Sea Minerals Reduce Dysbiosis, Improve Pasture Productivity and Plant Morphometrics in Pasture Dieback Affected Soils

Maria M. Whitton 1, Xipeng Ren 1, Sung J. Yu 1, Andrew D. Irving 2, Tieneke Trotter 1, Yadav S. Bajagai 1 and Dragana Stanley 1,*

1 Institute for Future Farming Systems, Central Queensland University, Rockhampton, QLD 4702, Australia
2 Coastal Marine Ecosystems Research Centre, Central Queensland University, Gladstone, QLD 4680, Australia
* Correspondence: d.stanley@cqu.edu.au; Tel.: +61-7-4923-2079

Abstract: Pasture dieback (PD) is a grassland deteriorating syndrome resulting in grass loss and weed expansion in Australian pastures, with current estimates indicating that over four million hectares are affected. PD creates financial losses to the industry by reducing animal carrying capacity and producing poor-quality feed, resulting in diminished productivity. After more than a decade since PD first appeared in Australia, the causes and effective treatments are still unknown. Suggested causes include soil microbiota dysbiosis, pathogens, insects, climate change and overuse of chemical fertilisers. Sea minerals have been suggested as capable of improving plants’ yield, quality, taste, and nutritional value, but were never brought into conventional practice as an alternative to chemical fertilisers. Here, we investigated the capacity of sea minerals to improve grass health and yield of PD-affected soil. The replicate plots were treated with water or with 4 mL/m² of commercially available sea mineral product to investigate the soil chemistry profile, plant morphometrics, pasture productivity, soil microbiota profile, and microbiota-nutrient interactions. Sea mineral application significantly increased total dry matter 20 weeks after a single application, translating to an additional 967 kg/ha; this benefit was still present at 498 kg/ha six months post a single application. Sea mineral application improved soil microbiota by boosting beneficial taxa while reducing genera associated with arid and toxic soils. Additionally, sea mineral application increased the number of grassroots up to six months post a single application. Our data suggest the benefits of sea mineral application to damaged, unproductive or exhausted soils could be further explored as a natural, affordable, and non-toxic alternative to chemical fertilisers.

Keywords: pasture; sustainability; sea minerals; grass; dieback; soil; microbiota

1. Introduction

Since the industrial revolution, anthropogenic activities have rapidly increased greenhouse gasses (GHG), mainly CO₂, CH₄, N₂O [1], the pollution of water (industrial, mining, agricultural) [2–5], and soils (overuse of fertilisers, mining) [6–11], thus generating global warming and climate change. Changing weather patterns, increasing temperatures, radiation, salinity, drought, and flooding worldwide, significantly impacted the agriculture industry, threatening the food security of an ever-growing human population.

By adopting regenerative and sustainable practices to improve soils, agriculture practices can adjust to new conditions and try to reverse the damage caused by the overuse of chemicals and traditional farming habits [12]. Regenerative agriculture has two main objectives: to repair soil health and biodiversity, which are diminished due to monoculture in most farming practices [13]. It is well established that plant biodiversity can assist the ecosystem during extreme climate events. In controlled multisite biodiversity and drought grassland trials, Kreyling and co-authors suggested that species richness does not improve resistance but instead promotes recovery [14], where the most diverse...
ecosystems went so far as showing overcompensation. The phenomenon of climate change is inevitably increasing the severity and occurrence of droughts. In a study by Wright et al. [15] the majority of species declined in prolonged drought when grown in monoculture, but were not affected when grown in a high diversity mixture [15], suggesting that the benefits of biodiversity become more apparent during challenging years. Similarly, extreme rainfall events, especially when combined with drought [16], decrease biomass productivity, but although the response to extreme rainfall and drought periods was independent of species richness and diversity, mixtures were more biomass productive than monocultures [16].

In addition to improving extreme climate response, plant richness and biodiversity affect and interact with the soil microbial communities. There is a positive correlation between plant and soil microbial diversity, and plant evenness leads to more pronounced positive correlations between bacterial and plant richness [17]. Rich soil microbiota provides plant communities with nutrient cycling, decomposition and climate regulation [18]; thus, loss of plant richness leads to the functional decline of the microbial community affecting the plants’ ability to thrive. The microbial composition also plays a role in soil multifunctionality, independently from richness [18].

