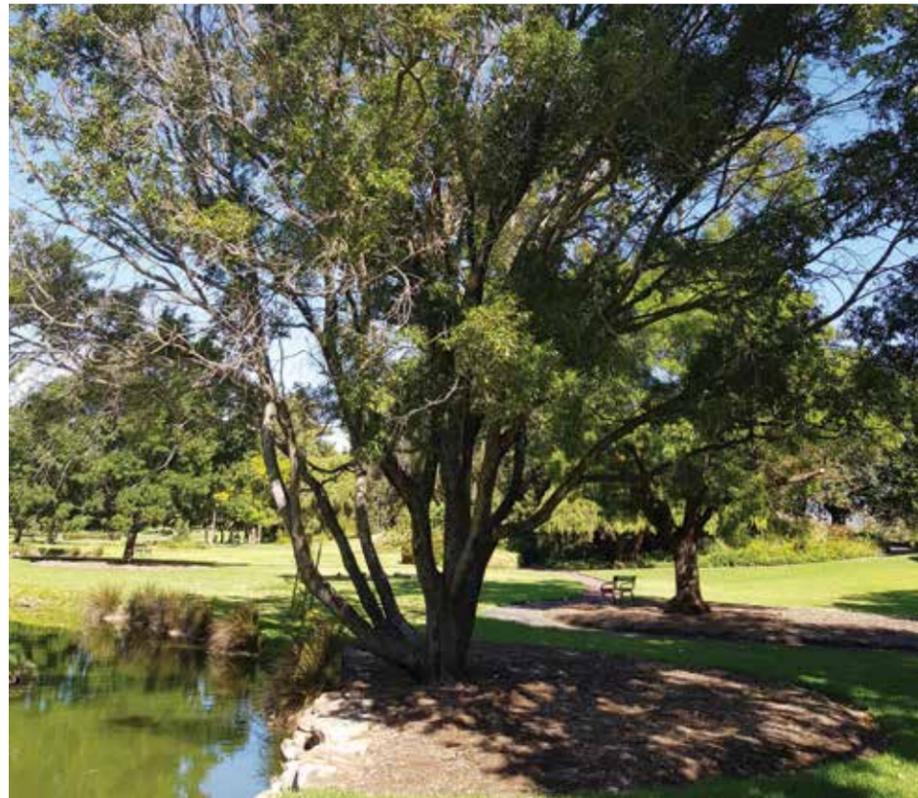


SOIL HEALTH, TREE HEALTH

WORDS & IMAGES | MATTHEW R. DANIEL - GLOBAL URBAN FOREST, DR DAN TERAVEST - OURSCI - MICHIGAN, USA

VEALE GARDENS/WALYU YARTA - (PARK 21) - PART 3

This case study examines the application of advanced tree management techniques to rehabilitate a population of trees experiencing decline in Urban Forestry. These management techniques focus on enhancing soil health by improving soil physical structure, chemistry and biology. The efficacy of this approach was evaluated using innovative sensing technologies to better understand the links between soil health and tree health and to quantify the productivity of trees in Urban Forestry.



Following up to PART 3 of this Case Study, which ran in *The Australian Arbor Age* April/May 2019 issue, we are now evaluating the results in terms of soil chemistry, soil biology, mycorrhizal colonization, soil respiration and compaction and wildlife predation.

Results

3.1 Soil Chemistry

Independent laboratory assessment of eight trees was conducted throughout the three years of the project. In 2015, Trees #6 and #31 were the focus with Tree #31 providing healthy a control throughout the PHC intervention period.

Soil chemistry is complex and shifts in one nutrient will affect other nutrient concentrations. In the initial investigations in 2015 and 2016 there were multiple issues associated with the chemistry at Veale Gardens ranging from nutrient deficiencies to nutrient toxicities based on desired levels.

