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Exterminating Bohemian knotweed (*Reynoutria* × *bohemica*) propagules using thermal treatment

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Key words: knotweed; reynoutria; fallopia; polygonum; invasive

Abstract
Invasive knotweeds in North America, including Japanese knotweed (*Reynoutria japonica*), Giant knotweed (*Reynoutria sachalinensis*), and Bohemian knotweed (*Reynoutria* × *bohemica*), cause significant environmental, structural, and economic damage worldwide. These impacts have placed knotweeds among just 37 plants on the International Union for the Conservation of Nature's list of the 100 worst invasive species. Despite their profound effects, most research has focused on specific treatment methods rather than exploring the plants' physiological vulnerabilities. Our study seeks to address this gap by investigating the thermal limitations of *Reynoutria* species. Specifically, we aim to identify the temperatures and exposure durations needed to achieve 100% mortality in knotweed rhizomes and seeds. Preliminary results show that temperatures of 150°C or higher can result in complete seed mortality within 60 minutes. These findings can inform various treatment methods, including incineration, composting, microwave radiation, thermal desorption, and other novel thermal approaches.

Introduction
Knotweeds, including Japanese knotweed (*Reynoutria japonica*), Giant knotweed (*Reynoutria sachalinensis*), and their hybrid, Bohemian knotweed (*Reynoutria* × *bohemica*), have caused significant economic and ecological damage worldwide. Lowe et al. (2000) identified Japanese knotweed as one of the world's 100 "worst" invasive species on a list encompassing plants, animals, fungi, and microbes.

These challenges underscore the need for effective prevention and management strategies for invasive knotweeds. Conventional methods, such as herbicide application and mechanical treatments, have shown limited success, with only herbicide treatments consistently achieving results (Hocking et al. 2023). However, even herbicide treatments require multiple applications over several years to ensure eradication. In construction projects where knotweeds are prevalent, excavation is often necessary to prevent long-term damage to foundations or paving. Disposal of excavated material remains a challenge; while deep burial is commonly used (McHugh 2006), it is not a reliable long-term solution since knotweed propagules can remain dormant in soil for up to 20 years (Parkinson and Mangold 2010).

Excavating contaminated soils presents an opportunity to apply off-site treatments. This study aims to establish a foundational understanding of the effectiveness of heat treatments on knotweed propagules. Specifically, the objective is to determine the temperature and duration combinations required to achieve complete mortality of Bohemian knotweed seeds and rhizomes. Bohemian knotweed, the most prolific and widely distributed of the three species (Grimsby and Kesseli 2010; Gaskin et al. 2014), is the focus of this study. Rhizomes, which account for most of the plant's establishment potential (Gowton et al. 2016), are a primary concern, but seeds are also included due to their emerging role in natural establishment, driven by the substantial seed production observed annually (Beerling et al. 1994; Bram and McNair 2004).

Methods

Bohemian knotweed rhizomes and seeds were collected on October 23, 2023, from a site near the municipal library in Mission, BC, Canada. Rhizomes were excavated and cut to 10 cm lengths before being sealed in plastic bags placed in a cooler for transport. Seeds were collected by severing dead seed heads, which were then stored in brown paper bags and later cold stratified.

Rhizomes were culled to exclude any that were obviously dead or excessively damaged. Samples lighter than 10 g were also removed. An average weight was calculated for the remaining rhizomes, and those that varied by the greatest degree were removed. To further limit the effect of third variables on treatment efficacy and analysis, samples were separated into four weight classes. Samples were then block randomized by weight class to assign treatment using formulas in Microsoft excel. Each treatment contained an even number from each weight class to ensure a weight distribution reflecting that of the overall population in each treatment.

Rhizomes were treated at three different temperatures for three different time periods. Temperatures of 100, 125 and 150°C over periods of 10, 20 and 60 minutes were used for this trial. Each treatment had nine replicates of four rhizomes for a total of 36 rhizomes per treatment. Each replicate of four rhizomes was fed through the oven separately in all cases. After treatment, rhizomes were planted in well-draining 4" pots with 600 ml of a 1:1 mixture of potting soil and sand. Rhizomes were left to grow for a period of 30 days and observed for signs of growth. As a control 36 untreated rhizomes were also planted before treatment (initial viability). After 30 days of growth, each rhizome was excavated and observed for growth. Presence of root or shoot material was determined as confirmation of survival. An ANOVA and Tukey test were conducted using R software, which looked to determine statistical significance of treatment as it related to mortality when compared to untreated samples.