With shifting weather patterns and increased pollution, plants and soil are confronting multifactorial stress, and the stability of biomass products for the grazing industry is likely to face changing times. Although plants, soil and soil microbiota frequently face environmental stress, pollutants, pests, and diseases, often in the mild form, in combination, these relatively mild stresses can decrease the soil microbiome diversity, decomposition rate, water-stable soil, and soil respiration. Zandalinas et al. [19,20] also discovered that while individual stresses applied to seedlings had a minimal effect, their combination resulted in a decline in root growth, chlorophyll content and plant survival.

Pasture production is vital to the cattle and sheep industry as it provides the animal with the nutritional requirements to grow and produce good quality meat. Pasture-based beef production depends on the quality and availability of high nutritional value feed the animal can consume [21]. There are currently $13.5 billion worth of cattle and $4.3 billion worth of sheep in Australia [22], not counting $8.5 billion of livestock products that include wool and milk. Pasture dieback (PD) is a grass decaying syndrome affecting grass production across multiple climates and soil types, reducing animal carrying capacity and producing financial losses to the industry by diminishing pasture availability and producing poor-quality feed. PD appears first as yellowing or reddening of the tip of older leaves, spreading to the rest of the plant. Plant roots are undeveloped and stunted, with obstructed conduction vessels and lesions on root tissue where cellular damage was observed [23]. Around 2001, dieback was detected in different cultivars of buffelgrass in Queensland and, more recently, in New South Wales, Australia [23,24].

There are currently different speculations on the cause of PD, implicating eroded soils, damaged soil microbiota, loss of diversity, drought stress, ground pearls [24], mealybugs [25], reduced organic matter, deforestation, loss of biodiversity, poor soil conditions. Still, despite many suggested factors, a specific cause has not yet been identified.

Since the green revolution in the 1970s, using chemical fertilisers as a soil supplement has significantly increased crop production [19], food quality and profit and is now considered common practice. Plant nutrition is vital to deliver the correct nutrients for each plant stage to maximise plant production [26]. Mineral fertilisers are used to increase soil capacity to provide necessary nutrients for plants to grow and generate good-quality products. Minerals like nitrogen, phosphorus, potassium, magnesium and calcium are considered macronutrients, and micronutrients manganese, boron, zinc, copper, molybdenum, chlorine, and nickel, are essential for plant health and growth. They intervene in photosynthesis, the formation of sugars, starches, proteins, vitamins, enzymes, and cell wall formation [27]. Mineral-microbial complexes powerfully influence plant growth, soil health, microbial community and biodiversity [28].
The indiscriminate use of chemical fertilisers has created many environmental issues [29,30] caused by the leaching, runoff and evaporation of fertilisers in the rivers and sea, with unfavourable soil conditions usually constraining plant growth and nutrient supply. With regenerative agriculture and sustainable organic farming techniques, more natural ways to improve plant production are being pursued and implemented [31]. As many nutrients applied to crops end up in rivers and the sea [32], it is logical that the sea can act as a mineral reservoir.

Seawater has been used for decades as a source of essential minerals for cropping. Between 1960 and 1974, Dr Maynard Murray conducted a series of experiments using seawater as a soil amendment to provide plants with all necessary nutrients. In his book Sea Energy Agriculture, he states, “All essential nutrients can be supplied in proper proportions by a single dilute solution of seawater, plus nitrogen”. He reported an improved yield, quality, taste and nutritional value [33]. This makes sea minerals (SM) a potential, yet currently under-utilised, an avenue for organic, sustainable, regenerative agriculture [34,35]. Additionally, the sea minerals are a side product in salt production after the salt is removed, thus eliminating the salinity concern. They are also used in minute doses and present a less costly alternative to often destructive chemical fertilisers which are known to cause waterway and air pollution, toxicity to aquatic animals, chemical burn to crops, acidification of the soil and long-term destruction of the soil [36–44].

In this study, we investigated the benefits of applying sea minerals to pasture dieback-affected soil to investigate its capacity for long-term remediation and the effects on soil microbial communities.