Trees receiving PHC treatment displayed large increases in chemical properties ►

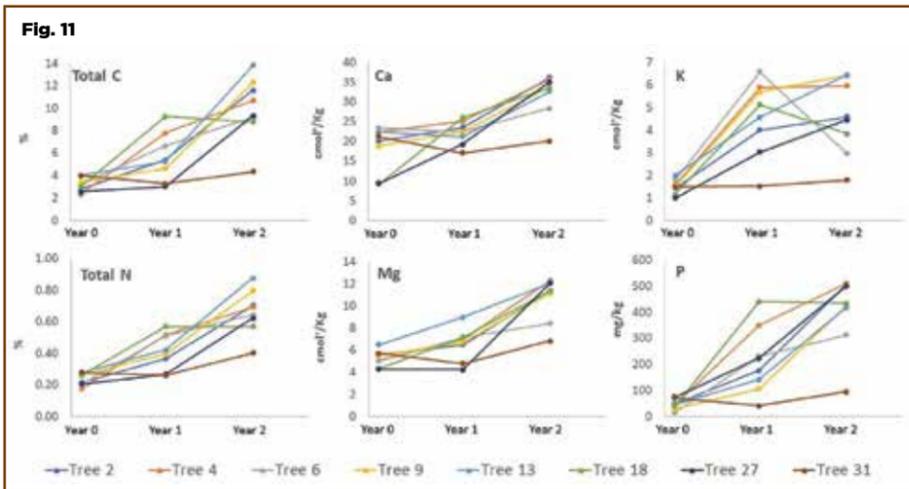
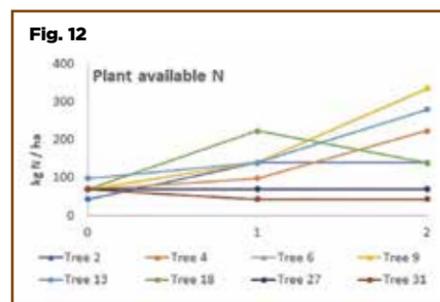


Figure 11: Soil chemical properties by year, total C and N, exchangeable Ca, Mg and K, and available P. Tree 31 did not receive any treatment, while the other seven trees all received treatment. Year 0 = samples in 2015 and 2016, prior to treatment; year 1 = 2017; and year 3 = 2018.

Figure 12: Plant available N by year. Tree 31 did not receive any treatment, while the other seven trees all received treatment. Year 0 = samples in 2015 and 2016, prior to treatment; year 1 = 2017; and year 3 = 2018.



baseline. There was also an increase in plant available N over time compared with the baseline tree in treated trees (Fig. 12).

In an ever-changing soil environment, it is important to understand all these nutrient pools and not just available soluble nutrient that is a common soil agronomic method. The shift in soil chemistry of the trees treated can be explained in some cases although it is important to understand this is a snap shot in time and additional adjustments will occur seasonally and over time. Another important factor is the interaction between soil biology and soil chemistry which is difficult to quantify.

compared with the untreated baseline (Fig. 11). For example, the amount of total C, total N, exchangeable Mg, and exchangeable K increased by an average of two or three-fold (100 – 230%) in trees receiving treatment while in the baseline the increases were 8%, 44%, 20%, and 21%, respectively. Available P increased by a factor of ten 2 years after treatment, but only increased 33% in the untreated

