

Brief Overview

Field trials plots at ShoEi were amended with various Biorock amendment formulations at varying application rates beginning in 2020. Soil samples were collected in 2024 to analyze if the microbial communities have been altered as a result of Biorock applications.

Compared to the control plot, the Biorock treated plot had higher bacterial richness (number of different species) in Biorock treated plot compared to control plot. The higher bacterial richness was associated with higher metabolic potentials related to the nitrogen cycle. Nitrogen fixation metabolic potential ($\text{N}_2 \rightarrow \text{NH}_3$) was 46% higher in the Biorock treated plots compared to the control plots, but denitrification ($\text{NO}_3 \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2$) was also 36% higher in the treatment plots. Nitrate reduction ($\text{NO}_3 \rightarrow \text{NO}_2 \rightarrow \text{NH}_3$) potentials were also 36% higher in the Biorock treatment plot compared to the control plot. There were also greater abundances of nitrogen fixing bacteria in the Biorock treatment plot compared to the control plot. This could be explained by the lack of synthetic nitrogen applied in the treatment plot which inhibits naturally occurring nitrogen fixing bacteria.

Methods and Test Protocols:

Field trial design.

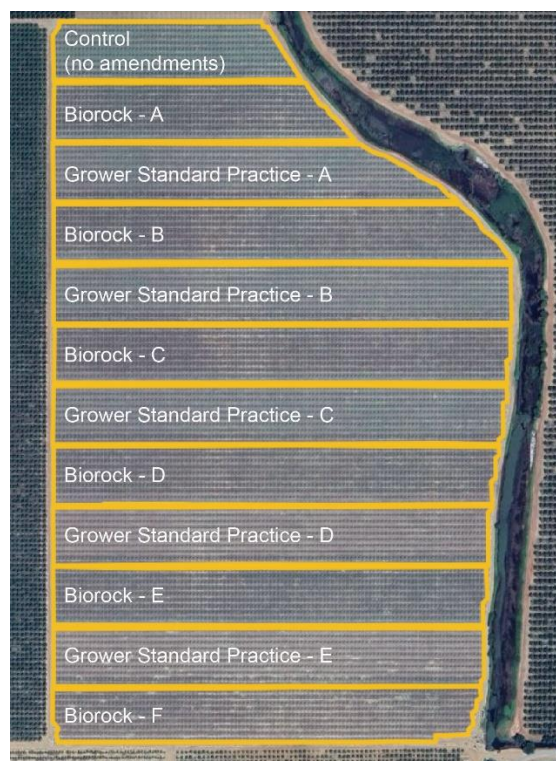


Figure 1. ShoEi almond field trial layout.

Table 1. ShoEi almond plot design and amendment application rates.

Plot ID	Soil Amendment	Application Rate
Control Plot	No soil amendments/fertilizer added	NA
Standard Plot A-E	Standard fertilizer application	UN-32 (30 lbs/acre) kelp (0.25 gal/acre) 2-17-17 fertilizer (1 gallon/acre) calcium (1 gallon/acre) Potash (500 lbs) Zinc (500 lbs) MAP (50 lbs)
Biorock Plot A	Legacy Biorock	1 ton (2,000 lbs) per acre
Biorock Plot B	New Foundation Biorock	1 ton (2,000lbs) per acre
Biorock Plot C	New Foundation Biorock (+) dormant fungal spore inoculant	1/2 ton (1,000lbs) per acre
Biorock Plot D	New Foundation Biorock (+) dormant fungal spore inoculant	1/4 ton (500lbs) per acre
Biorock Plot E	New Foundation Biorock (+) dormant full suite spore inoculant	1/4 ton (500lbs) per acre
Biorock Plot F	New Foundation Biorock (+) dormant full suite spore inoculant + basalt rock dust + Foundation Biorock	New Foundation+: 1 ton/2,000lbs/acre Basalt: 1 ton/acre Foundation: 1 ton/acre

Crop health and yield analysis.

The ShoEi almond trees are expected to reach maturity in 2025 when they are expected to produce their first harvest of almond nuts.

During crop growth, the control and field trial plots were routinely monitored using satellite imaging to assess growth with red edge spectra (NDVI, RECI, NDRE). The normalized difference vegetation index (NDVI) measures the biomass of crops that are actively photosynthesizing. The red-edge chlorophyll vegetation index (RECI) shows the photosynthetic activity of the crop leaves. The normalized difference red edge vegetation index (NDRE) is used to monitor crops during the maturity stage.

Soil bacterial community assessment.