2. Materials and Methods

2.1. Farm Description and Location

The experimental trial was performed in Garnant, Queensland. The property is 1500 ha of vertisol soil type, which carries up to 800 head of Bos indicus X Bos taurus cattle on an organic pasture. The paddock used in this trial was 38 ha, and has been affected by PD since 2017 when the prevailing Queensland bluegrass (Dichnthium sericeum), buffelgrass (Cenchrus ciliaris), black spear grass (Heteropogon contortus), Indian couch (Bothriochloa pertusa), kangaroo grass (Themeda triandra), green panic (Megathyrsus maximus) and forest blue grass (Bothriochloa bladhi subsp. glabra), started to die off in patches. By 2019 all grass species were replaced by common weeds and legume plants. By the time this trial was conducted, the paddock was overrun with weeds unpalatable to cattle, like wild sage (Salvia verbenaca L), woody plants such as currant bush (Carissa ovata and Carissa lanceolata), Indian couch (Bothriochloa pertusa), and native species of annual herbs, significantly reducing the pasture quality and cattle carrying capacity.

2.2. Experimental Design (Plots Description, Location, Treatments, Sampling)

The experimental design used in this trial was a randomised complete block with three replicates [45]. Each experimental plot was 5 m by 5 m square, with a 2 m buffer separating each treatment to avoid drift contamination while spraying the product. The sea mineral product (Olsson’s Liquid Sea Minerals) used in this trial (Table 1) was diluted at a ratio of 100 mL in 50 L (2 mL/L) of untreated water and applied at a rate of 50 L of diluted product per experimental plot. This results in the final application rate of 4 mL/m². Control plots (CTR) were treated with the same volume of water without SM.
Table 1. Olsson’s Liquid Sea Minerals typical analysis.

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total dissolved ions</td>
<td>32%</td>
</tr>
<tr>
<td>Sodium</td>
<td>2.87%</td>
</tr>
<tr>
<td>Chloride</td>
<td>19%</td>
</tr>
<tr>
<td>Potassium</td>
<td>1%</td>
</tr>
<tr>
<td>Aluminium</td>
<td>0.1 mg/L</td>
</tr>
<tr>
<td>Copper</td>
<td>0.09 mg/L</td>
</tr>
<tr>
<td>Manganese</td>
<td>6.78 mg/L</td>
</tr>
<tr>
<td>Boron</td>
<td>469.51 mg/L</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>0.05 mg/L</td>
</tr>
<tr>
<td>Barium</td>
<td>0.015 mg/L</td>
</tr>
<tr>
<td>Beryllium</td>
<td>0.01 mg/L</td>
</tr>
<tr>
<td>Calcium</td>
<td>10 mg/L</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>12 mg/L</td>
</tr>
<tr>
<td>Sulphur</td>
<td>17429 mg/L</td>
</tr>
<tr>
<td>Magnesium</td>
<td>12%</td>
</tr>
<tr>
<td>Zinc</td>
<td>1.85 mg/L</td>
</tr>
<tr>
<td>Lithium</td>
<td>17.1 mg/L</td>
</tr>
<tr>
<td>Titanium</td>
<td>0.13 mg/L</td>
</tr>
<tr>
<td>Uranium</td>
<td>0.041 mg/L</td>
</tr>
<tr>
<td>Vanadium</td>
<td>0.1 mg/L</td>
</tr>
<tr>
<td>Arsenic</td>
<td>0.01 mg/L</td>
</tr>
<tr>
<td>Mercury</td>
<td>0.01 mg/L</td>
</tr>
<tr>
<td>Selenium</td>
<td>0.01 mg/L</td>
</tr>
<tr>
<td>Cadmium</td>
<td>1.43 mg/L</td>
</tr>
<tr>
<td>Chromium</td>
<td>4.7 mg/L</td>
</tr>
<tr>
<td>Lead</td>
<td>0.6 mg/L</td>
</tr>
<tr>
<td>Nickel</td>
<td>0.6 mg/L</td>
</tr>
<tr>
<td>Silver</td>
<td>0.35 mg/L</td>
</tr>
<tr>
<td>Tin</td>
<td>0.01 mg/L</td>
</tr>
<tr>
<td>Nitrates-Nitrogen</td>
<td>0.1 mg/L</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>0.011% w/w</td>
</tr>
<tr>
<td>Sulphate</td>
<td>7.01%</td>
</tr>
<tr>
<td>Salt from chloride</td>
<td>31%</td>
</tr>
</tbody>
</table>

Soil samples were taken before the first treatment with SM and then weekly for 6 weeks, and after that, fortnightly until week 20. The method used was core soil collection [46], sampling at 15 cm depth using a T-bar, one sample per plot each time. Soil samples were kept on ice for transport and placed immediately at −80 °C.

