8). At Nortier, the microbial activity was also positively correlated with ammonium nitrogen.



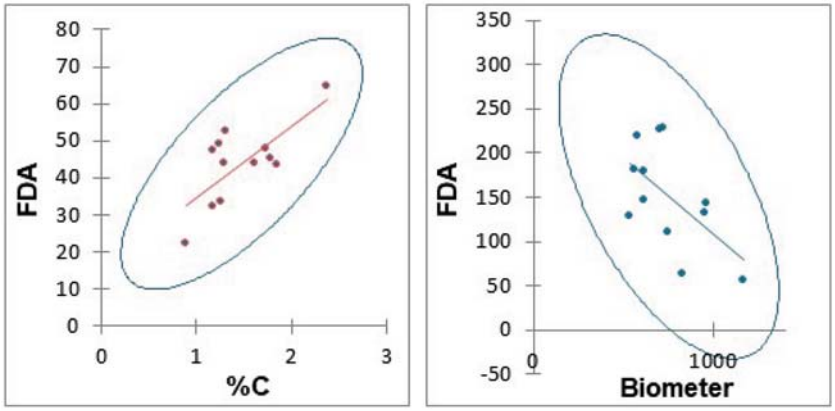
a: r = -0.643 p = 0.024 **b**: r = 0.694 p = 0.012

Figure 6: Langgewens – Wet soil aggregates showed negative correlation with (**a**) microbial activity (FDA) and positive with (**b**) microbial respiration ($SolvCO_2ppm$). Pearson's correlation coefficient (r) en *p* = specific significance of the coefficient.

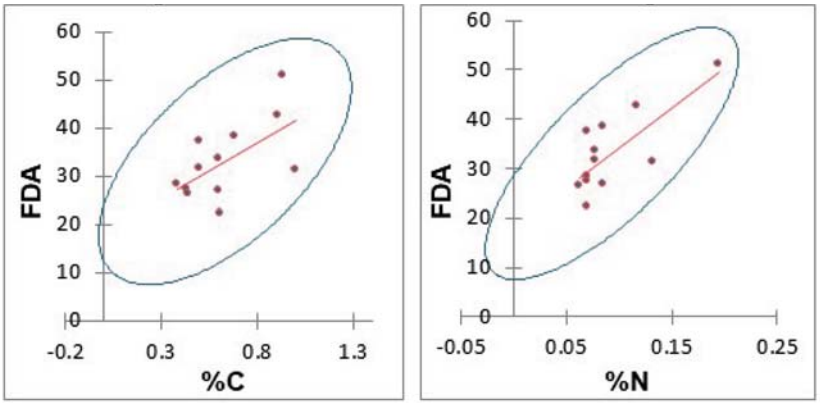


a: r = 0.601 p = 0.039 **b**: r = 0.781 p = 0.003

Figure 7: The log transformation of the logarithmic estimated population of protozoa (LogPopProt) correlated positively with (**a**) percentage ammonium nitrogen (%N) at Langgewens and with (**b**) microbial respiration ($SolvCO_2ppm$) at Outeniqua. Pearson's correlation coefficient (r) en *p* = specific significance of the coefficient.



a: r = 0.718 p = 0.009 **b**: r=-0.583 p= 0.047



c1: r = 0.603 p = 0.038 **c2**: r = 0.762 p = 0.004

Figure 8: Correlations of microbial activity (FDA) with other measurements as determined in the soil of the different farms. Langgewens (**a** = organic carbon, %C); Outeniqua (**b** = microbial biomass, Biometer) and Nortier (**c1** = organic carbon, %C; (**c2** = ammonium nitrogen, %N). Pearson's correlation coefficient (r) en *p* = specific significance of the coefficient.

The positive correlation between microbial activity and % organic carbon as found at Langgewens and Nortier (Figure 8a, c1), is explainable, since different fractions of organic carbon provide the energy sources for microbial metabolism (Hoyle et al. 2011). The negative correlation with aggregate stability at Langgewens (Figure 6a), could probably be due to the high inputs of agrochemicals as well as the 1-2 mm aggregate size that was measured, as was previously reported.

The negative correlation with microbial biomass (Biometer) at Outeniqua (Figure 8b), was unexpected, but could possibly be explained by the influence of the mulch plot that had no living plant material and lower carbon (Table 1) than the other plots on this farm. Hoyle et al., (2023) reported that microbial biomass reacted very quickly to changes in management. This correlation also tended to the negative at Nortier, with its low carbon and sparse vegetation, though this was not significant ($p = 0.81$).

At Nortier, the microbial activity also correlated positively with percentage ammonium nitrogen (Figure 8c2), while the positive trend in correlation at the other two farms were not significant (Outeniqua p = 0.098; Langgewens p = 0.375). This can probably yet again be explained by the absence of added agrochemicals at Nortier and less agrochemical input at Outeniqua than at Langgewens (Table I). Wang et al., (2022) reported a significant negative influence of long-term nitrogen fertiliser (as was the case at Langgewens and Outeniqua in this study) on the potential nitrogen cycling rates.

Microbial respiration (Solvita® CO_2-Burst)

At Outeniqua, microbial respiration, as determined by the Solvita® CO_2-Burst test kit, was significantly and positively correlated with active carbon (Figure 5b) and the log transformation of the estimated protozoan population (Figure 7b). At Langgewens, microbial respiration, as measured by the microBIOMETER®, correlated positively with the wet soil aggregate stability (Figure 6b).

These correlations are explainable, since active carbon is the readily utilisable form of energy for microbes (Breker, no date). More microbial food will lead to more respiration (SolvCO_2-Burst) and more microbes will serve as more food for the protozoa. The higher protozoa numbers will in turn also support the increase in respiration and active carbon, as the carbon that was trapped in their prey is made available to the soil and they themselves also respire. Stable aggregates are often associated with higher microbial activity and numbers, especially in the 1-2 mm size class (Yang et al., 2019), as was measured in this study.

Conclusion

During the evaluation of the microBIOMETER® and its simultaneous testing against other available soil health indicators, the following emerged throughout the process: each soil is unique and each test measures something different. There is no single test for soil health or quality that can universally be classified as the best. Even a test that can be classified as the best in a specific soil may mean little or nothing in another soil type, if there are different climatic conditions, vegetation or even if the samples were only taken at a different time of the year. The microBIOMETER® does measure aspects of soil health, as the correlation with other indicators shows.

It seems prudent to determine as many different aspects of a soil as possible and to only compare soils from the same area with the same history and use. Whatever measurements are made, the samples should also be taken on several occasions during the course of the season in order to obtain more information. Before and after management interventions (planting, spraying, fertilising), will also produce different results, but it can convey important information to the farmer.

The microBIOMETER® can make a major contribution to the monitoring of soil health, as it is one of the few tests currently available for soil health that can be carried out in the field and therefore measures other aspects than those tests where soil has to be transported and the test performed later in a laboratory. However, whether it can accurately determine microbial biomass was not evaluated in this study.
With more repetitions in the same type of soil and over seasons, it may be possible to identify a set of tests that works best for each type of soil/land use.

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