Soil samples were taken from Biorock plot E and the grower standard plot F and sent for 16S rRNA amplification genomic sequencing (BiomeMakers, Inc). Bacterial taxonomy was assessed for highly abundant species, and species that co-occurred in the raw material applied and those found in the treated soils.

Results

ShoEi almond trial taxonomy analysis.

Soil samples were collected from both a control plot and a Biorock treated plot from the ShoEi almond orchard trial plots. Genetic sequencing analysis found 20% more bacteria species in the Biorock treated plot compared to the control plot (Figure 2). After incorporating species abundances into a bacteria diversity index, it shows that the overall diversity is near equal in both plots. This tells us that the species abundances in the control plot are more evenly distributed, whereas the species in the Biorock treated plot contain more rare or unique species. This is further supported by the fact that there were only 93 species unique to the control plot (not found in the Biorock treated plot), yet there were 215 species that were unique to the Biorock treated plot (not found in the control plot).

Overall, this suggests that Biorock is both contributing novel species to the soil microbial community and fosters the growth and activity of other unique species likely through strengthened mutualism relationships.

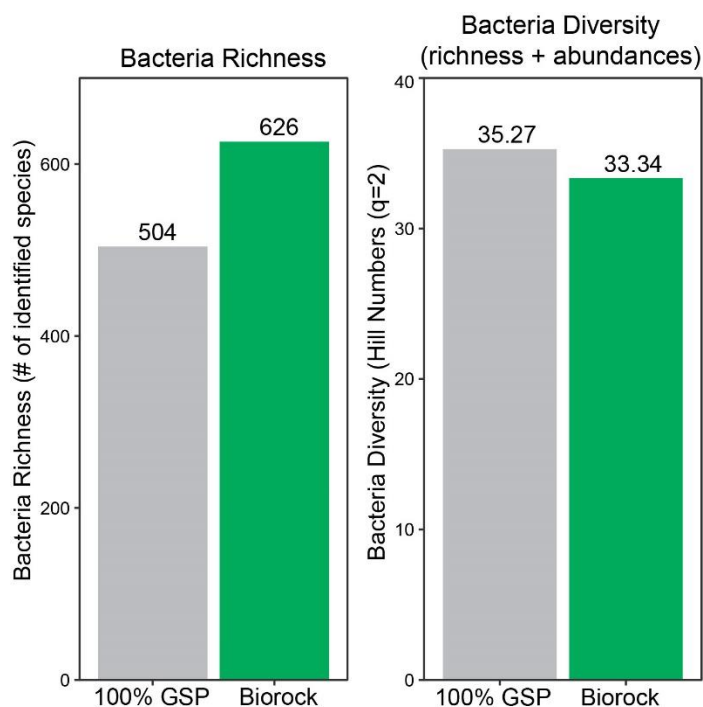


Figure 2. Bacterial richness and diversity from a control plot compared to a Biorock amended plot.

We further looked at what species were present in only the Biorock control plot and the raw material that was applied to the treatment plot to see which species were being imparted to the soil from the Biorock itself. We found nine species that were novel to the native soil coming from the Biorock material (Table 5). These species were generally found in low abundances in both the mix and the soil. However, most notably we found a *Sporosarcina* species that was highly abundant (18%) in the NFX-1 mix in the Biorock treated plots. This genus, as reported above, forms endospores and can survive harsh conditions. A species of the *Sporosarcina* genus is well-known for its ability to hydrolyze urea which in turn forms calcium carbonates in alkaline environments.

The *Rhodopseudomonas* genus is a photosynthetic bacterium that can fix nitrogen and denitrify with a very wide range of metabolic pathways and potentials (Larimer et al., 2004). *Hyphomicrobium facile* is capable of methylotrophic growth, meaning it can oxidize one-carbon compounds like methanol and methylamine into formaldehyde, which is then assimilated into biomass. By removing methanol from the soil, they are then inhibiting denitrification, or the release of nitrogen into the atmosphere as nitrous oxides.

Table 2. Bacteria species present in Biorock amended plot and raw material applied.

Phylum	Species	Biorock plot abundance (%)	Raw material NFX-1 abundance (%)
Actinobacteriota	<i>Saccharopolyspora</i> sp.	0.009	0.009
Actinobacteriota	<i>Nonomuraea</i> sp.	0.014	0.026
Actinobacteriota	<i>Sphaerisporangium</i> sp.	0.012	0.028
Bacteroidota	<i>Chryseobacterium</i> sp.	0.004	0.012
Firmicutes	<i>Sporosarcina</i> sp.	0.005	17.565
Firmicutes	<i>Herbinix</i> sp.	0.019	0.028
Proteobacteria	<i>Hyphomicrobium facile</i>	0.019	0.009
Proteobacteria	<i>Rhodopseudomonas</i> sp.	0.007	0.009
Proteobacteria	<i>Sulfurifustis</i> sp.	0.006	0.028

*None of these nine species were found in the adjacent control plot.