| NUTRIENT | DESCRIPTION OF CHANGE |
|------------------------------|---|
| Potassium | Increase in potassium is most likely due to the increased soil moisture and the liberation from clay particles that the microbiology is interacting with. Elevated potassium has been a response from the soil microbiology and it is recommended to monitor this as further changes occur over time. |
| Phosphorus | Total Phosphorus has increased from the PHC # 1 program, as the control tree is still deficient in Phosphorus. The increase in phosphorus may be the result of the compost and Compost Tea. Will need to investigate if manure was used in the production of the compost. The trees will take up the increased phosphorus over time although there is a limit each growing season. Iron and Zinc levels will need to be monitored in relation to the high Phosphorus levels. The fungal activity may also sequester some of the increased phosphorus. |
| Carbon/Organic Matter | Carbon levels have increased significantly due the application of compost and mulch and the increased microbial activity. The significant increase in Carbon in the soil is another example of how soil management can reduce carbon dioxide in the atmosphere substantially. The control tree (Tree #31) has a reduction in soil carbon in comparison. |
| Nitrogen | Nitrogen levels have increased due to the increase in soil beneficial bacteria and predation of bacteria on protozoa and making Nitrogen available for plant uptake. |
| Calcium | Total Calcium has increased. The high calcium levels are most likely due to the elevated soil pH and microbial activity. High calcium levels may bond with phosphorus and reduce the calcium, although phosphorus is also high so is not an issue with this nutrient relationship. Calcium is the king of nutrients and essential for new cell growth (stems, leaves and roots) Calcium will also be taken up by fungi so it may not be toxic as the soil biology is increasing and other nutrients are in excess. |
| Magnesium | Total Magnesium has decreased due to the increase in calcium, calcium: magnesium ratio (C: Mg) A conventional agronomic response to this issue would be to add lime to increase calcium, although Calcium levels are already elevated and once lime has been applied it cannot be removed if it causes issues. Adding lime now is not recommended. Further monitoring is required as microbial growth is also adjusting the chemistry. |
| Sodium | An important reduction in sodium levels can be explained by the increase in average rainfall over the past two years enabling salt to be leached from the soil profile. It may also be the case that increased humus levels in the soil have bonded with the sodium molecules and reduced the impact of salt damage to soil biology and the plants root systems. |
| Selenium | Selenium has increased. |

| NUTRIENT | DESCRIPTION OF CHANGE |
|-----------------|--|
| Copper | Total copper has increased and decreased within the different trees tested. Copper will affect the microbial growth in soil. Copper increase may be the result of decomposing organic matter as the park is not sprayed with copper fungicides such as an orchard. Toxic copper levels can affect uptake of Iron, Nitrogen, Phosphorus and Zinc, although all these nutrients are also elevated. Elevated Zinc levels reduce copper uptake in plants, so this contradiction is unusual. Toxic copper levels will also reduce root activity. Copper is generally low in alkaline soils, so this is also unusual as the pH is around 8. As the fungi levels are increasing the elevated Copper levels appear to not be affecting fungal growth but may be a means of explaining the lack of Mycorrhizal infection of the roots tested. Investigate recycled water mulch and compost further. |
| Sulphur | Sulphur has increased with the trees treated. This is most likely from the compost or mulch as sulphur did not come from PHC # 1 liquid treatments. Sulphur is a secondary macronutrient in soil and important to plant function in desired levels. The sulphur levels appear to have increased and cannot be explained other than possibly from air pollution absorbed into the mulch or compost. |
| Chromium | Chromium is elevated in the samples in 2016 and can affect plant growth, photosynthesis and a variety of uptake in nutrients. Elevated levels of chromium are dangerous to humans if inhaled or ingested. Chromium can be reduced by fungal activity such as Mycorrhizae. Chromium and Mycorrhizae will need to be monitored further to determine future status in relation to rates of mycorrhizal infection. |

Table 2 provides an in-depth analysis of the shifts in soil chemistry in 2016. In 2017, soil chemical parameters were shifting in many directions (Table 3). However, there appears to be a clear shift in total nutrient status between the treated trees and the untreated control tree. To date, these shifting patterns are positive and further monitoring is required to establish if it will result in more sustainable soil health for the trees treated at Veale Gardens/Waylu Yarta (Park 21).

Further analysis is required to determine the extent of the issues presented although as the soil physical status and soil microbiology improves so will the chemistry. The use of recycled water and mulch may present further issues in understanding the variation of soil chemistry results and requires further investigation to determine the long-term effects and management outcomes. Further analysis and soil testing are required to determine true patterns.