Seed trials were similar to those of the rhizomes, but used time increments of 10, 20, 30, 40, 50, and 60 minutes, as more samples were available. Assignment of treatment was fully random, and seeds were placed in 15 mL centrifuge tubes filled with 10 mL of water, where they were allowed to grow over 30 days. Presence of a radicle was used as an indication of viability.

Results

Rhizomes had high initial viability at 97%. Viability by treatment can be seen in Fig. 1. Increases in treatment period and temperature resulted in a decrease in viability, with zero viability observed after 60 minutes at all temperature treatments, and at 20 minutes when treated at 150 degrees. All treatments, except treatment at 100 degrees Celsius for 10 minutes, had a significant effect when compared to controls.

Initial seed viability was 78%. Seed viability by treatment can be visualized in Fig. 2. Viability dropped with intensity of treatment, but there were some anomalous results – namely the 11.11% viability seen in treatments of 125 degrees Celsius for 60 minutes. There is also an unexpected 2.78% viability for samples treated at 125 degrees Celsius for 40 minutes. All treatments at 150 degrees Celsius resulted in seed mortality.

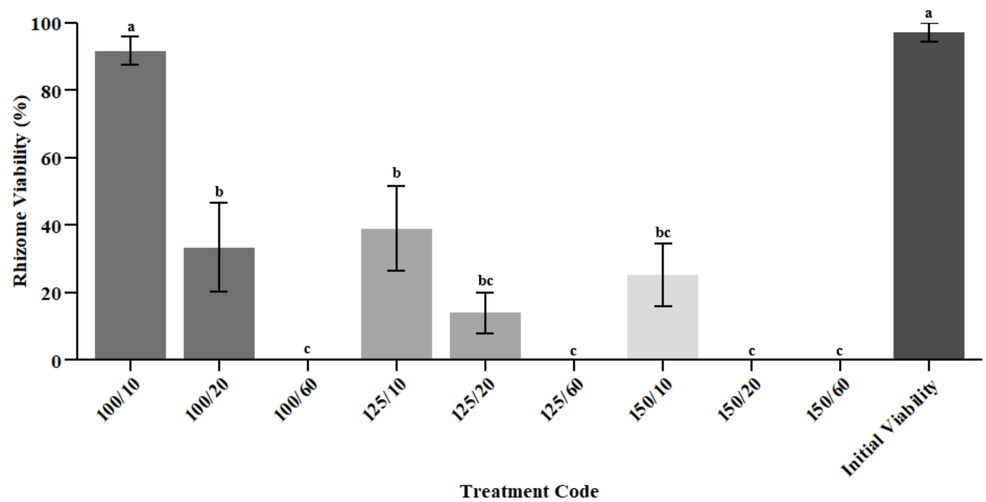

Fig.1: Viability of bohemian knotweed rhizomes based on thermal treatment with correlations. Treatment codes indicate temperature(°C)/time(minutes) except in case of Initial Viability (control), where no treatment took place. Columns are shaded based on temperature of treatment.