Species in the *Sporosarcina* genus are known for microbial induced calcium carbonate precipitation (MICP) via urea hydrolysis (Lapierre et al., 2020). *S. pasteurii* produces urease, which hydrolyzes urea into ammonia and carbon dioxide, a process which increases pH. As pH increases, calcium carbonates are formed when calcium ions are present (which are in abundance in basalt-based rock dust) (Ghosh et al., 2019). Species in this genus are often used in environmental engineering to “cement” soils with calcium carbonates as well as sequestering carbon in soils. Species in the *Rhodopseudomonas* genus are capable of nitrogen fixation and act as bioremediation species removing soluble cadmium from soils (Su et al., 2024). Species from the *Chryseobacterium* genus can promote plant growth and have been explored as a biocontrol agent (Jung et al., 2023).

ShoEi almond trial bacteria metabolic potential.

Analysis of the metabolic potential of the control and Biorock treated plots at the ShoEi field trial shows an overall increase in nitrogen cycle functions. The potential of nitrogen fixation increased by 46% in the Biorock treated plots. In general, there is a 40% increase in nitrogen related metabolic functions in the Biorock treated plots. The increases in denitrification and nitrogen respiration potentials are not ideal. However, keep in mind that metabolic potential just means the identified bacteria only have the capability to do these functions, and does not indicate whether or not the functions are actually being performed.

Quite honestly, these results are surprising given how large the increases in nitrogen related function are in the Biorock treated plots compared to the control. Further tests to determine enzyme activity or rates of nitrogen fixation etc should help inform us whether or not these metabolic potentials are being actualized in the field.

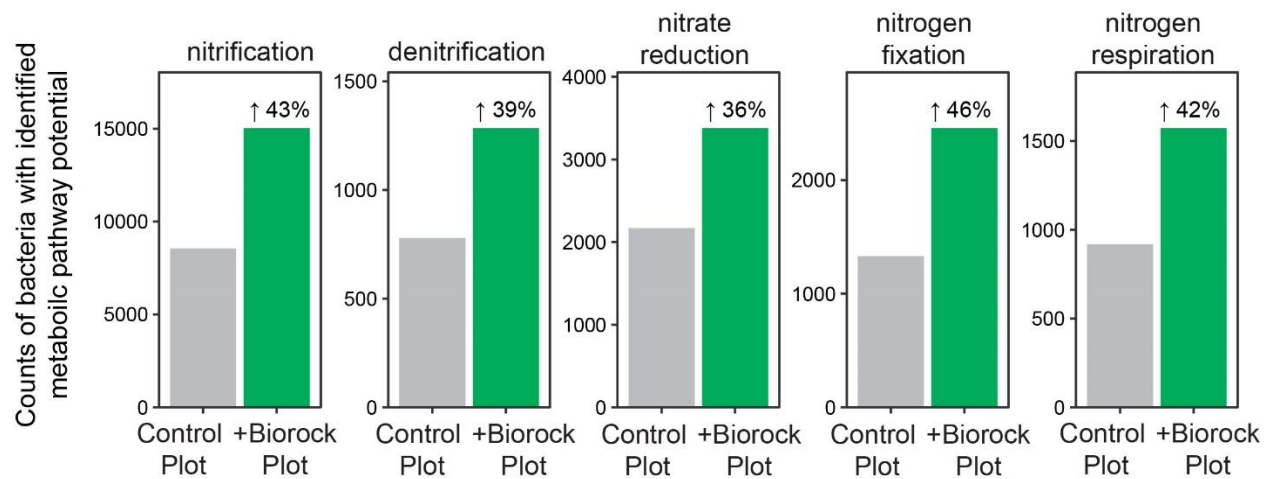


Figure 3. Summary of nitrogen related metabolic potentials from the grower standard practice and Biorock treated plots at the Shoei Almond trial. Nitrification is aerobic ammonia oxidation and nitrite oxidation, produces nitrate. Denitrification is the dissimilatory reduction of fixed nitrogen compounds (NO_3^- , NO_2^- , and N_2O) to dinitrogen (N_2). Nitrate reduction is the conversion of nitrate to ammonia. Nitrogen fixation is the conversion of dinitrogen gas to ammonia. Nitrogen respiration consists of dissimilatory reduction, nitrate and nitrite respiration, denitrification, ammonification and anammox.