The PHC treatments used in this study did not have a noticeable impact on soil pH, which remained elevated (> 7.5) in all of the tested trees, regardless of treatment (Fig. 13).

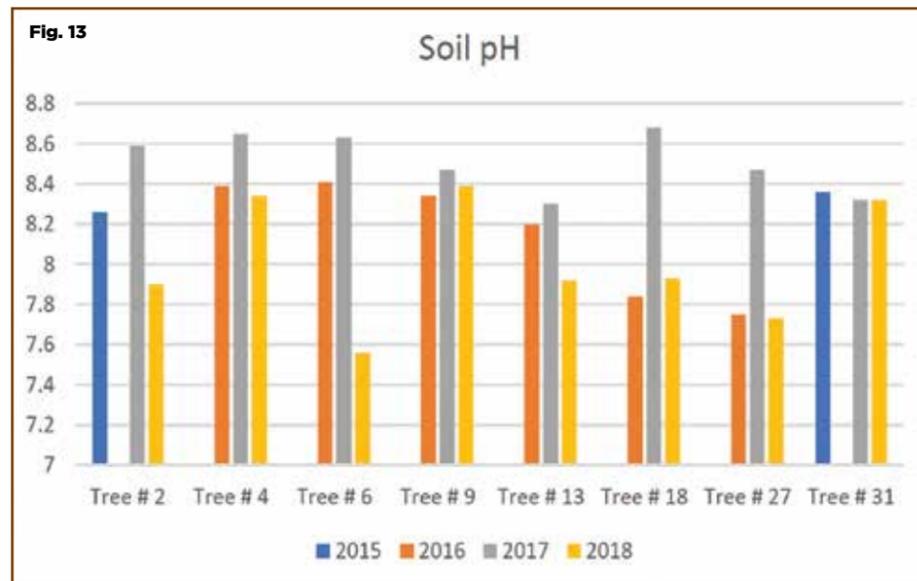


Figure 13: Soil pH is elevated. A healthy range for this soil type of Clay Loam is ph. 6.5

In summary, there were noticeable positive shifts in soil chemistry in the treated trees, suggesting that PHC # 1 and # 2 increased soil nutrition, which should lead to greater nutrient uptake in treated trees and better tree health outcomes. The reduction in sodium (salt) from the recycled

water is most likely a result of rainfall leaching, although increased soil carbon and humus from organic amendments and the resulting increase in microbial activity provides a buffer to sodium related issues because it is attracted to the humic and fulvic acid molecules.

| NUTRIENT | TREATED TREES | UNTREATED CONTROL TREE |
|------------------|----------------------|------------------------|
| Potassium | Significant increase | No change |
| Potassium | Significant increase | Decreased |
| Nitrogen | Significant increase | Decreased |
| Sulphur | Significant increase | No change |
| Sodium | Significant increase | Decreased |
| Carbon | Significant increase | Decreased |

Table 3: Comparative shift in soil chemical parameters between treated trees and the untreated control in 2017.

Increased root zone activity due to improved soil fertility

The root zone area of each tree had the sod removed prior to the application of compost and mulch.

The addition of organic materials and liquid amendments stimulated a significant amount of new healthy root growth and activity. This new root development was observed growing beneath the mulch layer and can be declared as new root mass (Fig.14), which increases the trees Root: Shoot ratio significantly.



Figure 14: Undisturbed soil surface with fine fibrous root material visually observed at the soil surface. Note that the 250 mm layer of carbon mulch material has almost fully decomposed leaving a healthy humus material and new soil.