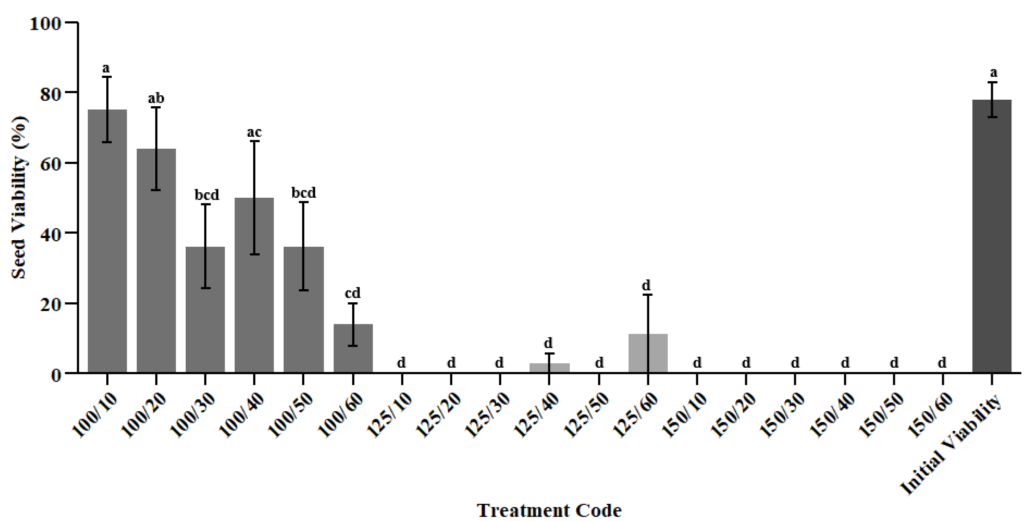

Fig.2: Viability of bohemian knotweed rhizomes based on thermal treatment with correlations. Treatment codes indicate temperature(°C)/time(minutes) except in case of Initial Viability (control), where no treatment took place. Columns are shaded based on temperature of treatment.

Discussion

Rhizome mortality under heat treatment exhibited a clear pattern of reduced viability with increasing temperature and application duration, suggesting that knotweed rhizome viability can be significantly diminished with relatively low energy input.

In contrast, results for seed viability under thermal treatment were less consistent. The expected decline in viability with increasing treatment intensity was not observed, with an unusual spike in viability at 125°C for 40 minutes. This anomaly is likely due to human or technical error, as all four surviving seeds came from the same experimental replicate, which showed 100% survival. Additionally, the inconsistency may stem from the lack of measures to ensure uniformity in the seed sample population, a step that was taken with rhizomes. Future trials could benefit from improved sample uniformity and increased sample sizes. Despite these challenges, initial seed viability fell within the 55–100% range reported in the literature (Forman and Kesseli 2003; Bram and McNair 2004), validating the novel viability assessment methodology used in this study, which also proved more cost-effective than conventional methods.

This research provides an initial understanding of the effects of thermal treatment on knotweed propagules but was limited by sampling location. Bohemian knotweed's high genetic diversity (Gaskin et al. 2014) suggests that the efficacy of thermal treatment may vary with location and seasonal factors. Additionally, knotweed propagules are often mixed with soil in practical applications, highlighting the need to study the impact of soil composition and moisture on treatment efficacy. Large rhizome and stem fragments could also limit treatment effectiveness.

Despite these limitations, results suggest that temperatures as low as 150°C, applied for as little as 60 minutes, can ensure the mortality of both seed and rhizome propagules. Although in-situ treatments using fire have been ineffective for killing vegetative propagules due to the plant's high water content (Child and Wade 2000), externally generated heat through incineration, composting, microwave radiation, or emerging technologies like thermal desorption may offer viable alternatives.

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Passive vs active restoration to improve soil health of old potato production circles in the Leipoldtville Sand Fynbos, South Africa

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Key words: brush packing; soil phosphorous; soil organic carbon; indigenous plants

Abstract

Potato production is the main land use in the endangered Leipoldtville Sand Fynbos vegetation type on the west coast of South Africa. Many fields have been abandoned and their restoration is important to conserve this vegetation type. These abandoned fields are subject to wind erosion and have high soil phosphorous levels due to fertilisation. We addressed the question "Does active restoration enhance soil health faster than natural processes?" We selected three sites, all in sandy soils with high soil phosphorous levels (35–63 mg/kg) compared to the surrounding natural vegetation (7–11 mg/kg). Cultivation had ended 5–7 years previously and sites were in different states of recovery. Seven treatments were applied, including planting of indigenous species, an initial rye mix consisting of cereal rye, lupins and serradella, and brush packing using branches from invasive trees packed in a single layer at a density of 50–80% soil cover, in various combinations, as well as a control. Soil samples were collected to determine changes in phosphorous levels using citric acid analysis, organic carbon using the Walkley-Black method, and microbial diversity using Biolog EcoPlates. Due to drought from 2017–2020 the initial rye mix established poorly and had little impact on the soil-P levels. Phosphorous levels decreased over time at two of the sites but increased significantly at Site 3, adjacent to active croplands. Organic carbon increased over time at Site 2 and Site 3. At Site 1, with the least natural plant cover, organic carbon only increased in the treatments that included brush packing. At all sites there was a significant increase in soil microbial diversity, but at Site 1 it was better in treatments with brush packing.