For comparison and calculation of plant dry matter, samples were taken before treatment and at week 20 of the trial using a quadrat randomly thrown twice in each plot and cutting all plant material inside the quadrat using scissors as close to the ground as possible. The samples were then dried in an oven at 60 degrees for 24 h, and the weights were recorded. Samples were taken six months after the trial (week 48 after treatment), after the rainy season and cattle grazing. A new grass and sage sample from each plot were taken at week 20 and week 48 after the treatment to observe differences in plant morphometrics.

Soil minerals from the collected soil samples were analysed using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). All minerals in the detection rate of this methodology were analysed, including Total carbon/nitrogen Al, B, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Zn, pH, Electric Conductivity (EC). The soil sample analysis was outsourced to an external laboratory.
2.3. Sequencing Data Analysis

Molecular analysis of soil microbial communities was done by extracting soil DNA. DNA extractions were performed using a commercial extraction kit (DNeasy PowerSoil Pro Kit) and following the manufacturer’s recommended protocol. After DNA extraction, library preparation was performed as previously described amplifying the V3–V4 region of the 16S molecule. The sequencing was outsourced to Azenta Life Sciences (Suzhou, China). The forward primer was 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and the reverse primer was 806R (5'-GGACTACHVGGGTWTCTAAT-3') and the better read with Phred quality score of >20 across the whole trimmed length was used.

The raw sequencing data were demultiplexed using Cutadapt [47]. Microbiota analysis was done with Quantitative Insights Into Microbial Ecology 2 (QIIME 2) [48] software. After the import into QIIME2 and the basic quality check, sequences were trimmed and filtered so that the lowest Phred quality score was 32, median 39 and maximum 39. Error correction and chimera checking was performed using Dada2 [49] and recommended protocols. ASV level data were binned into OTUs at 98% sequence identity. After removing all samples with less than 2000 sequences and rarefying the data table to 2000, the final dataset had 62 samples and 1040 OTUs. The results were further analysed using a range of R packages, including Microeco, Phylosmith and Phyloseq. Microeco package was used to integrate ecological data, such as mineral profiles, into microbiota analysis, while Phylosmith and Phyloseq were used to visualise alpha and beta diversity. Primer-e v7 was also used to further analyse and plot the soil microbiota data. Plant data, including dry matter and plant morphometrics, was analysed and presented using GraphPad Prism v9. Raw sequences are publically available on Sequence Read Archive (SRA) database under accession number PRJNA887675.

3. Results

3.1. Soil Chemistry Profile Was Mostly Unaffected

Soil chemistry profiles were measured on all plots at week 0, before SM application as a baseline, and 12 weeks post single application. There were no significant differences in any measured parameters between CTR and SM in week 0 before application or at week 12 (Figure 1). Although the same temporal trends were followed in both groups, the concentration of Al significantly \((p = 0.049)\) decreased, and S \((p = 0.007)\) and N \((p = 0.041)\) significantly increased in SM from week 0 to week 12. Electric conductivity and Ni significantly increased from week 0 to week 12 in both CTR \((p = 0.028\) and \(p = 0.031)\) and in SM plots \((p = 0.05, p = 0.023\) for EC and Ni, respectively). Another noticeable trend was a marginal increase in Pb, S, C, N, and Fe in CTR and SM from week 0 to week 12.
3.2. Impact of SM on Plants

The application of SM significantly \((p = 0.013)\) increased the total dry matter between week 0 and week 20. CTR plots also increased during the first 20 weeks but not significantly (Figure S1 of the Supplementary Materials). Six months post-application, SM plots had higher total dry matter than the control. There was less grass and comparable quantities of dicots with much more litter in SM plots (Figure S2 of the Supplementary Materials). The litter here embodies dried grass and dicots.

Plant morphometric analyses were performed at week 20 (Figure 2) and six months post SM application (Figure 3). At 20 weeks post-application, there was a significant increase \((p = 0.044)\) in the total number of roots in the grass and a marginal yet noticeable drop in all measured parameters in wild sage. Six months post-application, the total number of roots was still significantly higher \((p = 0.027)\) in the SM grass, while in sage, the total number of roots reduced in week 20 was now significantly higher \((p = 0.039)\) in SM-treated plots. The length of the youngest leaf and longest root was also reversed from reduced in week 20 to marginally increased six months post SM treatment, with seed heads consistently noticeably lower in the SM group (Figure 3).
Figure 2. Plant morphometric measurements at week 20. Statistical significance is indicated with an asterisk ($^* = p < 0.05$). Abbreviations: LL = longest leaf, YL = youngest leaf.
Figure 3. Plant morphometric at six months post single application of SM or the same amount of water (CTR). Statistical significance is indicated with an asterisk (* = p < 0.05). Abbreviations: LL = longest leaf, YL = youngest leaf.