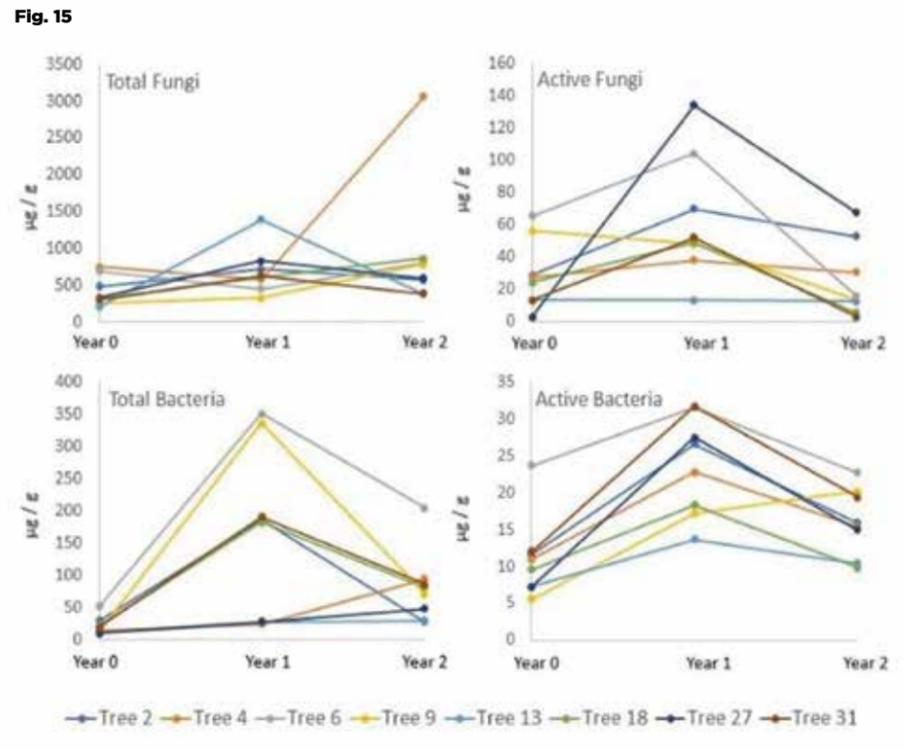


Figure 15: Total and active fungi and bacteria by year. Tree 31 did not receive any treatment, while the other seven trees all received treatment. Year 0 = samples in 2015 and 2016, prior to treatment; year 1 = 2017; and year 3 = 2018.

Figure 17: Possible *Coprinus niveus* observed during PHC application mobilisations in 2017 and 2018 fungal fruiting body blooms were observed regularly. A diversity of species was observed sometimes in unusually high summer temperatures such as 41 degrees Celsius.



The amount of total and active fungi and bacteria did not consistently increase over the three years of monitoring results (Fig. 15). On the other hand, there was a noticeable increase in protozoa under the treated trees over time compared with the non-treated baseline (Fig. 16).

While the laboratory analysis may have been a mixed bag of results, the visual indicators of soil microbial activity were astonishing. In January 2017 there was a fungal bloom and production of fruiting bodies (mushrooms) during the summer months and on consecutive days above 38 degrees Celsius. This is an unusual yet positive event and can only be explained because of the rapid increase to fungal activity caused by the intensive PHC works (Fig. 17,18).

Mycorrhizae

During PHC mobilisations in 2017 and 2018 146 Kg of Mycorrhizae spores were applied between 30 trees. The methodology used for mycorrhizal

Soil Biology

Soil biological activity is very sensitive to soil moisture content, compaction and soil chemistry. For this reason, there was a high degree of variability in the soil biological activity data. However, despite this variability, there were some very positive shifts in biological activity compared to the baseline (prior to PHC treatment) to note. In 2017, after the first application treatments, the soil biology of the treated trees increased 1741% compared with the increase of 273% of the control tree.