There was an improvement in overall soil health over time. Abandoned fields with the least natural cover benefited most from restoration actions.

Introduction

The Fynbos biome is recognized as one of the global hotspots of diversity. It ranks among the world's 25 most threatened biodiversity hotspots (Mittermeier et al. 2011). Vast expanses of this once-pristine habitat have succumbed to permanent agriculture, with crops, potatoes, and rooibos tea plantations replacing natural vegetation. The biome's transformation is further exacerbated by inappropriate fire management practices, livestock grazing, invasion by alien plant species, and overexploitation of natural resources (Mucina and Rutherford 2006).

Leipoldtville Sand Fynbos is an arid, endangered ecosystem with a mediterranean climate and acidic, sandy soils (Mucina and Rutherford 2006). Less than 45% of its natural vegetation remains, the rest is cultivated lands of which 27% is under pivot irrigation and used for potato production. As input costs rise and soil diseases proliferate, many of the fields are being abandoned and left to recover through natural succession, which is a very slow process in drylands. The abandoned fields are prone to wind erosion and years of fertilisation has led to high soil phosphorous levels compared to the surrounding natural vegetation. These high phosphorous levels inhibit the establishment of Fynbos species (Hawkins et al. 2010), since Fynbos soils are acidic and nutrient-poor, especially in terms of nitrogen and phosphorous levels (Richards et al. 1997). Therefore, some form of active restoration is necessary to ameliorate the soil condition, reduce wind erosion, and initiate the establishment of natural vegetation. We addressed the question "Does active restoration enhance soil health faster than natural processes?"

Methods

The study was done at three sites in the Leipoldtville Sand Fynbos on the west coast of South Africa. This winter rainfall area receives an average annual rainfall of 263 mm. All sites are characterized by deep sandy soils with high phosphorous levels (29–62 mg/kg) and low organic carbon levels (0.07–0.20%) compared to soil phosphorous levels in the adjacent natural vegetation that are below 10 mg/kg. Cultivation ended 5–7 years previously and sites were in different states of recovery.

The same experimental design and layout were implemented at all three sites, following a randomised block design with four replicates and seven treatments, which consisted of a control and a mix of the following treatments:

1) To combat the high phosphorous levels an initial rye mix (R), consisting of cereal rye, lupins and serradella, was planted in 2017 and 2018. Minimum soil disturbance was done with a tine implement to prepare the soil, and after sowing, the soil was rolled to ensure good seed-soil contact. Due to a drought in 2017 the planting was repeated in 2018. The plants were cut down and removed before seed set to remove the phosphates from the trial.

2) To combat wind erosion, brush packing (B) was done with branches from local invasive trees in 2020, which also provided organic matter. The branches were packed in a single layer at a density of 50–80% soil cover. Wind speed reached 11 m/s at Redelinghuys and Eland's Bay sites, where signs of wind erosion was the most visible.

3) Lastly cuttings (P) from three species indigenous to the area were made and planted in the plots to increase the species diversity on the trial.

Composite soil samples were collected at the start (June 2017) and end (October 2023) of the study to determine changes in phosphorous levels using citric acid analysis, changes in organic carbon using the Walkley-Black method (The Non-affiliated Soil Analysis Work Committee 1990), and changes in microbial diversity using Biolog EcoPlates (Lee et al. 2020).

Data was analysed using ANOVA, Fischer's Least Significant Difference test and Principal Component Analysis.

Results

The study area experienced a drought from 2017 until 2020. This resulted in the poor establishment of the initial rye mix and it had no significant impact on the soil phosphorous levels (plant-available phosphorous). However, the phosphorous levels decreased significantly over time at two of the sites ($p < 0.01$) but increased significantly at Eland's Bay ($p = 0.0056$) (Fig. 1a). There were no significant differences between the treatments for each site over time.

The soil organic carbon (SOC) increased significantly over time at the Eland's Bay and Sandberg sites ($p < 0.001$) (Fig. 1b), with no significant difference between the treatments at both sites. At Redelinghuys, with the least natural vegetation cover on the abandoned land and the worst establishment of planted cuttings, the organic carbon only increased significantly (LSD = 0.0702) in the Brush packing treatment (Fig. 1b).

At all three sites there was a significant increase over time in soil microbial diversity ($p < 0.001$), as seen on the first axis of the PCA, indicating an improvement in the overall soil health (Fig. 2).