Individual species identified as nitrogen-fixing species in the Biorock treated plots were generally higher compared to the control plot. The increase in the number of species with nitrogen-fixing metabolic capabilities is directly correlated with the increase in nitrogen-fixing metabolic potential shown in Figure 3.

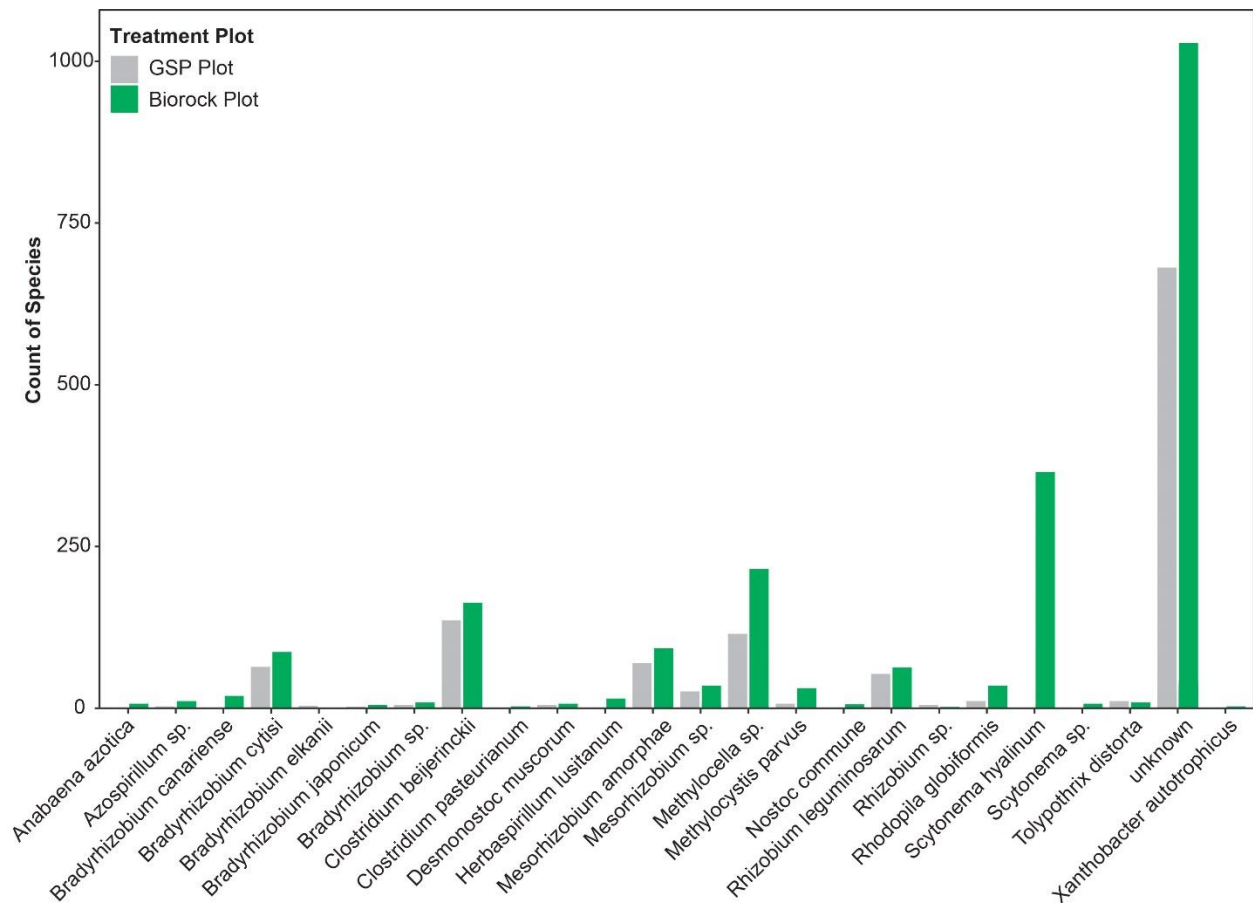


Figure 4. Positively identified nitrogen-fixing species found in the grower standard practice and Biorock treated plots at ShoEi orchards.

Plant growth indices.

Vegetation growth indices monitored with the Sentinel 2 satellite imaging for the growing season (April-October) of 2024 showed no statistical differences across the three treatment groups. However, the NDVI, NDRE and ReCI indices were generally higher in the Biorock treatment plots compared to the control plot which received no amendments.