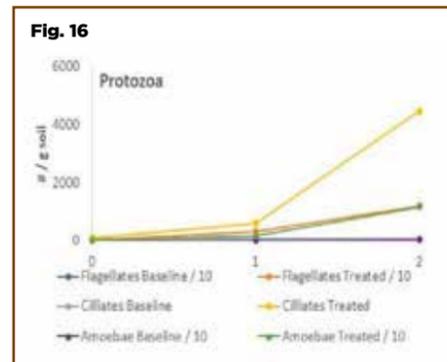


Figure 16: Comparing the mean of the treated trees to the baseline by protozoa class and year. Tree 31 values served as the baseline. Year 0 = samples in 2015 and 2016, prior to treatment; year 1 = 2017; and year 3 = 2018.

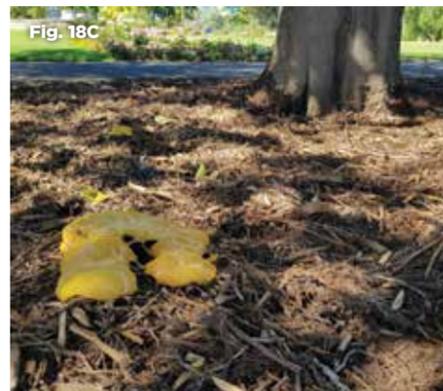


Figure 18: Fruiting Body (Mushroom) activity: A) Possible *Leptota sp* (saprophytic) if white spored or *Laccaria sp* (mycorrhizal) if pink spored; B) Possible *Mycena sp* if white spored or *Coprinus niveus* if black spored; C) *Fuligio septa* a bacterial feeding slime mould (beneficial) organism indicating the rapid increase in bacterial growth; D) *Mycena sp* if white spored; E) Visual mycelium activity, healthy mycelium with new roots growing through it.

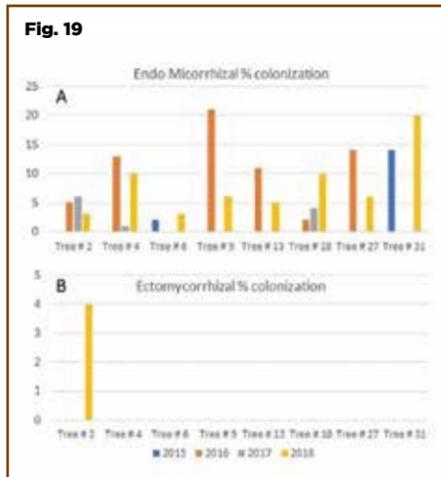


Figure 19: Colonization by A) Endo-Mycorrhiza and B) Ecto-Mycorrhiza by individual tree. In image A): The presence of mycorrhizal fruiting bodies (mushrooms), observed during the data collection strongly suggest activity is occurring and were not observed in previous visits around the same time of year. The increased soil copper levels and elevated sodium may also be inhibiting mycorrhizal colonisation and growth.

colonization laboratory assessment is direct microscopy fluoresces staining. Based on this method, Endo-Mycorrhizae colonisation does not appear to have increased, and in some instances, it decreased (Fig. 19A). Ecto- Mycorrhizae colonisation was only observed in Tree 2



Figure 20: Scleroderma cepa, ectomycorrhizae fruiting bodies observed at site tree number # 2 although is likely to be from roots of tree # 3 which is Pinus sp.

(Fig. 19B). This is not of major concern as the sampling methodology is random and the mycorrhizae spores that were applied during PHC # 1 may have been dormant in the soil profile and are yet to infect the increasing root mass that has been observed and documented.

As with the soil biological activity presented above, there was a disconnect between the laboratory data and visual observations made in the field. In this case, Mycorrhizal activity was observed during data collection in September 2017 (Fig. 20).

It is important to understand which species of trees have relationships with which mycorrhizae group.

As per Figure 20, *Scleroderma cepa* ectomycorrhizal fruiting bodies were observed on tree number # 2. Tree 2 is an *Ulmus sp* and not an and it is assumed that the root of this tree was collected as the root systems overlap.