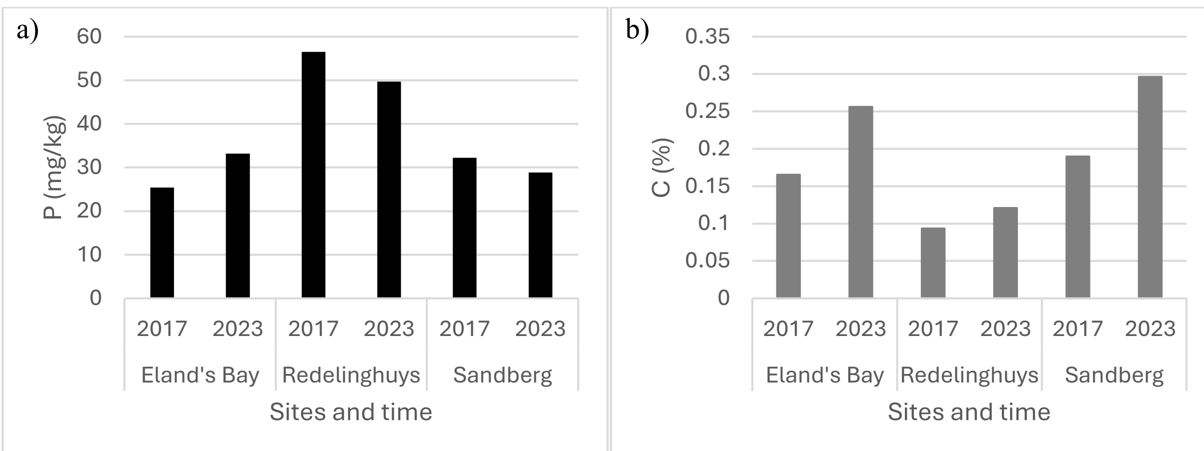

Fig. 1 Soil phosphorous levels (a) and organic carbon content (b) in 2017 and 2023 at Eland's Bay, Redelinghuys and Sandberg sites, South Africa.

Discussion

Hawkins et al. (2010) found that a cover crop mix of oats and lupins decreased the soil-P levels by 10–30% because of their fast growing rate. In the case of this study, the below average rainfall received in 2017 and 2018 resulted in no germination of the initial rye mix sown in 2017, and a poor growth rate of those plants that did establish in 2018. Therefore, the expected outcome was not achieved. According to Prasad and Chakraborty (2019), soil phosphorous is mostly lost through erosion and not leaching from the soil. At Sandberg soil-P was most probably removed from the soil by the plants established in each of the plots, as it had the best plant cover of all the study sites and no visible signs of wind erosion, with the maximum wind speed less than 9 $m.s^{-1}$. The wind at Redelinghuys and Eland's Bay sites reached a speed of up to 11 $m.s^{-1}$. At Redelinghuys, with visible signs of wind erosion, and the least plant cover, soil-P was lowered over time because of the wind erosion. At the Eland's Bay site, part of the trial area was covered by soil from an adjacent, actively used cropland caused by wind erosion.

This is most probably the reason for the increased soil-P over time. The other study sites did not have any active croplands in the surrounding area.

More plants established at Eland's Bay and Sandberg sites lead to an increase in the SOC in all the treatments over time supporting similar results reported by Qiu et al. (2018). At Redelinghuys the provisioning of branches on the soil assisted in the establishment of plants and an increase in SOC. The increase in SOC can lead to improved soil water retention and in dry areas, such as Leipoldtville Sand Fynbos, can result in improved vegetation growth (Li et al. 2024). This is likely to be beneficial during dry periods as the availability of soil water is a limiting factor in arid ecosystems (Qiu et al. 2018).

The improvement in the soil microbial diversity at all sites indicated an improvement in the soil health. Soil microbes are responsible for the decomposition of organic matter, nutrient transformation, plant health maintenance and the degradation of toxic compounds in the soil amongst others (Lee et al. 2020). This provides ecosystem services essential for humans, such as food production, climate regulation, and the provision of clean water (Adhikari & Hartemink 2016; Pulleman et al. 2012).