3.3. Soil Microbiota Responded to SM Application

The microbial community was dominated by the members of the phylum Actinobacteria which comprised more than 50% of sequence reads in all of the samples (Figure S3 of the Supplementary Materials). This was followed by Firmicutes, Proteobacteria, Chloroflexi and Acidobacteria, trailed by a range of other phyla shown in Figure S3. At a genus level, the most abundant known genera included the most plentiful Rubrobacter, 67–14, Bacillus, Conexibacter, Gaiella, Solirubrobacter and others (Figure 4). Figure 4 shows the local contribution of individual samples to beta diversity and the top 20 genera.

In terms of alpha diversity, we investigated a range of richness and diversity measures, including Observed features, Chao1, ACE, Shannon, Simpson, InvSimpson, Fisher and Coverage. Neither of the measures showed significant differences between CTR and SM or any other variables. We then investigated temporal variation in all alpha measures to find no significant alterations in any sampling time points. Figure 5 shows temporal variations in Alpha diversity parameters in CTR and SM groups with typically no significant differences between CTR and SM treatments over the duration of the experiment. Prolonged drought occurred from the application at week 0 to week 5, with significant rain events at weeks 5 and 7 with no further rain after week 8. The temporal diversity graphs show that diversity drop due to drought started to recover after week 8, three weeks after the first and one week after the second major rainfall in both CTR and SM plots.
Figure 4. Local Contribution to Beta Diversity (LCBD) bar chart at the genus level. The larger circle above the sample indicated a higher contribution to differences between CTR and SM groups.

Figure 5. Temporal alterations in alpha diversity measures. Changes in Chao1, Shannon and Simpson index are shown every week from weeks 0 to 16.
3.4. Multivariate Analysis

Multivariate analysis was performed to estimate the overall changes in the microbial community. PERMANOVA analysis (Weighted Unifrac) using a mixed effects design (Primer 7e) showed a marginal effect of overall community change by Treatment (CTR vs. SM \( p = 0.119 \)); and a significant influence of Plot \( p = 0.014 \) and Time \( p = 0.015 \). Investigating Treatment (CTR vs. SM) as a Time independent variable (all CTR vs. all SM samples) suggested that the differences between CTR and SM are highly significant \( p < 0.001 \). Pooling temporal data across the time would be justified here since the Treatment-Time interaction \( p \)-value using both weighted and unweighted Unifrac distance was higher than 0.3 [50]. We then performed a Simper analysis at genus level data (Primer 7e) to identify the genera driving the differences in beta diversity between CTR and SM (Table 2). To tease out observed changes in microbiota community between CTR and SM at a genus level, we extended this analysis to biomarker discovery tool using Random Forest, Metastats and LefSe (all data shown in Table S1 of the Supplementary Materials) to find that the outputs highly matched between the methods and with Simper results (Table 2). The selected genera responding to SM supplementation are shown in Figure 6.

Using Primer 7e we investigated the change in microbial community structure against temporal separation between soil samples collected in CTR and SM plots. While observed temporal trajectories of soil microbiota appeared different between CTR and SM treatments (Figure S4), there was clearly substantial variation in trajectory over time for each treatment, which likely obscured any clear interaction in the PERMANOVA analysis. Despite major rain events, severe temperatures and other environmental challenges that occurred over the 16 weeks, there were no differences in the microbiota development timeline that would suggest that any of these events affected CTR or SM communities differently (Figure S4 of the Supplementary Materials).