There have been recent statements by some leading experts in the Arboriculture industry that Mycorrhizal inoculums are not effective. This is problematic as mycorrhizal inoculums are a complex soil remediation concept. One component that makes mycorrhizal inoculums different from the use of chemical or organic fertilizers is that they are biological based and require a symbiotic connection with the plant root. Fertilizers provide available nutrients for root uptake or microbial food for microbial stimulation in soil. The Veale Gardens/Walyu Yarta PHC Project has identified that although some mycorrhizal activity was measured there was not a significant increase overall. There are multiple reasons for this in this case study.

- Elevated soil compaction levels
- Highly elevated Copper and sodium levels in soil
- Low root shoot ratios of established trees
- Extreme heat events

Establishing mycorrhizal connections in some cases has been successful, the nursery industry is an example. Because nursery soil medium and plant roots are accessible, applying spores directly to active root mass is highly effective. Another important reason nursery tree's develop mycorrhizal associations

effectively is the age of the tree.

Applying inoculums to established declining trees is difficult for two main reasons.

- Determining active root zones of trees is subjective
- High soil compaction levels prevent healthy root activity and inoculation of microbial spores
- Extreme heat events reduce soil moisture rapidly and increase soil temperature to levels difficult for beneficial soil microbes to flourish

The science of soil health focusing on soil microbiomes including mycorrhizae associations is relatively recent and evolving at a rapid rate. Those who have assisted in the development of these biological sciences are highly specialized, dedicated and experienced professionals. It is important for industry to acknowledge their message that mycorrhizal associations are crucial to healthy plant growth although key soil health

parameters will limit the effectiveness of developing biological activity. The use of inoculums such as mycorrhizae should not be discouraged. It is important to inform industry of the required soil health parameters that enable the effective use of mycorrhizal inoculums to provide better outcomes. Knowledge of how to define, investigate and measure soil health will assist industry to use these inputs successfully.

Soil Respiration

Soil respiration was measured from a subset of eight trees and cross referenced with laboratory assessments and direct microscopy to determine if it is a viable methodology to use in determining soil biological activity. The advantage of this approach is that it is low-cost, requiring very few tools or laboratory experience to conduct the tests. One disadvantage of the test is that it is non-specific, meaning that it is measuring the CO₂ mineralized by all the micro-organisms in the soil and does

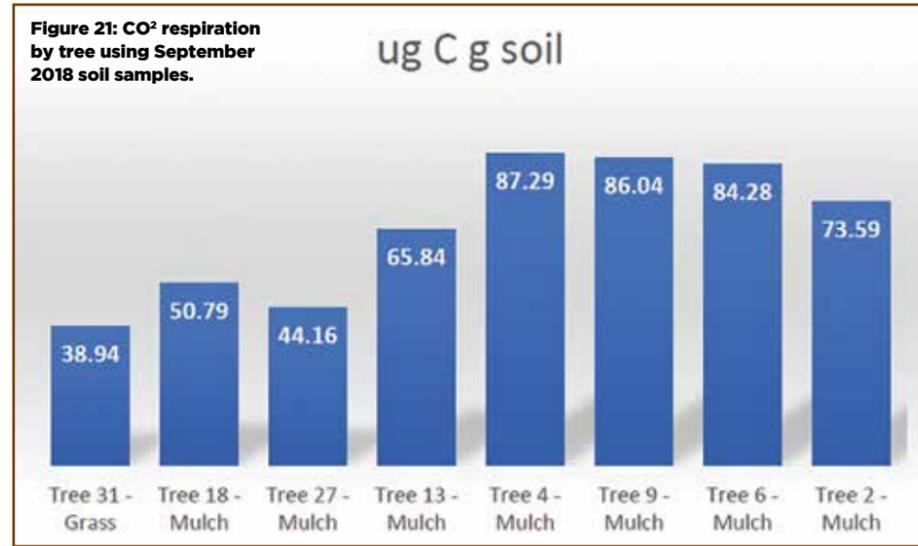
not allow the user to differentiate between bacteria, fungi or protozoa.