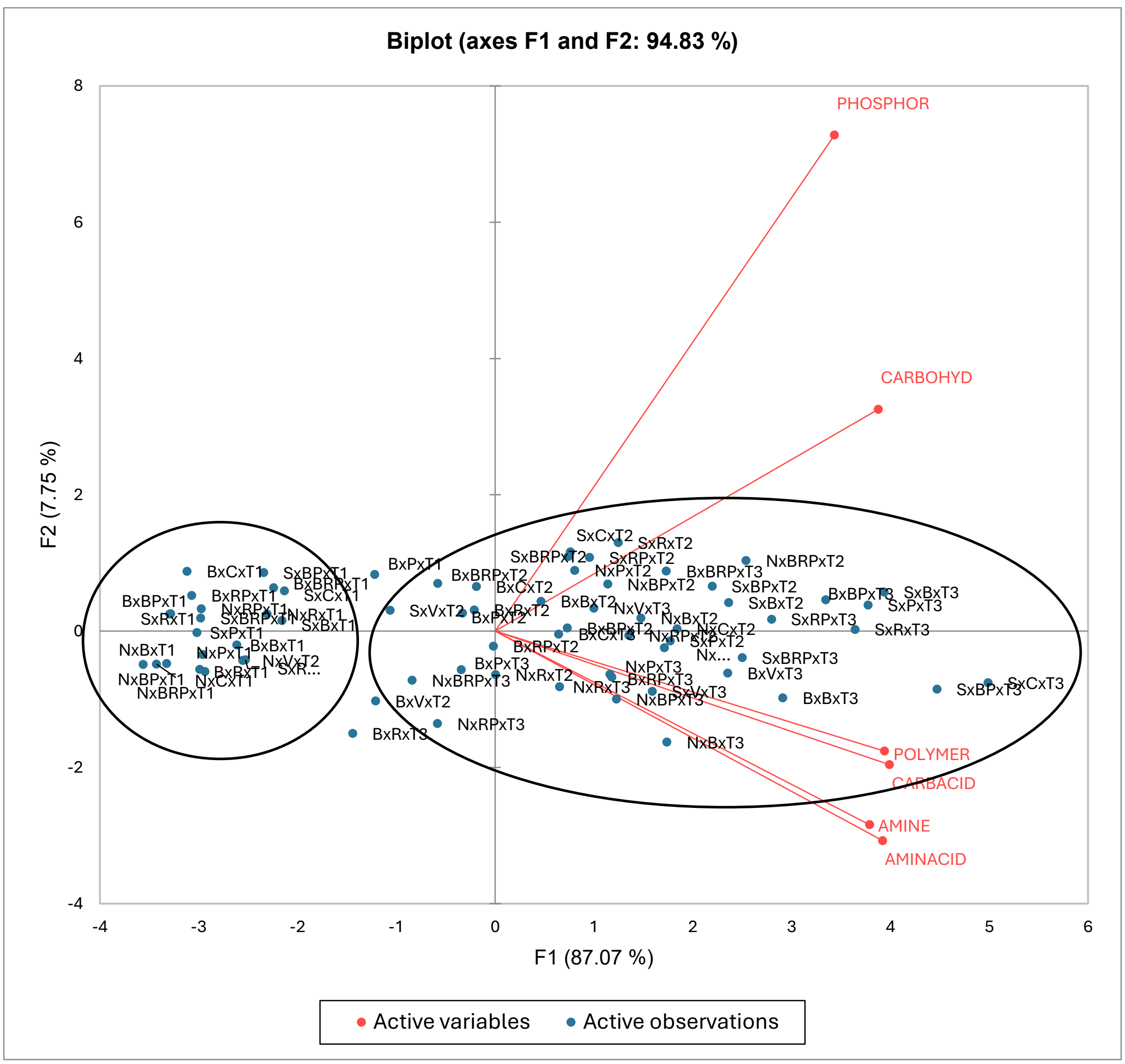

Fig. 2 Principal component analysis (PCA) of soil microbial diversity in 2017 (T1), 2021 (T2) and 2023 (T3) in the different treatments at Redelinghuys (B), Eland's Bay (N) and Sandberg (S). C = control; B = brush-packing; R = initial rye mix; P = planting indigenous species.

Conclusion/Implications

There was an improvement in overall soil health over time. Abandoned fields, such as the Redelinghuys site with the least natural cover benefited the most from the restoration actions.

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