<table>
<thead>
<tr>
<th>Genus</th>
<th>Contribution%</th>
<th>Cumulative%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus</td>
<td>7.14</td>
<td>7.14</td>
</tr>
<tr>
<td>Rubrobacter</td>
<td>5.86</td>
<td>13</td>
</tr>
<tr>
<td>Unknown Gaiellales</td>
<td>4.14</td>
<td>17.14</td>
</tr>
<tr>
<td>Unclassified Gaiellales</td>
<td>3.7</td>
<td>20.83</td>
</tr>
<tr>
<td>Unknown Bacillales</td>
<td>3.35</td>
<td>24.18</td>
</tr>
<tr>
<td>67-14</td>
<td>3.23</td>
<td>27.41</td>
</tr>
<tr>
<td>Unclassified Micromonosporaceae</td>
<td>2.83</td>
<td>30.24</td>
</tr>
<tr>
<td>Acidothermus</td>
<td>2.41</td>
<td>32.64</td>
</tr>
<tr>
<td>MB-A2-108</td>
<td>2.37</td>
<td>35.01</td>
</tr>
<tr>
<td>Micromonospora</td>
<td>2.24</td>
<td>37.25</td>
</tr>
<tr>
<td>Unclassified Xanthobacteraceae</td>
<td>2.2</td>
<td>39.45</td>
</tr>
<tr>
<td>Solirubrobacter</td>
<td>2.19</td>
<td>41.64</td>
</tr>
<tr>
<td>Gaiella</td>
<td>2.18</td>
<td>43.82</td>
</tr>
<tr>
<td>Conexibacter</td>
<td>2.03</td>
<td>45.85</td>
</tr>
<tr>
<td>Planosporangium</td>
<td>1.87</td>
<td>47.72</td>
</tr>
<tr>
<td>Candidatus Udaeobacter</td>
<td>1.83</td>
<td>49.55</td>
</tr>
<tr>
<td>Geodermatophilus</td>
<td>1.82</td>
<td>51.37</td>
</tr>
<tr>
<td>Unclassified Gaiellales</td>
<td>1.71</td>
<td>53.08</td>
</tr>
<tr>
<td>Jatrophihabitans</td>
<td>1.65</td>
<td>54.73</td>
</tr>
<tr>
<td>TK10</td>
<td>1.47</td>
<td>56.2</td>
</tr>
<tr>
<td>Unclassified Kineosphoriaceae</td>
<td>1.45</td>
<td>57.65</td>
</tr>
<tr>
<td>Pseudonocardia</td>
<td>1.3</td>
<td>58.95</td>
</tr>
<tr>
<td>JG30-KF-CM45</td>
<td>1.27</td>
<td>60.22</td>
</tr>
</tbody>
</table>

Table 2. Simper analysis shows the genera that are contributing to the dissimilarity between CTR and SM. The average dissimilarity between CTR and SM was 38.87%.
Figure 6. Genera responding to SM treatment identified using biomarker discovery tools and Simper analysis. All genera are highly significantly altered (p < 0.001), as shown in Table S1 in the Supplementary Materials and Table 2.
3.5. Microbiota-Environment Interactions

Although there were no major alterations in mineral profile post SM application, but rather delicate changes in particular minerals, the mineral and soil chemistry parameters influenced the abundance of a range of genera (Figure 7 unweighted Unifrac and Figure S5 weighted Unifrac at a phylum level) differently in CTR and SM treated plots. In addition to mineral microbiota interactions, the mineral profile, particularly Fe reduced Shannon and Simpson diversity (Figure S6 of the Supplementary Materials). Figure 7 shows interactions of genera and minerals, pointing at subtle differences in response to minerals in CTR and SM-treated plots. Here, we were interested in identifying opposite trends. For example, Gaiella was significantly negatively correlated with EC, N and C only in CTR, however, the same negative yet marginal correlation was also observed in SM plots, noticeable but failing to make the significance threshold, as the same overall response. On the other hand, Rb41 was significantly negatively correlated with P in CTR but marginally positively correlated with SM plots. Microbispora was significantly positively correlated with Mo in CTR but marginally negatively correlated with Mo in SM plots.

Figure 7. Mineral and genera interactions differed between CTR and SM plots. The figure shows the Pearson correlations of the taxa and mineral concentrations using the Unweighted Unifrac distance matrix. Statistical significance is indicated with an asterisk (* = p < 0.05, ** = p<0.01 and *** = p<0.001).