Soils collected in 2018 were analysed and those results are presented in Figure 21. The lowest soil respiration was recorded in tree #31, which was the untreated control. Therefore, it appears that the addition of compost, mulch, AACT and microbial stimulants did result in an overall increase in soil respiration. Likewise, comparing soil respiration to protozoa from laboratory tests (Fig. 22), there appears to be a correlation between soil respiration and protozoa populations in this case study.

There may be modifications to this method that can allow for more targeted analysis of certain microbial groups. For example, adding a substrate specific to a specific group (e.g. fungi or bacteria) and measuring the CO₂ burst may offer a low-cost way to estimate the activity of soil micro-organisms.

These methodologies need to be tested in future case studies. ▶

Figure 21: CO² respiration by tree using September 2018 soil samples.



ducks. Hundreds of aeration holes from microarthropod predation by local birdlife was observed in the TPZ of the 30 trees. Worms were observed in the new improved soil conditions and are an indication of increased soil microbiology.

Disclaimer

Note: Any soil analysis or observation taken and recorded in this report will only ever capture the status of the soil and vegetation on that day. It must be emphasized that changes of sometimes considerable magnitude can be expected in response to normal seasonal and extreme weather responses and some management actions. This means that outcomes as anticipated with the available evidence collated may be unpredictable, so regular recording of the soil and vegetation using a Soil Health Card or VSA and VTA or TREE HEALTH CALCULATOR 1.0 is essential, with the taking of photos always encouraged to record a history of change. G.U.F warrants that the methods adopted in its programs are largely a practical application of many years of experience in Plant Health Care together with scientifically verified management directives and measures through numerous sensors which are continually improved as new research findings come to hand.

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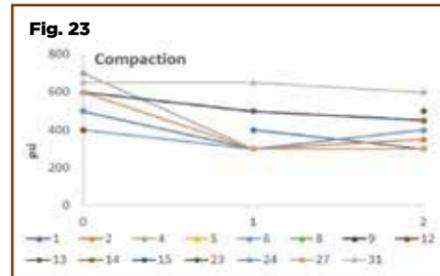
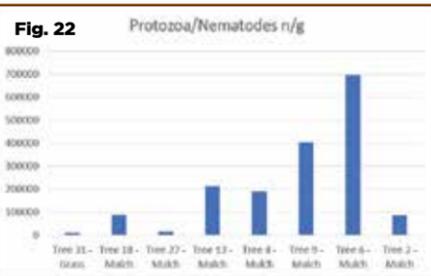


Figure 22: Protozoa / Nematodes (population / g soil) using September 2018 soil samples for laboratory analysis.

Figure 23: Soil compaction by tree and year. Tree 31 did not receive any treatment, while the other seven trees all received treatment. Year 0 = samples in 2015 and 2016, prior to treatment; year 1 = 2017; and year 3 = 2018.

Soil Compaction – a physical parameter

In addition to soil chemical and biological parameters, a penetrometer was used to measure soil compaction as an indicator of soil physical structure. Soil compaction decreased over time in all the treated trees that were measured, while compaction remained much higher in the untreated baseline (Fig. 23). Penetrometers are an indication device and the measures can be affected by the users-familiarity with the tool and measurement protocol, making interpretation of penetrometer data subjective. In this case study, all penetrometer measurements were taken by Matthew Daniel, reducing measurement variability. The development of more user-friendly penetrometers that standardize measurement processes and data capture would increase the value of compaction measurements in Urban Forestry.

Wildlife Predation on Soil Microarthropods

Local bird life had a noticeable impact on the soil in the TPZ. Ibis appeared to be the most effective soil aerators as they had longer beaks than the local



Figure 24: An interesting effect of the rapid increase in soil microbiology was the increase in microarthropod predation from birds. This became a valuable free resource of the birds aerating the soil medium. Soil health has assisted ecosystem function.