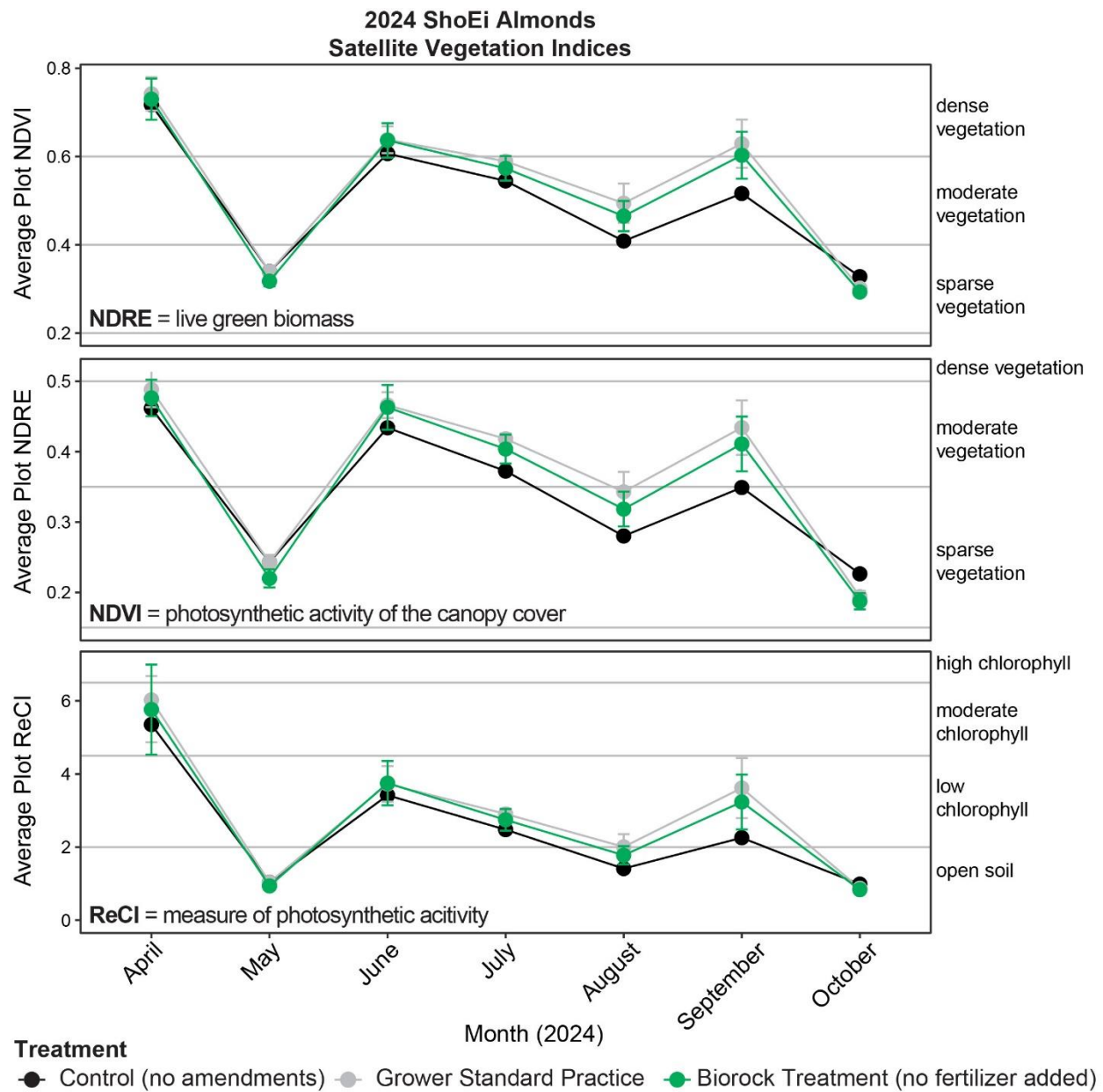


Figure 5. NDVI, NDRE, ReCI vegetation indices from the control, grower standard practice and Biorock treatment plots.

Summary

Bacteria taxonomic analysis shows that after multiple years of Biorock addition, the bacterial richness is higher in the Biorock plot compared to the grower standard practice plot. Higher bacterial richness was associated with greater nitrogen fixing metabolic potentials as well.

Future Directions

Biorock has recently been re-applied in late 2024 to the treatment plots. We aim to take soil samples from all of the plots to identify differences in soil chemical and biological properties. This

includes soil organic carbon concentrations, pH, CEC, and concentrations of trace metals. We further aim to assess changes in microbial genomic differences with the intent of linking these chemical and biological changes to crop yield.