4. Discussion

The soil chemistry profile was unaffected by SM application which was an expected result as the dilution rate of 4 mL/m² delivers a minimal amount of product, and it was intended to affect the soil microbial community rather than act as a soil amendment. Dry matter increased significantly in SM-treated plots compared to CTR twenty weeks post
application. SM plots had a slightly lower dry matter than control before the SM was applied at week zero, however, by week 20, SM treated plots showed a significant increase in the dry matter while control plots did not. At week 48 (6 months post-application of SM), the dry matter was still significantly higher than in control plots. After separating components of dry matter, an increase in in the litter was observed. This could be due to the ungrazed older plants drying due to the hot weather. A higher amount of litter will inevitably increase the organic matter in the soil. Organic matter and mineral transformation into the form available to the plant is a critical element of soil health and vital for regenerative and sustainable farming practices. This transformation is driven by a healthy soil microbial community, whose different microorganisms are responsible for transforming organic matter into plant available nutrients, disease and pest control and many other benefits [51]. The single low application in this experiment boosted biomass, but more work is needed to determine SM concentration and application rate that sustains higher biomass. This then may also lead to improved nutrient cycling back into the soil, and a faster improvement of soil health.

A single application of SM did not produce changes in the dicot weed (sage) at week 20, but at week 48, six months post application, sage exhibited a marginal improvement in the length of younger leaves and roots and a significant improvement in the total number of roots over control plants. Critically, there was a significant improvement in the total number of roots in the grass at week 20, which was still significantly higher six months post application. This is promising because PD affects grass roots by stunting, diminishing the root size and necrotising the root tissue [23].

In this study, the microbial community was dominated by phyla essential to preserving soil health, able to metabolise and degrade organic matter materials like cellulose, hemicellulose, chitin, and pectin. They also intervene in nitrogen, sulphur, carbon, phosphorus, and many more nutrient cycles. The two most dominant phyla included Actinobacteriota and Firmicutes. Actinobacteriota (over 50% of total abundance) is a known degrader of cellulose, hemicellulose, and pectin. It is also proven capable of removing contaminants like heavy metals and pesticides and producing antibiotics [52,53]. Members of Firmicutes, the second most abundant phylum, have a critical role in plant disease control [54–56].

Genera increased by SM application (Figure 6) included Bacillus, whose species can secrete Indole-3-acetic acid (IAA), the most common naturally occurring growth hormone in plants [57,58], and strengthen the plant against drought stress [59,60]. Bacillus can also protect plants from insect attacks [61] and survive in heavy metal-contaminated soils while promoting plant growth [62]. SC-I–84 genus, which was also significantly higher in SM plots, is associated with high levels of P and N, assisting plants in coping with drought and converting N into the available form [63,64].

Micromonospora species produce antibiotics, assist biocontrol [65], have antifungal and growth promotion properties, and help root ecology [66–68]. Amycolatopsis has antibiotic-producing species [69,70]. Streptomyces has biocontrol abilities, improve salt tolerance, and produce antibiotics [71–73]. Microbispora species are cellulyotic and hemicellulolytic antibacterial [74–76]. Actinoallomurus produces bioactive metabolites, antibiotics [77,78].

Further into the SM increased genera, we find TK10 genus of Chloroflexi, whose species are associated with organic carbon and nitrogen, accelerating C decomposition. Mycobacterium can degrade polycyclic aromatic hydrocarbons in contaminated soils [79–81]. RB41 maintains metabolic and biogeochemical functions in low nutrient or stressed soils [82], and assists carbon flow through bacteria respiration [83,84]. Solibacter species break down organic carbon and participate in Nitrite-Nitrate reduction [85]. Bryobacter species are involved in degrading complex organic compounds [86]. Sphaerobacter is a thermophilic, lignin and lignocellulose degrading species in soil [87,88]. Cohnella species inter-
vene in N fixation [89] and are radiation tolerant [90]. *Bradyrhizobium* intervenes in N fixation and soil fertility [91]. *Jatrophihabitans* are endophytes of plant roots, sensitive to heavy metals in soil [92,93], and it has the potential to be beneficial [94].

Overall, a low single SM application increased the range of beneficial microbial genera capable of supporting the plant against various stresses, promoting growth, improving soil quality, and providing endophytic assistance to the plants. On the other side, genera decreased by SM application include 67-14 genus of *Solirubrobacteriales*—*Gaellales* associated with Pb and Zn content in soil [95], which positively reacts to K [96]. *Rubrobacter* is temperature and radiation tolerant [97]. *Gaiella* intervenes in the N cycle [98]. MB-A2-108 species increase with afforestation, increasing soil organic carbon and declining with straw mulching [99,100]. *Geodermatophilus* Gamma and UV radiation resistant, these species are characteristic of arid and poor soils [101,102]. *Pseudonocardia* species are associated with heavy metal pollution in soil and have antifungal properties [64,103,104]. JG30-KFCM5 denitrifying species are beneficial for tobacco growth in reduced N conditions [105,106]. *Rokubacteriales* are involved in N respiration [61]. *Luedemannella* species mediates nutrients and energy fluxes between bacteria in drought conditions [107]. Thus, the bacteria reduced in SM include those associated with very poor, arid and heavy metal polluted soils, as well as radiation and temperature tolerant species.

SM application altered the correlations of minerals and taxa. For example, in Figure 7, *Jatrophihabitans* is significantly (*p* < 0.01) positively correlated with Ca concentrations in CTR plots, while in SM plots, it shows a marginal negative correlation. As another example, *Actinophycocyla* is significantly (*p* < 0.001) positively correlated with Mo in SM plots, while there are no correlations in CTR plots. This trend extends to both weighted and unweighted Unifrac distance data. This can indicate that Mo, for example, can increase the abundance of *Actinophycocyla* only in the presence of SM application. Thus, interactions of soil nutrients with taxa could be one of the mechanisms of the SM-driven alterations of the microbial community.

5. Conclusions

The application of a low concentration of sea minerals significantly increased total plant dry matter 20 weeks after a single application, which would translate to 967 kg/ha more dry matter than in untreated control plots. This trend continued with a marginal increase six months after the application, with an additional 498 kg/ha. SM application significantly boosted the number of roots six months post application, which could be driven by the significant alterations in microbial community and soil nutrients-microbiota interactions. Our data demonstrate the benefits of SM application to damaged, unproductive or exhausted soils and its capacity to restore and improve soil microbiota with minimal intervention. Expanding the research into sea mineral agricultural applications, started by Murray and Valentine [33], is long overdue, especially considering the damage artificial fertilisers do to agricultural soils. On the other hand, measuring the concentration of minerals before and after SM application confirmed no significant difference in any of the minerals or soil parameters between CTR and SM, 12 weeks after the application. This can eliminate the concerns that SM would negatively alter soil chemistry parameters and reiterate the benefits plants and soil microbiota can obtain from a very low level mineral supplementation.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su142214873/s1, Figure S1: Dry matter alterations 20 weeks post SM application; Figure S2: Six months after the single application, dry matter showed marginally increased total dry matter in SM compared to CTR, the same trend from the data at 20 weeks; Figure S3: Phylum level taxa; Figure S4: Temporal variation in microbiota maturation; Figure S5: Interactions of soil chemistry parameters with the Phylum level microbiota analysed separately in CTR and SM; Figure S6: Mineral profile influence on alpha diversity measures; Table S1: Biomarker Discovery–Genus Level.
Author Contributions: CRediT author statement: M.W.W.: Investigation, Methodology, Formal analysis, Writing—Original Draft. X.R.: Investigation, Methodology, Writing—Review and Editing. S.J.Y.: Investigation, Methodology, Writing—Review and Editing. A.D.I.: Methodology, Writing—Review and Editing. T.T.: Methodology, Writing—Review and Editing. Project administration. Y.S.B.: Investigation, Methodology, Formal analysis, Project administration, Writing—Review and Editing. D.S.: Conceptualisation, Methodology, Formal analysis, Funding acquisition, Writing—Review and Editing. All authors have read and agreed to the published version of the manuscript.

Funding: Dr David Tomlinson, provided funding towards the scholarship for MMW.

Institutional Review Board Statement: No animals were used in this trial.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data associated with this study have been deposited in the NCBI SRA sequencing database under the accession number PRJNA887675 (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA887675).

Acknowledgments: The data was analysed using the Marie Curie High-Performance Computing System at Central Queensland University. We acknowledge and appreciate Jason Bell’s help in all aspects of High-Performance Computing. We also wish to acknowledge continual support in our pasture dieback investigations by Fitzroy Basin Association and numerous local farmers. We also thank Robert Alder (Geo Leak Solutions), Mick and Noela Alexander for their continual advisory and in-kind help with the project.

Conflicts of Interest: The authors declare no conflict of interest.

References


54. Charalamous, A.; Grivogiannis, E.; Dieronitou, I.; Michael, C.; Rahme, L.; Apidianakis, Y. Proteobacteria and Firmicutes secreted factors exert distinct effects on *Pseudomonas aeruginosa* infection under normoxia or mild hypoxia. *Metabolics* 2022, 12, 449.


